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Short Communication

Spices Mixture Containing Garlic, Ginger and Nutmeg Has Protective Effects on the Kidneys and Liver of Cadmium Exposed Rats

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Abstract
Purpose: To determine the potential protective effect of spice mixture containing garlic, ginger and nutmeg on the liver and kidney of cadmium exposed rats.
Methods: Male albino rats (n=30) weighing 120 – 180 g, grouped into five (1-5) of 6 rats/group were studied. Group 1 (NC) rats were administered distilled water (1 ml) orally for 4 weeks and served as the negative control while group 2 (PC) rats were administered low dose (LD) cadmium (25 mg/kg body weight) orally for 4 weeks and served as positive control. Group 3 (TBE) rats were treated with spice mixture (SM); 300 mg/kg body weight orally for 2 weeks and then administered LD cadmium for 4 weeks. While group 4 (CET) rats were concurrently administered LD cadmium and SM for 4 weeks, group 5 (TAE) rats were administered LD cadmium for 4 weeks and then treated with SM for 2 weeks. The whole experiment lasted for 42 day after which the animals were sacrificed and blood collected for determination of biochemical parameters using standard procedures and techniques.
Results: Exposure to Cd produced greater increases in the liver function parameters. However treatment with SM significantly ($p<0.05$) reduced ALT in animals treated after exposure, AST and bilirubin in those treated before exposure and significant ($p<0.05$) increased serum albumin in animals treated before exposure to Cd. The altered renal function parameters and total serum cholesterol were restored to near normal values following treatment with SM.
Conclusion: It may be concluded that concurrent intake of garlic, ginger and nutmeg at culinary dose in the diet has both therapeutic and prophylactic effect at mitigating Cd toxicity and reaffirms the safely of spices combinations as being currently practiced.

Introduction

Cadmium (Cd) has been recognised as a common environmental pollutant associated with several adverse health effects such as carcinogenesis, hepatotoxicity, renal impairment as well as disruption of normal endocrine and reproductive functions.[1-6] It has been proposed that Cadmium mediates its cytotoxicity via several mechanisms including induction of apoptosis, ischemia, inflammation and oxidative stress.[4,5] Compelling evidence also suggests that cadmium may cause toxicity by displacing important divalent metals ions such as zinc, selenium, calcium, and copper from their binding site.[7,8] Once binds to the active site, Cd tends to mimic the action of these essential metal ions which are required for normal functioning of key enzymes and several biological activities. In support of this role, some divalent metals such as zinc, selenium and manganese have been shown to antagonize and prevent cadmium-induced cytotoxicity.[7-9]
Several studies have investigated a number of agents which have potentials to counteract cadmium-mediated toxicity. Some of these agents including blueberry, curcumin, caffeic acid phenethyl ester, etc have been shown to be effective in preventing cadmium toxicity.[10,11] For instance, studies have shown that ginger (*Zingiber officinale)* has ameliorative effect against cadmium-induced liver and kidney injury in rat models.[12,13] Again, Eteng *et al*.[14] have reported reversal of cadmium induced toxicity following dietary supplementation with garlic, ginger and cabbage in male Wister rats. On the other hand, apart from its protective effect on oxidative stress in hypercholesterolaemic rats reported by Hassanen,[15] nutmeg has been associated with hepatotoxic activity and intoxication.[16,17]
Considering that spices are usually used as mixtures and based on the fact that garlic, ginger and nutmeg have protective effects against oxidative stress, apoptosis, ischemia and inflammation which are implicated in cadmium-induced toxicity, this study aims to investigate the potential protective effect of spice mixture containing

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garlic, ginger and nutmeg on the liver and kidney of cadmium exposed rats.

Materials and Methods

Chemicals

Cadmium chloride was purchased from Sigma Aldrich (LLC. USA).

Animals

Male Wistar albino rats (n=30), weighing 120 – 180 g were used in this study. The rats were divided into 5 groups (6 rats / group). The animals were acclimatized to the animal room condition for at least a week prior to the commencement of the study. The rats were maintained in an environment with 12-hour light/dark cycle, temperature ranging from 25 – 27 °C and humidity of 55 – 60%. The rats were provided with rat pellets and water *ad libitum*.

Preparation of spices mixture extract

Fresh ginger rhizomes (*Zingiber officinale*), garlic bulbs (*Alluim sativum Linn*) and nutmeg (*Myristica fragrans*) were purchased from Abakpa main market in Abakaliki, Ebonyi State. The spices were peeled and washed with water. Fifty grams (50.0 g) of each spice was pounded together and thereafter soaked overnight in distilled water. It was later filtered and the filtrate was allowed to stand for 24 hours at room temperature (25-32°C) for sedimentation. At the end of 24 hours, supernatant was decanted and the sediment was dried under sun to obtain the crude extract. The extract was concentrated to dryness at 60 °C using electric oven. It was carefully evaporated to dryness on water bath at 40 °C. The extract was kept in a refrigerator (-20 °C) until used.

Experimental protocol/animal treatment

(i). Group 1 (NC): Rats were administered distilled water (1 ml) orally for 4 weeks and served as the negative control.
(ii). Group 2 (PC): Rats were administered low dose (LD) cadmium (25 mg/kg body weight) orally for 4 weeks and served as the positive control
(iii). Group 3 (TBE): Rats were treated with spice mixture (SM); 300 mg/kg body weight orally for 2 weeks and then administered LD cadmium orally for 4 weeks
(iv). Group 4 (CET): Rats were concurrently administered LD cadmium and SM (300 mg/kg body weight) orally for 4 weeks.
(v). Group 5 (TAE): Rats were administered LD cadmium orally for 4 weeks and then treated with SM orally for 2 weeks
The experiment lasted for 42 days

Sample collection/preparation

At the end of 42 days, the animals were fasted overnight and sacrificed and blood samples were collected into plain bottles. The samples were allowed to clot and retract after which serum was isolated after centrifugation at 2000g for five minutes. Serum was stored in the refrigerator (-20 °C) until they were analysed.

Biochemical analyses

Serum samples were analysed for total cholesterol, liver and renal function parameters using commercial test kits from Randox Laboratories (UK). The tests were carried out in strict compliance with the manufacturers' instructions.

Data analysis

Basic and inferential statistics were performed using Statistical Package for Social Sciences (IBM SPSS® ver. 20) for Windows®. Results were expressed as mean ± standard deviation. Differences in parameters among the groups were determined by Post-Hoc one-way analysis of variance (One-way ANOVA) with p values < 0.05 considered as significant.

Results and Discussion

The present study determined the effect of spices mixture containing garlic, ginger and nutmeg on the liver and renal functions parameters in cadmium exposed rats. The significant elevation in liver enzymes observed in the present study (Figure 1) reaffirms the hepatotoxicity of cadmium.[18] Elevation of liver enzymes in Cd-exposed animals has been attributed to leakage from the liver cytosol into the blood stream. Although the exact mechanism by which Cd mediate its toxicity is not completely understood, it has been found that Cd promotes lipid peroxidation in the liver soon after exposure by displacing iron and copper from intracellular sites and subsequently resulting in the initiation of Fenton reaction.[19] Other mechanisms of Cd toxicity include modification of biomolecles, modulation of DNA repairs and genotoxic consequences, antagonism of zinc and impairment of p53 protein[20] involved in suppression of cancer.

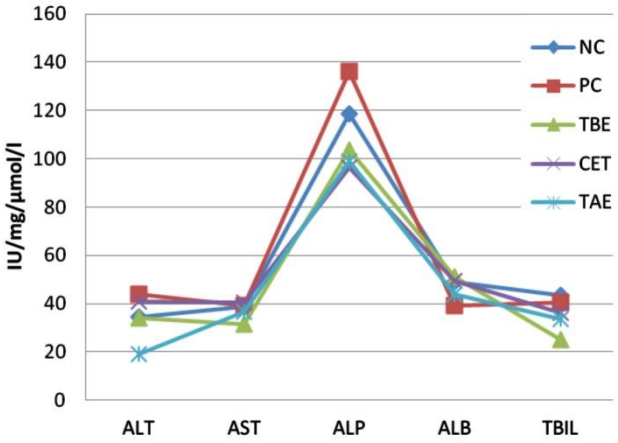

Figure 1. Effect of aqueous extract of spices mixture containing garlic, ginger and nutmeg on liver function parameters of cadmium exposed rats
***NC**: Negative control; **PC**: Positive control; **TBE**: Treatment before exposure; **CET**: Concurrent exposure and treatment; **TAE**: Treatment after exposure.*
ALT: Alanine aminotransferase; ***AST:*** Aspartate aminotransferase; ***ALP:*** Alkaline phosphatase; ***ALB***: Albumin; ***TBIL***: Total bilirubin

However, the significant reduction of serum ALT in animals treated with spices mixture after exposure to Cd, AST and bilirubin in animals treated with spices mixture before exposure to Cd and elevation of albumin in animals treated after exposure to Cd observed in the present study (Figure 1) suggest that spices mixture (SM) has potentials of ameliorating cadmium toxicity. It also suggests that SM exerts both therapeutic and prophylactive effect against cadmium-induced liver damage. These ameliorative effects of SM on Cd-induced liver damage have been attributed to antioxidant properties of spices. For instance, flavonoids and sulfur-containing compounds (diallyl sulfide, trisulfide and allylcysteine), the two main classes of antioxidant components have been reported in garlic.[21] Moreover, ginger has been found to contain zingerone, gingerdiol, zingibranes, gingerols and shegaols (all polyphenols), which also posses antioxidant activity.[22] Thus, the protective effect exerted by the spices mixture may in part be attributed to the biological active ingredients present in this mixture. The therapeutic and prophylactive effects proposed for spices mixture in the present study is in agreement with the findings of Egwurugwu *et al.*,[13] where it was concluded that cadmium detoxification by ginger was more effective therapeutically than prophylactically. For instance, it has been previously reported that dietary supplement containing ginger, garlic and cabbage, which have antioxidant properties reversed the alterations in biochemical parameters induced by Cd.[14]

The significant alterations in the renal function parameters of Cd exposed animals observed in the present study (Table 1) is in line with the renal toxicity of Cd.[23] It has been observed that Cd induced renal damage is associated with degeneration and hypertrophy of epithelial cells and dialation of glomeruli and massive local haemorrhage of the renal tissues in kidney tubules.[24] The present study however did not include histological examination of various organs, which would have confirmed these changes earlier attributed to Cd toxicity. Interestingly, the mechanism of Cd-induced kidney damage is thought to be related to increased oxidative stress.[25]

Table 1. Effect of aqueous extract of spices mixture containing garlic, ginger and nutmeg on serum total cholesterol and renal function parameters of cadmium exposed rats

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Total cholesterol (mg/dl)
NC	6.80±0.55^{a}	2.90±0.25^{a}	0.50±0.20^{a}	155.80±11.03^{a}
PC	8.57±1.02^{b}	12.33±1.30^{b}	2.00±0.70^{b}	231.67±17.93^{b}
TBE	8.27±0.59^{b}	2.77±0.57^{a}	1.30±0.80^{a}	135.97±40.11^{a}
CET	10.77±0.75^{c}	7.07±1.22^{c}	5.63±0.50^{c}	242.00±19.16^{c}
TAE	6.53±1.12^{a}	5.00±1.84^{d}	0.60±0.44^{a}	239.00±5.30^{d}

***NC**: Negative control; **PC**: Positive control; **TBE**: Treatment before exposure; **CET**: Concurrent exposure and treatment; **TAE**: Treatment after exposure.*
Values are expressed as means ± standard deviation. Values with different superscripts along the column were significantly different ($p<0.05$).

The restoration of altered renal function parameters following administration of spices mixture (SM) (Table 1) is in corroboration of earlier findings,[12,13] although in these studies, ginger alone was administered. It was however observed in these studies that ginger expressed an antagonistic action on Cd toxicity. The authors attributed this action to the high contents of antioxidants in ginger, which makes it a free radical scavenger.

Although exposure to Cd caused elevation of serum total cholesterol which either administration of SM concurrently or after exposure to Cd had no effect at ameliorating, administration of SM before exposure to Cd caused restoration of serum total cholesterol to almost the value in the non-exposed animals. This observation corroborated earlier findings of Ugwuja *et al.*[26] who observed that concurrent administration of garlic and ginger at culinary dose exerts beneficial effects on plasma glucose and lipids in health and diseases.

Although several studies have examined the potentials of individual spices in ameliorating Cd toxicity,[12,16,24] the present study seems to be the first to examine the role of spices mixture in mitigating Cd-induced organ damage. The present findings is therefore a true reflection of what is obtainable in practice, as spices are usually consumed as mixtures of two or more spices used to improve food flavour.

Conclusion

It may be concluded that spices mixture containing garlic, ginger and nutmeg possesses both therapeutic and prophylactic effect against Cd-induced organ damage. Intake of garlic, ginger and nutmeg concurrently at culinary dose in the diet is therefore recommended as an alternative way at mitigating Cd toxicity.

Ethical Issues

The project was approved by the ethics committee of Tabriz University of Medical Sciences.

Conflict of Interest

The authors report no conflict of interest.

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