Effect of Aframomum melegueta on carbon tetrachloride induced liver injury

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ABSTRACT

This present study was performed to evaluate the hepatoprotective effect of Aframomum melegueta seeds, used as spicy in the traditional conditions. The hepatoprotective activity of the seeds of A. melegueta at 100 and 150 mgEq.pp/kg, administered orally, was assessed using carbon tetrachloride-induced (CCl4) liver damage (1 ml/kg bw 1:1 CCl4 and corn oil). The results obtained from this study show that A. melegueta produced a significant decrease in serum transaminases and alkaline phosphatase. At the dose of 150 mg Eq.pp/kg, A. melegueta induced a significant increase in serum total proteins. Liver and serum antioxidant potentials were also significantly increased. Those results indicate that A. melegueta seeds protect the liver against toxicity induced in this study.

INTRODUCTION

Aframomum melegueta, (Roscoe) K. Schum (Zingiberaceae), also named Melegueta pepper, alligator pepper, or Guinea pepper; is an herbaceous plant cultivated or growing in clearings of rain forest in the tropical areas of West Africa mainly for the importance of its seeds. The seeds are known to have strong aromatic and pungent odor, peppered taste, prickling and slightly bitter (Iwu et al., 1999). The seeds of this plant are generally called "grains of paradise" because they are very appreciable like spices and they are ingredients for foods preparation and several medicinal formulations for traditional healers in the West Africa. Throughout the South of Nigeria (Simmons, 1956), Benin and Togo the seeds are used for magical and in the practices of divination. A. melegueta is one of the most medicinal plants in Africa used in ethnomedicinal plants. Investigations have shown that the seeds are used traditionally for constipation, rheumatism and fever (Ajaiyeoba and Ekundayo, 1999; Fernandez et al., 2006).

Previous studies had indicated that the extracts of the seeds are used to treat diarrhea and others gastro-intestinal disorders, snake bite and intestinal worms and pains (Kokwaro, 1993; Akendengue and Louis, 1994; Rafatullah et al., 1995). Traditionally, aqueous extract of the seeds is applied topicaly for abscesses and joint distortions. Pharmacological investigations have demonstrated that the seeds have anti-ulcer, antimicrobial and cytoprotection effects (Rafatullah et al., 1995; Galal, 1996). Antibacterial and anti fungal effects result from 6-paradol and 6-shogoal, compounds found in the seeds (Galal, 1996). The seeds of A. melegueta contain also gingerol, inhibitor of prostaglandins and leukotriens synthesis, justifying the anti-inflammatory effect of the plant. Aqueous extract of A. melegueta has aphrodisiac effect (Kamchouing et al., 2002). Moreover this extract had been shown to reduce significantly abdominal contraction induced by acetic acid in mice (Umukoro and Ashorobi, 2001). This study has later confirmed by analgesic effect of the aqueous extract of the plant (Umukoro and Ashorobi, 2007). Unpublished oral report indicates that, in rural areas, people use the seeds to warm their body and for alleviate joint pains. Traditional healers use the seeds of A. melegueta into the formulation of many medicines because they think that these seeds potentiate the effects of bioactive compounds of medicinal plants.

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Previous investigation showed that the seeds of *A. melegueta* inhibit significantly the activities of human microsomes CYP 3A4, 3A5 and 3A7 in vitro (Agbonon *et al.*, 2010) confirming that those seeds may have bio enhancer effects on drugs metabolized by CYP 3A enzymes. Traditional medicines contain many unknown compounds that may affect liver physiology. *A. melegueta* seeds, CYP 3A inhibitors, are associated to others medicinal plants organs in polyherbal medicines formulation, this practice may potentiate the effects of plants products and increase the risk of toxicity for the liver. For this purpose, we hypothesize that hepatic protection of *A. melegueta* seeds may be very useful when these seeds are associated to others plants organs in the traditional medicine formulations. The aim of the present investigation is to study the effect of *A. melegueta* seeds on the hepatotoxicity induced by carbon tetrachloride in Wistar rats.

**MATERIAL AND METHODS**

**Plant materials**

The dried fruits of *Aframomum melegueta* were purchased from local market called Totsi (West of Lomé -Togo). The fruits were identified by a well known traditional healer and certified by the Laboratory of Botany and Vegetal Ecology, Faculty of Sciences, University of Lomé.

**Animals**

Wistar rats (150-200g) of either sex used in the present investigation were provided by animals facilities of the Laboratory of Physiology/Pharmacology of Natural Substances in the Faculty of Sciences, University of Lomé (Togo). The animals are housed in cages (Five rats per cage) at ambient temperature and humidity with a 12 hours day-light cycle, with free access to food and water.

**Chemicals**

TPTZ (2,4,6-tripyridyl-s-triazine) and DPPH were previously obtained from Avocado Research Chemical (Canada) and Sigma-Aldrich (Canada) with the assistance of the Professor John T Arnason (University of Ottawa, Ontario, Canada); Silymarin and quercetin were obtained from Sigma Chemical (St. Louis, MO, United States). Carbon tetrachloride was obtained from BDH Chemicals (Poole, England). Potassium Chloride was obtained from Fisher Bioblock Scientific (Illkirch, France). Iron sulphate, iron chloride, acetic acid, and sodium acetate were analytical grade and purchased from BDH, France.

**Product preparation**

The dried seeds of *Aframomum melegueta*, extracted from mature fruits, were ground into fine powder which was dissolved in distilled water daily before administration to animals.

**Experimental**

Hepatoprotective effect of *Aframomum melegueta* seeds on Carbon tetrachloride (CCl₄)-induce liver damage was carried out, according to the method described by Agbonon and Gbeassor, (2009). Wistar rats were divided into five groups of five animals: a control group (corn oil), a group with CCl₄ plus water, a group with CCl₄ plus *A. melegueta* seeds at 100 mg-Eq.pp/kg (milligram equivalent of plant product), a group with CCl₄ plus *A. melegueta* seeds at 150 mg-Eq.pp/kg and a group with CCl₄ plus Silymarin. The control group was injected intraperitonealy corn oil at 1 ml/kg followed by oral administration of water at 0, 5, 10 and 20 hours. All the four remaining groups were injected intraperitonealy carbon tetrachloride at 1 ml/kg mixed in an equal volume of corn oil (1:1). At 0, 5, 10 and 20 hours after CCl₄ injection, animals were administered orally water (CCl₄ + water groups), the solution of powder of *A. melegueta* seeds at 100 and 150 mg Eq. pp/kg (CCl₄ plus *A. melegueta* seeds groups) or Silymarin at 60 mg/kg (CCl₄ plus Silymarin group). Experimental protocols were based on World Health Organization Guidelines for the care and use of laboratory animals, and use of the animals was approved by the Ethics Committee of Université de Lomé, a branch of the National Ethics Committee.

**Blood and liver analysis**

At 24 hours after CCl₄ injection, all the animals were sacrificed under ether anesthesia. Blood was collected for biochemical analysis, and samples of the liver were removed and placed in a cool KCl solution (1.5%). The liver samples were subsequently homogenized (2 g of liver in 5 ml of KCl solution). The liver homogenates and blood were centrifuged at 3000 rpm corresponding to 1107g force for 15 min using electric centrifuge (Shimadzu Scientific Corporation Tokyo, Japan). The supernatants of liver homogenates were used for ferric reducing activity of plasma (FRAP) determination (Nair *et al.*, 2007; Agbonon and Gbeassor, 2009).

**Liver toxicity**

The concentrations of Aspartate aminotransferase (AsT), Alanine aminotransferase (AIt), Alkaline Phosphatase (AIP), total protein and total bilirubin were measured in the sera using commercial kits purchased from Human GmbH. D-65205 (References 12012 for AIT and 12011 for AST), Wiesbaden, Germany. Data are expressed in international units (UL⁻¹).

**In vivo antioxidant potential**

Antioxidant potential of sera and liver homogenates from control and CCl₄ treated were performed using FRAP (Ferric reducing activity of plasma) method. Briefly, 900µl of a daily working reagent (prepared by mixing 25 ml of acetate buffer, 2.5 ml of 10 mM Fe³⁺-TPTZ in 40 mmol of HCl, and 2.5 ml of FeCl₃·6H₂O at 20 mmol/l) was mixed with 30µl of samples (serum or liver homogenate) and 90µl of distilled water. The change in absorbance at 593nm was measured when the blue Fe³⁺-tripyridyl-s-triazine (Fe²⁺-PTZ) compound formed from colorless, oxidized Fe³⁺ (Nair *et al.*, 2007). Calibration curves were generated from aqueous solution of FeSO₄ at different concentrations (125µM, 250µM, 500µM, 1000µM and 2000µM).
In vitro antioxidant effect

Antioxidant capacity of *A. melegueta* seeds was evaluated in vitro by using the DPPH method described by Mcune and Johns (2002). Briefly, 0.25 ml of a methanol solution of seeds powder at different concentrations (50-1000μg of powder/l) was mixed with 1.5ml of DPPH at 100μmol/l. After 10 min, the change in the absorbance was determined at 517nm. Quercetin was used as a positive control. The antioxidant effect of the extracts was estimated by the decrease in the absorbance of the test solution and compared with the positive control. The DPPH radical inhibition was calculated as follows:

\[
\text{DPPH radical inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100\%
\]

Statistical analysis

Results are expressed as mean ± SEM (n = 5). Statistical analysis was performed using ANOVA one-way followed by Tukey’s multiple comparison. p-values less than 0.05 were considered statistically significant.

RESULTS

Effects of *aframomum melegueta* on CCl₄-induced liver toxicity

Administration of CCl₄ produced hepatotoxicity by increasing significantly (p < 0.001) the serum level of Alt (alanine aminotransferase) and AsT (aspartate aminotransferase), compared to the control group (Corn oil). Administration of *A. melegueta* seeds at the doses of 100 and 150 mg Eq.pp/kg induced significant (p < 0.001) decrease of AsT and Alt (Table 1). Serum level of alkaline phosphatase is also decreased significantly (p < 0.05) in the same groups as indicated in table 1. In CCl₄-treated alone animals, serum level of total proteins is significantly decreased in comparison to corn oil-treated and *A. melegueta*-treated groups.

In *A. melegueta*-administered groups, there is dose-dependent significant (p < 0.05) increase in serum level of total proteins. At the dose of 150 mg Eq.pp/kg, serum protein is the same level compared to control group. CCl₄-induced non-significant (p > 0.05) increase in serum level of total bilirubin in comparison to control group and *A. melegueta* seeds treated groups (Table 2). Those results indicate that *A. melegueta* seeds protect the liver against toxicity induced in this study.

In vivo and in vitro antioxidant effects of the seeds

The concentration of Fe⁺² was reduced significantly (p < 0.001) when animals were injected with CCl₄ compared to the control animals. *Aframomum melegueta* seeds at 100 and 150 mg Eq.pp/kg have increased significantly the concentrations of Fe⁺² in both the serum and in the liver homogenate indicating that these seeds have increased antioxidant potential in vivo. In vitro assays

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### Table 1 Concentration of trasaminases and alkaline phosphatase in serum of rats injected CCl₄ and treated with *Aframomum melegueta* seeds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT</th>
<th>AsT</th>
<th>AIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil + water</td>
<td>46.4 ± 11.5</td>
<td>125.8 ± 15.0</td>
<td>215.4 ± 48.7</td>
</tr>
<tr>
<td>CCl₄ + water</td>
<td>371.2 ± 61.1*</td>
<td>592.1 ± 21.3*</td>
<td>759.8 ± 52.6</td>
</tr>
<tr>
<td>CCl₄ + AMS 100 mg Eq.pp/kg</td>
<td>167.0 ± 23.3</td>
<td>255.4 ± 42.9*</td>
<td>396.4 ± 35.7***</td>
</tr>
<tr>
<td>CCl₄ + AMS 150 mg Eq.pp/kg</td>
<td>126.8 ± 17.8*</td>
<td>239.6 ± 35.7*</td>
<td>311.0 ± 53.3***</td>
</tr>
<tr>
<td>CCl₄ + sylimarin 60 mg/kg</td>
<td>141.4 ± 24.9*</td>
<td>192.2 ± 11.8*</td>
<td>359.0 ± 68.2***</td>
</tr>
</tbody>
</table>

Each data represents the mean ± S.E.M. for five animals per group; with *p < 0.01 when compared to Corn oil group; **p < 0.001 and ***p < 0.05 when compared to CCl₄ alone group (ANOVA followed by Tukey’s multiple comparison).

### Table 2 Concentration of total protein and total bilirubin in serum of rats injected CCl₄ and treated with *Aframomum melegueta* seeds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Protein (g/l)</th>
<th>Total Bilirubin (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil + water</td>
<td>70.51 ± 3.52</td>
<td>3.68 ± 0.66</td>
</tr>
<tr>
<td>CCl₄ + water</td>
<td>48.12 ± 3.29*</td>
<td>5.64 ± 0.68</td>
</tr>
<tr>
<td>CCl₄ + AMS 100 mg Eq.pp/kg</td>
<td>59.61 ± 1.67*</td>
<td>4.83 ± 0.89</td>
</tr>
<tr>
<td>CCl₄ + AMS 150 mg Eq.pp/kg</td>
<td>70.32 ± 1.86*</td>
<td>3.84 ± 0.89</td>
</tr>
<tr>
<td>CCl₄ + sylimarin 60 mg/kg</td>
<td>55.98 ± 1.99*</td>
<td>4.86 ± 1.10</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. for five animals per group; with *p < 0.01 when compared to Corn oil group; **p < 0.05 when compared to CCl₄ alone group (ANOVA followed by Tukey’s multiple comparison).

### Table 3 Effect of *Aframomum melegueta* seeds on antioxidant potential of plasma in rats injected CCl₄

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of Fe⁺² using FRAP assay (μM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil + water</td>
<td>1248 ± 113.1</td>
</tr>
<tr>
<td>CCl₄ + water</td>
<td>644.8 ± 96.44*</td>
</tr>
<tr>
<td>CCl₄ + AMS 100 mg Eq.pp/kg</td>
<td>1114.49 ± 87.3*</td>
</tr>
<tr>
<td>CCl₄ + AMS 150 mg Eq.pp/kg</td>
<td>1057.57 ± 183.87*</td>
</tr>
<tr>
<td>CCl₄ + sylimarin 60 mg/kg</td>
<td>1169.14 ± 122.23*</td>
</tr>
</tbody>
</table>

Data represents the mean ± S.E.M. for five animals per group; with *p < 0.01 when compared to Corn oil group; **p < 0.05 when compared to CCl₄ alone group (ANOVA followed by Tukey’s multiple comparison).

### Table 4 Effect of *Aframomum melegueta* seeds on antioxidant potential of liver homogenate in rats injected CCl₄

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of Fe⁺² using FRAP assay (μM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil + water</td>
<td>1910.89 ± 75.88</td>
</tr>
<tr>
<td>CCl₄ + water</td>
<td>897.87 ± 299.4*</td>
</tr>
<tr>
<td>CCl₄ + AMS 100 mg Eq.pp/kg</td>
<td>1676.4 ± 89.52*</td>
</tr>
<tr>
<td>CCl₄ + AMS 150 mg Eq.pp/kg</td>
<td>1526.79 ± 140.17*</td>
</tr>
<tr>
<td>CCl₄ + sylimarin 60 mg/kg</td>
<td>1640.04 ± 242.47#</td>
</tr>
</tbody>
</table>

Data represents the mean ± S.E.M. for five animals per group; with *p < 0.01 when compared to Corn oil group; **p< 0.001 and ***p < 0.05 when compared to CCl₄ alone group (ANOVA followed by Tukey’s multiple comparison).
using DPPH method indicates that the seeds of A. melegueta have antioxidant effect by reducing synthetic free radical DPPH. The IC₅₀ is 74.36 ± 0.28 μg/ml of A. melegueta powder, whereas the IC₅₀ of quercetin is 17.19 ± 0.10 μg/ml.

DISCUSSION
The aim of the present investigation was to study the hepatoprotective effect of A. melegueta seeds use currently to alleviate many pathological conditions alone or in combination with many others medicinal plants organs by traditional healers in their receipt formulation. The main results may be summarized as follow: A. melegueta seeds reduce significantly liver injury induced by carbon tetrachloride in wistar rats; they have antioxidant effects both in vitro and in vivo. Carbon tetrachloride induced liver injury it valid experimental method (Ikatsu et al., 1998, Lee et al., 2008), used currently to evaluate potential hepatoprotective effect of medicinal plants (Agbonon and Gbeassor, 2009; Mahmoud et al., 2012, Xie et al., 2012). When administered to rats, carbon tetrachloride is bio-transformed into trichloromethyl, a powerful free radical that induces liver injury by CYP 2E1 (Ikatsu et al., 1998). This injury affects currently hepatocytes by increasing intracellular enzymes such as alanine aminotransferase and aspartate aminotransferase in plasmatic liquid. More alkaline phosphatase concentration is increased in the plasma and others hepatic physiology such as protein synthesis and conjugation are affected. A. melegueta seeds, administered orally four times after carbon tetrachloride injection, reduced significantly transaminases and alkaline phosphatase concentration in the plasma. This effect may bypass through inhibition of CYP 2E1. Previous investigation has demonstrated that ethanolic and aqueous extracts of A. melegueta seeds inhibit CYP 3A4, CYP 3A5 and CYP 3A7 (Agbonon et al., 2010). This effect may reduce the bioavailability of trichloromethyl which is the main cause of liver injury. It is well known that CYP 2E1 inhibition or absence of production may be useful in carbon tetrachloride induced liver injury. In CYP2E1 knockout mice, carbon tetrachloride injection did not induce liver damage (Wong et al., 1998; Avasarala et al., 2006). Trichloromethyl derived from CCl₄ metabolism by CYP2E1 is powerful free radical that destroys liver cells membrane. The present investigation has shown that A. melegueta seeds have antioxidant effect. This property may contribute to reduce the toxicity of trichloromethyl in liver injury through free radical scavenging or reduction. A. melegueta seeds inhibit C - reactive protein synthesis, the COX-2 enzyme (Dybas and Raskin, 2007) and prostaglandins formation (Umukoro and Ashorobi, 2007). Those anti-inflammatory effects, previously demonstrated by the presence of gingerols in the seeds, may contribute to prevent liver injury induced by carbon tetrachloride. It is well demonstrated that antioxidant compounds are good hepatoprotectors. Moreover, A. melegueta seeds seem to restore others hepatic functions such as protein synthesis and conjugation. A. melegueta seeds are used for medicinal and moreover for food purposes in Africa and many others tropical countries. They are considered as safe plant organ; however administration of high doses of these grains of paradise may induce liver toxicity by increasing alkaline phosphatase concentration in the plasma as previously shown (Ilic et al., 2010).

CONCLUSION
The present study has demonstrated that A. melegueta exhibits hepatoprotective effect. This hepatoprotective effect of A. melegueta seeds may contribute to reduce potential toxicity of many unknown compounds present in traditional medicines; and justify systematic involvement of those seeds in traditional polyherbal formulations. Further investigations are needed to evaluate the effect of these seeds on the bioavailability of current drugs used in ours countries.

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