Ameliorative potential of *Aframomum melegueta* extract in cadmium-induced hepatic damage and oxidative stress in male Wistar rats

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**ABSTRACT**

The present study was undertaken to explore the ameliorative potential of aqueous extract of *Aframomum melegueta* (AM) on cadmium-induced hepatic damage in rats. Toxicity was induced by daily administration of 200 mg/L cadmium: Cd (Cd as CdCl₂) in the animals’ main drinking water for 21 days. Lipid peroxidation (LPO), catalase (CAT), glutathione peroxidase (GPO) and superoxide dismutase (SOD) activities were determined in the liver while total protein, albumin, direct bilirubin and total bilirubin concentration as well as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were monitored in the serum and histological examination was carried out. Exposure to cadmium resulted in various alterations in all the evaluated parameters. Treatment with AM (200 or 400 mg/kg bw) extract showed a significant (P <0.05) reversal effect that mitigated the deleterious effect of cadmium. Results of the histological examination also support the above findings. The results suggest that aqueous extracts of *A. melegueta* when administered orally, could ameliorate cadmium-associated oxidative stress in male Wistar rats in a dose dependant manner via its free radical-scavenging mechanism which could be linked to the synergetic effects between the bioactive constituents present in the extract.

**INTRODUCTION**

Cadmium is ubiquitous in nature and to a great extent; it is concentrated in the food chain due to its high soluble nature compared to other toxic heavy metals, it is not degradable; consequently it is easily transported from soil to plants which animals and humans largely depend on for survival (Gallagher et al., 2010; Del Pino et al., 2014). Some of the routes of cadmium intake involve the lungs, intestines and skin. There are no exact mechanism for the excretion of cadmium in humans, so it bioaccumulates in various tissues especially kidneys, lungs, pancreas and liver (Haidry and Malik, 2014; Rahman, 2007); where it contributes significantly in the pathogenesis of oxidative dysfunction in various animal and human organs notably the kidney, brain, testes, heart and liver (Sarkar et al., 2013). Oxidative stress resulting from excessive generation of free radicals, especially reactive oxygen species (ROS) is the basic mechanism of cadmium toxicity. However, cadmium is unable to induce ROS generation directly since it’s a non-fenton metal (Haidry and Malik, 2014). It induces oxidative stress indirectly via displacement of redox-active metals and makes use of their transport systems. It also depletes redox scavengers and inhibits antioxidant enzymes as well as electron transport chain (ETC) resulting in mitochondrial damage (Nair et al., 2013). In the liver, cadmium is taken into the hepatocytes where it binds to metallothioneins (MTs), glutathione (GSH) and other proteins or peptides and form new complexes. Hydroxyl radical is the most reactive and damaging radical formed; which initiates cellular damages and lipid peroxidation (LPO) even at extremely small concentrations (Haidry and Malik, 2014; Abdel Moneim et al., 2014; Prabu et al., 2012; Mitra et al., 2012; Nair et al., 2013). The
global health impact and socioeconomic burden associated with oxidative stress arising from various alteration patterns in hepatic antioxidant status is of great concern for researchers in the last few years. Damaging consequences of oxidative stress can be prevented by employing medicinal plants and spices containing natural antioxidants that can inhibit the generation of free radicals and prevent/delay progressions in oxidative stress and damage, thereby conferring protection to the liver (Flora, 2009).

*Aframomum melegueta* (Alligator pepper) is one of such plants having both medicinal and nutritive values. It is popularly used as herbal remedy against a wide range of ailments, both in Nigeria and several other countries of the world. Detailed phytochemical screening carried out revealed that aqueous extract of *A. melegueta* seeds contains hepatoprotective and free radical scavengers such as alkaloids, phenols, flavonoids, saponins and tannins in appreciable quantities (Nwozo and Oyinloye, 2011). The present work demonstrates that aqueous extract of *A. melegueta* at a dose of 200 or 400 mg/kg bw possess hepatoprotective and antioxidative potential to ameliorate cadmium induced toxicity and oxidative stress in animal models.

**MATERIALS AND METHODS**

**Chemicals**

All the chemicals and reagents were purchased from Sigma Chemical (St Louis, MO, USA) and Merck (Germany). All kits (Randox assay kits) were purchased from ABJ Chemicals (Lagos, Nigeria).

**Plant material and extract preparation**

Fruits of *Aframomum melegueta* were purchased from Bodija market (Nigeria). Identification and authentication were previously carried out by Nwozo and Oyinloye, 2011. One kilogram of seeds from air-dried fruits was washed with distilled water and air-dried. The dried seeds were pulverized into统一 powder using an electric blender (25 – 28°C). The pulverized seed was extracted by maceration in distilled water (200 g/1000 ml) for 72 hours. The aqueous extract was filtered and the filtrate was freeze-dried to yield a yellowish brown extract. The lyophilized extract was carefully scraped (into a clean sample bottle and stored in a refrigerator at 4 °C for further use. Portions of the lyophilized extract used for this experiment were weighed and reconstituted in distilled water daily, just before administration to the animals.

**Animals and experimental design**

Twenty-four male albino (Wistar strain) rats were obtained from the Animal house of the Department of Biochemistry, College of Sciences, Afe Babalola University, weighing between 103 g and 157 g. The animals were allowed access to feed and water *ad libitum* for a period of fourteen days, for their acclimatization prior to the commencement of the experiment. The animals were kept in well ventilated cages at room temperature (28° – 30 °C), and under controlled light cycles (12 h light/12 h dark). All procedures were carried out in accordance with the conventional guidelines of the National Institutes of Health (Maryland, USA) for experimentation with animals and protocol approved by the Institutional Animal Care and Use Committee of Afe Babalola University. The rats were randomly distributed into four groups of six animals each. Group 1: served as the control and consisted of animals fed with standard rat pellet and distilled water only. Group 2: consisted of animals fed with standard rat pellet and cadmium only (cadmium control). Group 3 consisted of animals fed with standard rat pellet, cadmium and *A. melegueta* extract (200 mg/kg), while Group 4 consisted of animals fed with standard rat pellet, cadmium and *A. melegueta* extract (400 mg/kg). *A. melegueta* (AM) extracts (200 and 400 mg/kg bw) was given orally by gavage for 21 days. Cadmium was administered daily (200 mg/L Cd as CdCl₂) in the animals’ (Groups 2, 3 and 4) main drinking water per day for 21 days to induce toxicity (Layachi and Kechrh, 2012).

**Preparation of blood and tissue homogenates for biochemical analyses and histological examination**

The experiment lasted for 21 days, on day 22th; the animals were sacrificed 24 hours after the last dose. Blood samples were collected and allowed to coagulate at room temperature. The clear, non-haemolysed supernatant sera were quickly removed and stored at -20 °C for subsequent analysis. Liver samples were quickly excised and washed in ice-cold 1.15 % KCl solution, dried using filter paper and weighed. They were then homogenized in 4 volumes of 56 mM Tris/HCl buffer (pH 7.4) containing 1.15 % potassium chloride and centrifuged at 10 000 x g for 15 min. The supernatant was collected and stored at -20 °C until needed for assays. Small pieces of liver sections were fixed in 10 % formal saline and processed for paraffin embedding. Sections of 4-6 μm thickness were cut and stained with hematoxylin and eosin (H and E) and observed under light microscope for histopathological changes (Lilli, 1965).

**Biochemical assays**

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation by following the method of Varshney and Kale (1990). Catalase (CAT) activity was determined by adopting the method described by Sinha, 1972. The level of SOD activity was determined by the method of Misra and Fridovich (1972). Glutathione peroxidase (GPx) was assayed by the method of Hafeman *et al.*, 1974. Serum total proteins concentration was determined according to the method of Henry (1964) while albumin concentration was determined in serum according to the method of Doumas *et al.*, (1971). Direct bilirubin concentration was also determined in serum according to the method of Walter and Gerade (1970) while total bilirubin was determined according to Schmidt and Eisenburg, 1975. Activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the method of Reitman and Frankel (1957), using commercially available diagnostic kits (Randox Laboratories, UK).
Statistical analysis

The values were presented as means ± SD of different groups. Differences between the mean values were estimated using one-way analysis of variance (ANOVA). The results were considered statistically significant when p < 0.05.

RESULTS

Effects of cadmium exposure and Aframomum melegueta on total protein, albumin, direct bilirubin and total bilirubin

The effects of cadmium exposure and A. melegueta (200 or 400 mg/kg bw) treatment on total protein, albumin, direct bilirubin and total bilirubin are depicted in Figure 1. Serum total protein and albumin levels were decreased significantly p < 0.05 in cadmium-administered rats as compared with the control, whereas significant p < 0.05 increases were recorded in levels of serum total and direct bilirubin in cadmium-treated rats compared with the control. The treatment of cadmium-administered rats with A. melegueta (200 or 400 mg/kg bw) ameliorate this changes in a dose dependent manner.

Effects of cadmium exposure and Aframomum melegueta on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities

Figure 2 shows the effects of cadmium-exposure as well as the preventive effects A. melegueta (200 or 400 mg/kg bw) on serum aminotransferase activities. Exposing animals to cadmium resulted in severe hepatotoxicity, as indicated by the significant p <0.05 elevation of serum AST and ALT activities compared with the control animals. Consistent with these modifications, in a dose dependent manner, administration of A. melegueta prevented the elevations observed and restored the activities of AST and ALT near normal levels when compared with the control animals.

![Figure 1](image1.png)

Fig. 1: Effects of cadmium exposure on total protein, albumin, direct bilirubin and total bilirubin. Values are mean of six animals ± SD. *The mean is significantly different compared to control at P<0.05, and † mean is significantly different compared to cadmium-only group.
The protective role of *A. melegueta* on cadmium-induced oxidative liver injury is presented on Figure 3. A significant p<0.05 increase of MDA content associated with a corresponding and concomitant reduction in the activities of catalase, superoxide dismutase and glutathione peroxidase was witnessed in the hepatic tissue homogenate of rats exposed to cadmium. Administration *A. melegueta* (200 or 400 mg/kg bw) significantly p<0.05 mitigate and reversed the elevated MDA levels as well as significantly p<0.05 ameliorating the activities of catalase, superoxide dismutase and glutathione peroxidase in hepatic tissue homogenate of rats exposed to cadmium compared with the control animals.

**Histological examination of liver sections**

Histological examinations revealed that cadmium-exposure resulted in severe hepatic damage as indicated by degeneration of hepatocytes associated with periportal hepatic necrosis as well as cellular infiltration by mononuclear cells and fatty infiltration. *A. melegueta* treated rats, however, showed marked improvements in the degree of various alteration patterns as presented in Figure 4.
DISCUSSION

Cadmium (Cd), a non-fenton metal; is an important environmental pollutant present in soil, water, air and food. Its intracellular accumulation induces oxidative stress leading to hepatocellular damage via displacement of redox-active metals, depletion of redox scavengers, inhibition of anti-oxidant enzymes and inhibition of the electron transport chain resulting in mitochondrial damage (Nair et al., 2013; Adiele et al., 2012; Patra et al., 2011). In the present study, the significant decrease in the serum total protein and albumin of animals exposed to cadmium can be attributed to impairment in hepatocyte functions causing decreased cytochrome P-450 activity and inhibition in protein metabolism in the liver (Ibiam et al., 2013; Asagba, 2010). Whereas, the increased serum level of direct bilirubin and total bilirubin in the present study is a clear indication of hepatic dysfunction which correlates with the oxidative damage in the liver due to oxidative stress (Renugadevi and Prabu, 2010; Kowalczyk et al., 2003). Administration of AM extract showed a significant reversal effect, which is in agreement with the result from similar studies (Zhang et al., 2015; Elgaml and Hashish, 2014).

The significant elevation witnessed in serum transaminases activity (AST and ALT) clearly indicates the loss of cellular integrity and the leakage of hepatic membrane. Hepatocellular injury associated with Cd-exposure is well established in literature (Baba et al., 2013; Lu et al., 2013). Both aminotransferases (AST and ALT) are mainly concentrated in the liver; ALT is localized solely in the cytoplasm, whereas AST is present both in the cytosol and mitochondria of hepatocytes (Haidry and Malik, 2014). Interestingly, the up-regulation of AST and ALT activities due to cadmium exposure was significantly declined following the concomitant administration of AM extract (200 or 400 mg/kg bw). The ability of AM extracts to ameliorate the elevated levels of AST and ALT, also confirms its protective role.

Furthermore, cadmium interference with cellular components led to increased generation of free radicals especially, reactive oxygen species (ROS) in Cd-exposed rats. This was evident by the noticeable elevation witnessed in the levels of lipid peroxides in liver. This finding is consistent with previous studies that have demonstrated that Cd-exposure was associated with elevation of thio-barbituric acid reactive substances (TBARS) in the liver (Ashour, 2014; Prabu et al., 2012; Srinivasan and Ramprasath, 2012). The disrupted LPO level was alleviated by administration of AM extract. Lipid peroxidation is one of the key manifestations of oxidative damage and has been found to play an essential role in the toxicity of many xenobiotics. Cd may induce damage directly by causing conformational changes of bio-molecules or modify specific binding sites. Added to this, Cd indirectly induces cellular damage; this is associated with metal driven generation of free radicals involving superoxide, hydroxyl radicals or nitric oxide, hydrogen peroxide and/or endogenous oxidants (Meena et al., 2014; Ognjanovic et al., 2008).

The data obtained in our study confirmed that Cd-exposure diminished the enzymatic antioxidant status in the liver. It has been shown that various antioxidants and antioxidant defense systems protect cells from Cd-induced toxicity (Ognjanovic et al., 2008). Generally, alteration in the antioxidant defense system enhances lipid peroxidation and oxidative stress. This defense system includes the enzymes SOD, catalase, glutathione peroxidase, glutathione-s-transferase as well as glutathione, which usually protect the cell against oxidative damage (Roopha and Padmalatha, 2012). Treatment with AM extract significantly restored liver CAT, SOD and GPx activities. These results were confirmed by the results obtained from the histopathological assessment of the liver which revealed that Cd-exposure stimulated hepatocellular injuries. In conclusion, oral administration of aqueous extracts of Aframomum melegueta could provide significant protection against cadmium-induced toxicity in male Wistar rats in a dose dependant manner via its free radical-scavenging activities. Therefore, in alleviating cadmium-associated oxidative stress; A. melegueta could be a promising nutritional-supplement.

Conflict of Interest: The authors declare that there is no conflict of interest.

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