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Full Length Research Paper

Effect of aqueous extract of scent leaf (*Ocimum gratissimum*) on carbon tetrachloride (CCl_4) induced liver damage in albino Wister rats

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The effect of aqueous leaf extract of *Ocimum gratissimum* was investigated in rat models of liver injury induced by carbon tetrachloride (CCl_4). Treatment of separate groups of rats with 2.5 ml/kg body weight of 5, 10 and 15% aqueous extracts of *O. gratissimum* for 3 weeks after establishment of CCl_4 induced liver damage, resulted in significantly ($p < 0.05$) less hepatotoxicity than with CCl_4 alone, as measured by serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. For serum alanine aminotransferase, activity decreased from 68.95 ± 21.38 U/l to 35.77 ± 1.48 U/l, while for aspartate aminotransferase, activity level decreased from 165.65 ± 17.75 to 110.10 ± 3.05 U/l and for alkaline phosphatase, activity level decreased from 364.65 ± 37.75 to 212.74 ± 15.27 U/l. The reduction though not statistically significant ($p < 0.05$) was dose dependent. Histopathological findings also suggest that treatment with aqueous extracts of *O. gratissimum* after establishment of CCl_4-induced liver damage significantly reduced and even reversed the liver damage in the rats. The results of the study indicate that *O. gratissimum* might be an effective plant hepatoprotector in the diet of patients with hepatopathies.

Key words: Aqueous extract, *Ocimum gratissimum*, hepatoprotector, hepatotoxicity, carbon tetrachloride.

INTRODUCTION

The use of herbal products for medicinal benefits has an important role in nearly every culture on earth. Herbal medicine was practised by the ancient people of Asia, Europe and the Americas (Wargovish et al., 2001). Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programmes. Many natural and artificial agents possessing anti-oxidative properties have been proposed to prevent and treat hepatopathies induced by oxidative stress (Lieber, 1997; Cervinkova and Drahota, 1998). There is increasing evidence for the hepato-protective role of hydroxyl and polyhydroxy-organic compounds particularly from vegetables, fruits and some herbs (Bass, 1999). *Ocimum gratissimum* is a widely used local plant in Nigeria for both nutritional and therapeutic purposes. Mostly a weed of roadsides and wasteland, but is also important in pastures. It is not a problem in cultivation. It prefers moist and fertile soils during growth, but will tolerate drought after flowering. *O. gratissimum* in the coastal areas of Nigeria is used in the treatment of epilepsy (Osifor, 1992), high fever (Oliver, 1980) and diarrhoea (Oliver, 1980; Sofowora, 1993), whilst in the savannah areas, decoctions of the leaves are used to treat mental illness (Abdulrahman, 1992). The whole plant is used as an antibacterial agent throughout West Africa (Iwu, 1993). Oboh (2004) reported the antioxidant and antimicrobial properties of *O. gratissimum*. The extracts of *O. gratissimum* exhibited antibacterial activity (Oforkansi et al., 2003). The liver is the key of metabolism, secretion and excretion and it is continuously and variedly exposed to xenobiotics,

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environmental pollutants and chemotherapeutic agents because of its strategic location in the body. Liver diseases are a world wide problem. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace currently used drugs of doubtful efficacy and safety. In this study, we report the effects of aqueous leaf extracts of *O. gratissimum* on carbon tetrachloride-induced liver damage in rats.

MATERIALS AND METHODS

Animals

Male Wister albino rats (100 – 150 g) of about three months old bred in the animal house of Biochemistry Department, University of Port Harcourt were used in this study. The animals were randomly selected and kept in 6 groups of three animals per group. Each group was caged separately. All animals were fed with commercial rat feed and distilled water *ad libitum*. The cages were cleaned daily and food and water changed daily. The animals were allowed to acclimatise for two weeks.

Chemicals

All chemicals used in the study were of analytical reagent grade.

Preparation of aqueous extract

The leaves of *O. gratissimum* were collected from Sagbama in Bayelsa State of Nigeria and were identified at the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

The leaves were sun dried, pulverized and sieved. A 25 g portion of the powdered leaf was weighed out and mixed with 250 ml of distilled water. The mixture was shaken and kept on the laboratory bench for 24 h before filtering. The filtrate was evaporated to dryness at room temperature in a rotary evaporator. From the stock of scent leaf extract, 5, 10 and 15% (w/v) solutions were prepared.

Carbon tetrachloride model for evaluation of antihepatotoxic activity

The CCl_4 model described by Obi et al. **(**1998), was used for scheduling the dose regimen. 0.5 ml/kg intraperitoneally of carbon tetrachloride diluted in vegetable oil (1:1) was employed for inducing liver damage.

Experimental procedure

The animals were assigned to one of six groups each of not less than three rats per group. Group I which served as control group was not treated with CCl_4, Group II received vegetable oil 0.5 ml/kg intraperitoneally, Group III received CCl_4: vegetable oil (1:1) 0.5 ml/kg intraperitoneally, Group IV received CCl_4: vegetable oil (1:1) 0.5 ml/kg + 5% *O. gratissimum* 2.5 ml/kg. Group V received CCl_4: vegetable oil (1:1) 0.5 ml/kg + 10% *O. gratissimum* 2.5 ml/kg, while Group VI received CCl_4: vegetable oil (1:1) 0.5 ml/kg + 15% *O. gratissimum* 2.5 ml/kg. At the end of treatment, once daily for 7, 14 and 21 days respectively, blood samples were collected by direct cardiac puncture and the serum seperated. The liver was received and part of the right lope was sliced, fixed in 10% buffered formaldehyde solution and used for histological examination.

Assessment of liver function

Biochemical analysis of the serum enzymes for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was by the method of Reitman and Frankel, 1957. Alkaline phosphatase (ALP) was assayed according to the method of REC (1972).

Statistical analysis

Results of the biochemical estimations are reported as Mean ± SD. Statistical analysis was performed using students t-test and $P \leq 0.05$ being considered statistically significant.

RESULTS

There was a significant ($p \leq 0.05$) increase in the level of serum aminotransferase and alkaline phosphatise activities in the CCL_4 treated rats when compared with the normal (control) rats (Table 1).

The intraperitoneal administration of CCl_4 to experimental animals brought about markedly increased serum aminotransferase and alkaline phosphatase activities (used for assessing liver function) in rats treated with CCl_4 only and with significantly lower activities of these enzymes in rats additionally treated with different doses (5, 10 and 15%) of *O. gratissimum* extract. The reduction of the ALT, AST and ALP activities by *O. gratissimum* extract was dose dependent though not statistically significant ($p > 0.05$) as seen in Table 2.

Table 3 shows that the effect of time administration of *O. gratissimum* extract on ALP, AST and ALT activities was statistically significant ($p < 0.05$). The liver of rats in groups 1 and 2 showed a normal architecture, cords of hepatocytes well preserved, cytoplasm not vacuolated, sinusoids well demarcated, no area of necrosis, no fatty change, no fatty degeneration and no area of infiltration by inflammatory cells. In carbon tetrachloride treated livers, drastic alterations were observed. Histopathological examination showed extensive fatty change, distended hepatocytes, vacuolated cytoplasm, compressed sinusoids, fatty degeneration, area of necrosis and infiltration by inflammatory cells. *O. gratissimum* – CCL_4 treated rats which are the test groups (4, 5 and 6) showed significant recovery.

There were some parameters in the test groups that were not only close to normal but even reverted completely to normal. These findings correlated with markedly increased serum aminotransferase and alkaline phosphatise activities in rats poisoned with CCl_4 only and with significantly lower activities of these enzymes in rats additionally treated with aqueous extracts of *O. gratissimum*.

Table 1. AST, ALT and ALP activities of normal rats and rats poisoned with carbon tetrachloride (CCL_4).

Parameters	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal (control)	35.10 ± 3.77	120.17 ±2.91	247.30±3.56
CCL_4 Treated	68.95 ± 21.38[a]	165.65 ± 17.75[a]	364.65±37.75[a]

Results represent the Mean ± SD of three estimations.
a = significantly different from the normal (control) group ($p < 0.05$).

Table 2. Effect of aqueous extract of scent leaf (*O. gratissimum*) on carbon tetrachloride (CCL_4) hepatotoxicity.

Experimental groups	ALT (U/l)	AST (U/l)	ALP (U/l)
Group 1 (normal)	35.10 ± 3.77[b]	120.17 ± 2.91[b]	247.30 ± 3.56[b]
Group 2 (Veg. oil)	35.30 ± 5.42[b]	122.58 ± 3.25[b]	214.24 ± 6.43[b]
Group 3 (CCL_4)	68.95 ± 21.35[a]	165.65 ± 17,75[a]	364.65 ± 37.75[a]
Group 4 (5% O.G + CCL_4)	37.31 ± 2.15[b]	112.55 ± 4.8[ab]	221.86 ± 15.71[ab]
Group 5 (10% O.G + CCL_4)	35.77 ± 1.48[b]	110.10 ± 3.05[ab]	212.24 ± 15.27[ab]
Group 6 (15% O.G + CCL_4)	40.09 ± 4.61[b]	120.04 ± 6.51[ab]	221.65 ± 17.94[b]

Results represent the Mean ± SD of three estimations. a = significantly different from the normal control group. b = significantly different from CCL_4 treated group.

Table 3. Effect of time (duration) of administration of aqueous extract of scent leaf (*O. gratissimum*) on carbon tetrachloride (CCL_4) hepatotoxicity.

	Period	ALT (U/L)	AST (U/L)	ALP (U/L)
Group 3 (CCL_4)	7 days	80.60±0.59[a]	177.50±0.54[a]	375.36±0.94[a]
	14 days	57.70±0.29[b]	157.00±1.30[b]	381.92±0.26[b]
	21 days	48.80±0.40[c]	145.00±0.10[c]	309.00±0.92[c]
Group 4 (5% *O.G* + CCL_4)	7 days	37.00±0.53[d]	114.00±0.28[d]	225.23±0.88[d]
	14 days	39.60±0.15[e]	116.46±0.36[e]	230.35±0.65[e]
	21 days	35.33±0.45[f]	107.20±0.36[e]	210.00±0.53[f]
Group 5 (10% *O.G* + CCL_4)	7 days	36.18±0.92[d]	113.20±0.20[d]	224.10±0.20[d]
	14 days	34.12±0.14[f]	107.10±0.22[f]	215.23±0.46[g]
	21 days	37.00±0.58[d]	110.00±0.53[g]	195.10±0.15[h]
Group 6 (15% *O.G* + CCL_4)	7 days	40.50±0.50[e]	121.38±0.20[h]	220.18±0.27
	14 days	35.30±0.45[f]	111.35±0.14[g]	209.76±0.07[f]
	21 days	44.48±0.14[g]	127.38±0.29[i]	235.00±0.48[i]

Results represent the Mean ± SD of three estimations. Means with different superscripts are significantly different from each other at $p < 0.05$.

DISCUSSION

Carbon tetrachloride (CCl_4) is one common hepatotoxin used in the experimental study of liver damage (Obi et al., 1998; Ulicna et al., 2003; Yan Jun Luo et al., 2004). CCl_4 treatment generates free radicals that trigger a cascade of events resulting in hepatic fibrosis. In this study, when treated with CCl_4, the liver exhibited drastic alterations, extensive fatty change, distended hepatocytes, compressed sinusoids, fatty degeneration, area of necrosis and infiltration by inflammatory cells as observed in the changes between Figures 1, 2 and 3. Figure 1 shows the slide of a normal liver. Cords of hepatocytes are well preserved, cytoplasm not vacuolated, sinusoids well demarcated, no area of necrosis, no fatty change, no fatty degeneration, while in Figure 2, we have a normal liver (treated with 0.5 ml/Kg vegetable oil). Here, cords of hepatocytes are distinct and essentially normal, no fatty changes are observed and cytoplasm is not vacuolated. On the other hand Figure 3

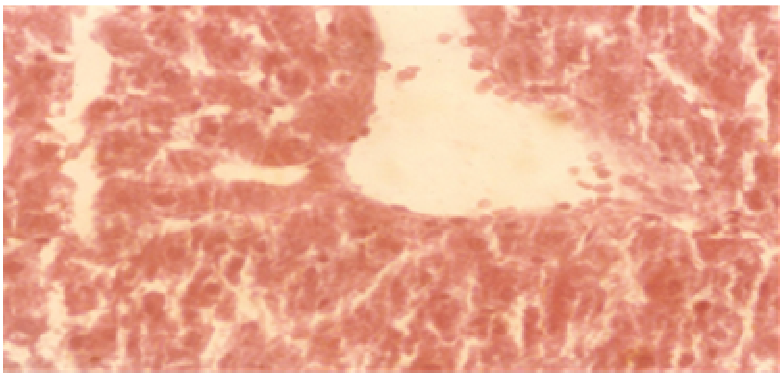

Figure 1. Liver (normal). Cords of hepatocytes well preserved, cytoplasm not vacuolated, sinusoids well demarcated, no area of necrosis, no fatty change, no fatty degeneration. Hematoxylin and eosin stained.

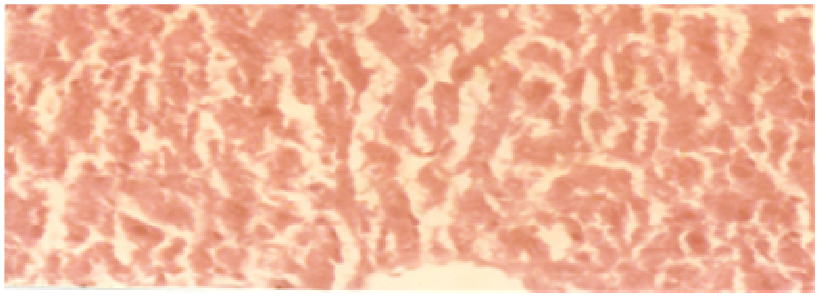

Figure 2. Liver (treated with 0.5 ml/Kg vegetable oil). Cords of hepatocytes are distinct and essentially normal, no fatty change, cytoplasm not vacuolated. Haematoxylin and eosin stained.

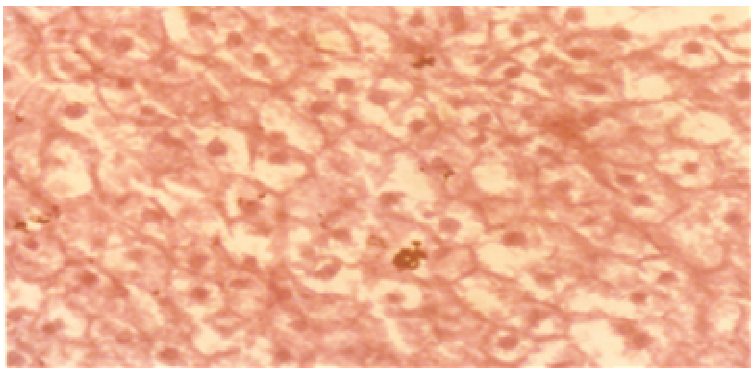

Figure 3. Liver (treated with 0.5 ml/Kg CCL_4). Enlargement of hepatocytes, prominent nucleoli. Although there is vacuolation, it is not as intense. There is fatty change Haematoxylin and Eosis stained.

shows a liver treated with 0.5ml/Kg CCl_4. Enlargement of hepatocytes and prominent nucleoli are observed. Although there is vacuolation, it is not as intense and there is fatty change. In addition, serum levels of ALT, AST and ALP were elevated. This is in agreement with the report by previous workers (Reinke et al., 1988; Obi et al., 1998; Ulicna et al., 2003; Yan Jun Luo et al., 2004). A primary consideration in the assessment of the efficacy of a potential therapeutic agent for hepatic injury (damage) is its effect on liver histology. The liver of the animals that were treated with CCl_4 (Group 3) had a high degree of fatty degeneration and fatty change. Scent leaf

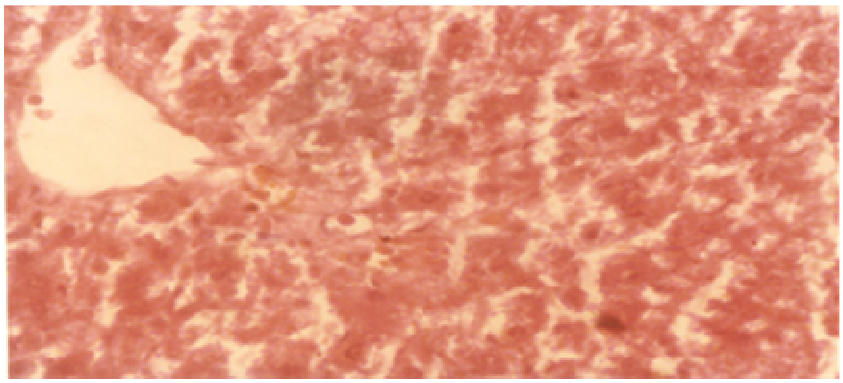

Figure 4. Liver (treated with CCL_4 + 5% *O.G*). There is no fatty change. Cords of hepatocytes are well preserved. Sinusoids well demarcated and no area of necrosis. It is essentially normal.

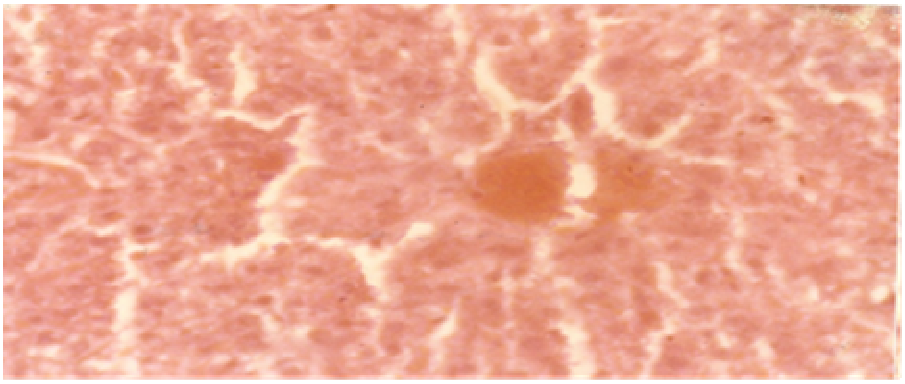

Figure 5. Liver (treated with CCL_4 + 10% *O.G*). Cords of hepatocytes are distinct and are essentially normal. No fatty change and cytoplasm not vacuolated.

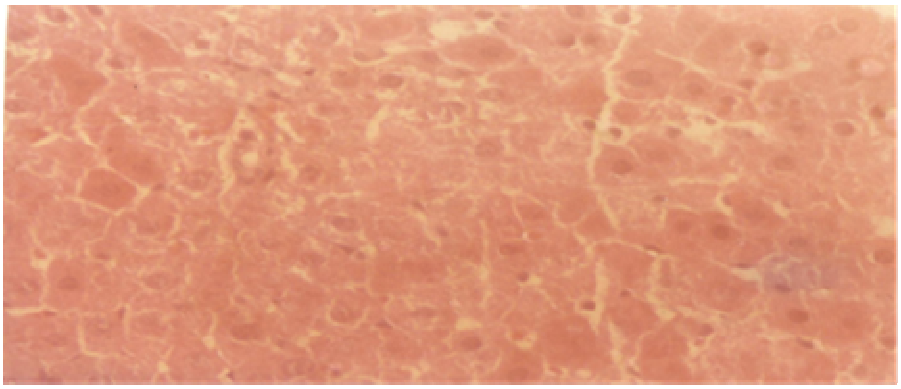

Figure 6. Liver (treated with CCL_4+ 15% *O.G*) .There is no fatty change. Cords of hepatocytes are well preserved. Sinusoids are well demarcated and no area of necrosis. It is just like the normal liver.

(*O. gratissimum*) extract in the dosage range administered to liver–damaged rats apparently accelerated the reversion of the liver damage (Figure. 4, 5 and 6) and lowered the high levels of serum ALT, AST and ALP activity when compared to rats treated with CCL_4 alone. The effect was time and dose dependent. Effriam et al. (2000) reported the presence of flavonoids and saponins in the leafs of *O. gratissimum,* while Effriam et

al. (2003) showed from histopathological studies that *O. gratissimum* can be used as an hepatoprotective agent. However, in this study the extract was not tried to see its effect on any hepatotoxic agent. Flavonoids are reported to exhibit antioxidant activity (Ramanathan et al., 1989) and are effective scavengers of superoxide anions (Robak and Grygleuski, 1988). The aqueous extract of *O. gratissimum* may have exhibited hepatoprotective activity due to its possible antioxidant property attributable to flavonoids. Interestingly, saponins especially terpene glycosides are reported to enhance natural resistance and recuperative powers of the body (Singh et al., 1991) and *O. gratissimum* has been shown to be a rich source of this compound (Prabhu et al., 2009)

In conclusion, our results indicate that treatment with *O. gratissimum* extracts after establishment of CCl_4 – induced liver damage significantly reduced and even reversed the damage in the rats. Hence *O. gratissimum* might be an effective plant hepatoprotector in the diet of patients with hepatopathies. However, there is a need for more studies in animal models for further confirmation, which is already in progress in our laboratory.

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