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Effect of *Aframomum melegueta* on carbon tetrachloride induced liver injury

Idoh Kokou[1], Karou S. Damintoti[2], Agbonon Amegnona[1,*], Adjrah Yao[2], Gbeassor Messanvi[1]
[1] Laboratoire de Physiologie/Pharmacologie des Substances Naturelles, Centre de Formation et de Recherche sur les Plantes Médicinales (CERFOPLAM), Université de Lomé BP 1515 Lomé- Togo.
[2] Laboratoire de Microbiologie et de Contrôle de Qualité des Denrées alimentaires, ESTBA, Université de LoméBP 1515 Lomé -Togo.

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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 (CCl_4) liver damage (1 ml/kg bw 1:1 CCl_4 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, pricking 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 ethnopharmacology for many purposes. Investigations have shown that the seeds are used traditionally for constipation, rheumatism and fever (Ajaiyeoba and Ekundayo, 1999; Fernandez *et al*., 2006).

** Corresponding Author*
Dr Agbonon Amegnona, PhD
E-mail: aamegnona3@gmail.com
Phone: (+228) 22 36 75 82

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 topically 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 (Kamtchouing 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.

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, Université de 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, Université de 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-triazin) 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 (CCl_4)-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 CCl_4 plus water, a group with CCl_4 plus *A. melegueta* seeds at 100 mg-Eq.pp/kg (milligram equivalent of plant product), a group with CCl_4 plus *A. melegueta* seeds at 150 mg-Eq.pp/kg and a group with CCl_4 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 CCl_4 injection, animals were administered orally water (CCl_4 + water groups), the solution of powder of *A. melegueta* seeds at 100 and 150 mg Eq. pp/kg (CCl_4 plus *A. melegueta* seeds groups) or Silymarine at 60 mg/kg (CCl_4 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 CCl_4 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 (AlT), Alkaline Phosphatase (AlP), total protein and total bilirubin were measured in the serums using commercial kits purchased from Human GmbH. D-65205 (References 12012 for AlT and 12011 for AST), Wiesbaden, Germany. Data are expressed in international units (UL^{-1}).

In vivo antioxidant potential

Antioxidant potential of serums and liver homogenates from control and CCl_4 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 Fe^{3+}-TPTZ in 40 mmol of HCl, and 2.5 ml of $FeCl_3$-$6H_2O$ 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 Fe^{2+}-tipyridyl-s-triazine (Fe^{2+}- PTZ) compound formed from colorless, oxidized Fe^{3+} (Nair *et al*., 2007). Calibration curves were generated from aqueous solution of $FeSO_4$ at different concentrations (125µM, 250µM, 500µM, 1000µM and 2000µM).

Table 1 Concentration of trasaminases and alkaline phosphatase in serum of rats injected CCl_4 and treated with *Aframomum melegueta* seeds.

Treatment	Serum Transaminases and alkaline phosphatase (IU/l)		
	AlT	AsT	AlP
Corn oil + water	46.4 ± 11.5	125.8 ± 15.0	215.4 ± 48.7
CCl_4 + water	371.2 ± 61.1*	519.2 ± 21.3*	759.8 ± 52.6
CCl_4 + AMS 100 mg Eq.pp/kg	167.0 ± 23.3	255.4 ± 42.9$^{\#}$	396.4 ± 35.7$^{\#\#}$
CCl_4 + AMS 150 mg Eq.pp/kg	126.8 ± 17.8$^{\#}$	239.6 ± 35.7$^{\#}$	311.0 ± 53.3$^{\#\#}$
CCl_4 + sylimarin 60 mg/kg	141.4 ± 24.9$^{\#}$	192.2 ± 11.8$^{\#}$	359.0 ± 68.2$^{\#\#}$

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 CCl_4 alone group (ANOVA followed by Tukey's multiple comparison).

Table 2 Concentration of total protein and total bilirubin in serum of rats injected CCl_4 and treated with *Aframomum melegueta* seeds.

Treatment	Total Protein (g/l)	Total Bilirubin (mg/l)
Corn oil + water	70.51 ± 3.52	3.68 ± 0.66
CCl_4 + water	48.12 ± 3.29*	5.64 ± 0.68
CCl_4 + AMS 100 mg Eq.pp/kg	59.61 ± 1.67$^{\#}$	4.83 ± 0.89
CCl_4 + AMS 150 mg Eq.pp/kg	70.32 ± 1.86^{d}	3.84 ± 0.89
CCl_4 + sylimarin 60 mg/kg	55.98 ± 1.99$^{\#}$	4.86 ± 1.10

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 CCl_4 alone group (ANOVA followed by Tukey's multiple comparison).

Table 3 Effect of *Aframomum melegueta* seeds on antioxidant potential of plasma in rats injected CCl_4

Treatment	Concentration of Fe^{2+} using FRAP assay (µM/L)
Corn oil + water	1248 ± 113.1
CCl_4 + water	644.8 ± 96.44*
CCl_4 + AMS 100 mg Eq.pp/kg	1114.49 ± 87.3$^{\#}$
CCl_4 + AMS 150 mg Eq.pp/kg	1057.57 ± 183.87$^{\#}$
CCl_4 + sylimarin 60 mg/kg	1169.14 ± 122.23$^{\#}$

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 CCl_4 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 CCl_4

Treatment	Concentration of Fe^{2+} using FRAP assay (µM/L)
Corn oil + water	1910.89 ± 75.88
CCl_4 + water	897.87 ± 299.4*
CCl_4 + AMS 100 mg Eq.pp/kg	1676.4 ± 89.52$^{\#\#}$
CCl_4 + AMS 150 mg Eq.pp/kg	1526.79 ± 140.17$^{\#\#}$
CCl_4 + sylimarin 60 mg/kg	1640.04 ± 242.47##

Data represents the mean ± S.E.M. for five animals per group; with $*p < 0.01$ when compared to Corn oil group; $^{\#\#}p < 0.001$ when compared to CCl_4 alone group (ANOVA followed by Tukey's multiple comparison).

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 517 nm. Quercetin was used to generate a standard curve for the 50% inhibition concentration (IC_{50}) determination.

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 CCl_4-induced liver toxicity

Administration of CCl_4 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 CCl_4-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. CCl_4-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 CCl_4 compared to the control animals. *Aframomum melegueta* seeds at 100 and 150 mg-Eq.pp/kg have increased significantly the concentrations of Fe^{2+} both in the serum and in the liver homogenate indicating that these seeds have increased antioxidant potential in vivo. In vitro assays

using DPPH method indicates that the seeds of *A. melegueta* have antioxidant effect by reducing synthetic free radical DPPH. The IC_{50} is 74.36 ± 0.28 µg/ml of *A. melegueta* powder, whereas the IC_{50} 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 CCl_4 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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