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Amelioration of carbon tetrachloride-induced hepatotoxicity by terpenoid extract from leaves of *vernonia amydgalina*

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Summary
Sesquiterpene lactone extract from the leaves of *Vernonia amygdalina* was tested for antihepatotoxic activity. Adult male rats were selected for the study. One group of rats was treated with toxic doses of carbon tetrachloride (CCl_4) the second group was pretreated with known concentration of terpenoid extract from leaves of *V. amygdalina*. One hour prior to receiving toxic doses of CCl_4, Kolaviron, a biflavonoid extract of the seeds of *Garcina kola* was used as a positive control.Serum enzymes, alanine amino transferase (ALT), ornithine carbamoyl transferase (OCT) that are known to be very sensitive to cytotoxic hepatic injury, and aspertate amino transferase (AST) that is particularly sensitive to carbon tetrachloride poisoning, were measured as indices of hepatotoxicity. The results obtained showed that there were reduction in the activities of serum ALT, AST and OCT from 20.57 ± 5.59, 10.46 ± 6.71 and 184.8 ± 10.45 in animals treated with toxic doses of CCl_4 to 3.40 ± 0.10, 3.95 ± 0.15 and 129.3 ± 12.10 in animal pretreated with terpenoid extract before CCl_4 intoxication, representing 83.5%, 62.3%, and 30% decrease respectively. These decreases were statistically significant ($P < 0.001, P < 0.05, P < 0.001$ respectively). From these results, it is concluded that sesquiterpene lactone extract from the leaves of *V.amygdalina* like kolaviron, a biflavonoid extract from the seeds of *G. kola* has antihepatotoxic activity in CCl_4-induced hepatic damage in rats.

Keywords : *Epatotoxicity, terpenoid, vernonia amygdalina.*

Résumé
Un extrait de sesoquiterpene lactone des feuilles de vernonia amygdalina a etc teste pour son activité anti hepatotoxique. Des rats males adultes ont etc traits avec des doses toxiques de carbone tetrachloride (CCL_4) et le second groupe était traite d'avance avec une concentration connue d'extrait de terperoide des femilles *V. amygdalinal.* Une heure avant de recevoir des doses toxiques de CCL_4, le klaviron, un extrait biflavonoide des grains de carcinal kola était utilisé comme controle positif. Le serum des enzymes, d'alamine amino transferase (ALT), l'ornithine carbamyl tranferase (OCT) qui sont connus comme produits tres sensibles onx blessures hepatiques cytotoxiques, et l'aspertate amino transferase (AST) qui est particulierement sensible a l'empoisonement due au carbone tetra chloride, ont ete mésures comme indice d'hepatotoxicite.

Les resultats obterus montrent qui il ya une reduction de l'activite du serum ALT, AST et OCT de. $20,57 \pm 5,59$, $10,46$ $6,71$ et $184,8$ $10,45$ chez les animaux troutes avec des doses toxiques de CCL_4 ā $3,40$ $0,10$, $3,95$ $0,15$ et $129,3$ 12. 10 chez les animaux traits prealablement avec des extraits de terperioide avant intoxication an CCL_4, representant 83, 5%, 62, 3% et 30% de decroissance respectivement. Cette decroissance était statistiquement significatif ($P < 0,001$, $P < 0,05$, $P < 0,001$ respectivement). A partir de ces resultats, nous ponvous conclure l'extrait de sesquiterpene lactone des femilles de *V. amygdalina* comme lkolaviron, un biflavonoide extrait des grains de *G. kola* a une activité antihepatotoxiue en degoit de CCL_4 d'induit hepatique chez les rats.

Introduction

Plants elaborate a seemingly endless number of organic compounds that vary tremendously in chemical complexity and functional significance. Among these are a large assemblage of structurally-complex and biogenetically related compounds called Terpenoids [1].

Three main sesquiterpene lactones (Vernodalin, Vernodalol and 11, 13 dihydrovernodalin) have been isolated from the leaves of *V. amygdalina* [2]. All these have been shown to have a number of biological activities including insect anti-feedant activity [2] and anticarcinogenic activity [3]. *V. amygdalina* is a valuable shrub that is widespread in East and West Africa [4]. In Nigeria, it is commonly known as "Bitter leaf" because the leaves and the stem have a bitter taste when chewed. *Vernonia* species are the sources of many local medicines.

This is in addition to its leaves being used as a popular vegetable for soups particularly among the Igbos of Southern Nigeria.Medical applications of *V. amygdalina* include its use as tonic and appetizer. It has also been reported as having been used for treatment of fever and pile (hemorrhoids) [4]. The leaves are usually macerated and the juice extracted consumed for the treatment of fever and pile. The seeds of *G. kola* are also used in Nigeria traditional medicine for therapy just like *V.amygdalina*. They are used for a broad spectrum of ailments including inflammatory disorders, such as gastrointestinal disorders, different liver diseases, dysentery and diarrhoea [4]. Phytochemical studies have shown that the seeds of *G. kola* contain biflavonoids [5] which have antihepatotoxic activity [6,7]. The antihepatotoxic effect of these biflavonoids may involve inhibitory action on the metabolism of the specific hepatotoxin (CCl_4) to its

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corresponding reactive metabolite in the liver [8]. Biflavonoid extract from the seeds of *G. kola*, which has been reported to the antihepatotoxic and have protective effect against CCl_4 induced hepatotoxicity in rat [7], was used in this work as the antihepatotoxicity standard.

Materials and methods

Subjects

Twenty-four male albino rats (Animal House, University of Ibadan) were selected for the study. The mean weight was 136.8 ± 41.35; range 100 -200g. These animals were grouped into four categories as follows:

Group 1 : Control rats (untreated)
Group 2 : Rats treated with toxic doses of CCl_4 alone.
Group 3 : Rats pretreated with Kolaviron, a biflavonoid extract from the seeds *G.kola*, 1 hr prior to receiving toxic doses CCl_4.
Group 4 : Rats pretreated with terpenoid extract from the leaves of *V. amygdalina*, 1 hr prior to receiving toxic doses of CCl_4.

Extraction of terpenoids from leaves of V. amygdalina

Extraction of Terpenoids from leaves of *V. amydalina* was carried out according to the methods described by Ganji *et al* [2]. Air dried leaves of *V. amygdalina* were grouped and extracted with diethyl ether. The extracts were concentrated under reduced pressure to thick oil. The crude material was chromatographed on 500g silical gel. After elution with 1.5L of chloroform, further elution with 1.5L of Chloroform: Methanol (99:1) resulted in the separation of the terpenoid fraction.

Extraction of kolaviron from seeds of G.Kola

Extraction of kolavoron from seeds of *G.Kola* was carried out using the methods described by Braide [7]. The seeds of *G. Kola* were grounded into coarse powder and air dried at room temperature. The dried seed powder was first extracted by using hexane. The biflavonoid fraction. Kolaviron, was obtained as a yellow-brown crystalline powder from the chloroform soluble/water insoluble fraction of the partitioned methanolic extract.

Drug administration

Toxic doses of CCl_4 (0.5ml/kg body weight, three times in a week) were administered intraperitoneally to test animals (groups 2,3 and 4). Groups 3 and 4 animals were, however, pretreated with 500mg/kg body weight of the extracts of *G. Kola* and *V. amygdalina* respectively, 1 hr prior to administration of toxic doses of CCl_4. The route of administration of the extract was oral.

Three hours after the last doses were administered on the animals, each animal was sacrificed. The blood was collected by heart puncture into clean, dry glass centrifuge tubes and allowed to clot. The blood was then centrifuged in the tubes for 10 minutes at 3000g in a MSE bench centrifuge. The serum obtained was used for the estimation of the serum activities of hepatic enzymes.

Serum enzyme assays

(i)Serum Alanine (ALT) and Aspartate aminotransferases(AST)

Alanine (ALT) and asparate amino transferases (AST) were assayed at 37°C using the modified method of Reitman and Frankel [9].

(ii) Serum Ornithine carbamoyl transferase (OCT)

Ornithine carbamoyl transferase (OCT) was assayed at 37°C using the method of Reichard [10].

Statistical analysis

Statistical analyses were performed using student t-test (unpaired) The 5% ($P < 0.05$) level of significance using the two-tailed 't' table was used to compare the calculated and critical 't' value from the table and thus statistical significance.

Results

Table 1 shows the mean activities of ALT, AST and OCT in all the four groups of rats.

Table 1: Activities of serum enzymes in rats (Mean ± 1SD)

	Serum ALT Activities (U/L)	Serum AST Activities (U/L)	Serum OCT Activities (U/L)
Group 1 (Control:Untreated rats) N=6	3.13 ± 0.20	3.63 ± 0.66	18.50 ± 1.20
Group 2 (CC14 Intoxicated rats) N=6	20.57 ± 5.59	10.46 ± 6.71	184.80 ± 10.45
Group 3 (Kolaviron pretreated rats) N=6	3.43 ± 0.53	3.83 ± 0.01	36.90 ± 6.20
Group 4 (Terpeniod pretreated rats) N=6	3.43 ± 0.01	3.95 ± 0.15	129.30 ± 12.10

ALT = Alanine amino transferase
AST = Aspartate amino transferase
OCT = Ornithine carbamoyl transferase.

Table 2 shows the comparative mean values for the activities of all the three enzymes in groups 2 and 4. The activities of ALT, AST and OCT were all significantly reduced in group 4 when compared with their corresponding activities in group 2 ($P < 0.001$; $P < 0.05$; $P < 0.001$ respectively).

Table 2: Statistical comparisons of serum enzyme activity in Groups 2 and 4 animals

	Group 2 N = 6	Group 4 N = 6	t - Test	P - Value
ALT (U/L)	20.57 ± 5.59	3.40 ± 0.01	7.50	<0.001
AST (U/L)	10.46 ± 6.71	3.95 ± 0.15	2.38	<0.05
OCT (U/L)	184.80 ± 10.45	129.30 ± 12.10	8.45	<0.001

The comparative mean activities of the three enzymes in group 3 and group 4 are shown in table 3. There were no significant differences in the activities of AST and ALT in the two groups. There was however a significant difference in the activity of OCT in the two groups ($P < 0.001$)

Table 3

Statistical Comparisons of serum enzymes activity in Groups 3 and 4 animals

	Group 3 N = 6	Group 4 N = 6	t - Test	P - Value
ALT (U/L)	3.43 ± 0.53	3.40 ± 0.01	0.14	NS
AST (U/L)	3.83 ± 0.71	3.95 ± 0.15	1.63	NS
OCT (U/L)	184.80 ± 10.45	129.30 ± 12.10	16.65	<0.001

NS = Not Significant

Discussion

Early works on the study of hepatotoxicity had established that the determination of the activities of hepatic enzymes released into the blood by damaged liver is one of the most useful tools in the evaluation of hepatic injury *in vivo* [11, 12]. This work, therefore employed the use of serum enzymes assay to detect and predict probable liver injury.

Three serum enzymes that are known to be very sensitive to cytotoxic hepatic injury and particularly the one induced by CCl_4 assayed : ALT, AST and OCT.

Elevations in activities of these enzymes in rats treated with CCl_4 had been demonstrated [13] and had been proven to be very sensitive indices of hepatocellular injury in rat [13].

The results showed that sesquiterpene lactone extract from leaves of *V.amygdalina* at a dose of 500mg/kg given 1 hr before CCl_4 intoxication reduced the alteration of parameters measured as indices of liver function and hepatotoxicity in rats. This influence is more pronounced for serum ALT activity (Table 1).

The liver injury associated with CCl_4 intoxication had been attributed to the production of free radicals by hepatic microsomes during the metabolism of CCl_4 [14]. This view is consistent with earlier hypothesis by previous investigators, relating CCl_4 induced hepatotoxicity to the formation of some toxic metabolites [15, 16].

Sesquiterpene lactone extract from the leaves of V. *amygdalina* like kolaviron, a biflavonoid extract of the seeds of *G. Kola*, has now been shown to have protective effect in the experimental model of CCl_4 induced hepatic damage. This is based on the observation that the terpenoid extract from *V. amygdalina* like kolaviron attenuated the increased activity of blood enzymes associated with hepatotoxicity induced by CCl_4. It is probable that the terpenoid extract from leaves of *V. amygdalina* have inhibitory action on the metabolism of CCl_4 to its reactive metabolism in the liver. Owing to the possible tremendous clinical implication of this observation, such as its possible use in treatment of viral hepatitis so common in this environment, further work is suggested.

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