

Antioxidants Roles of Some Plants Extracts in Cadmium-Induced Testicular Injuries

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Abstract

The effects of cadmium upon the testes were investigated in rats which injected subcutaneously with cadmium chloride (CdCl₂) (7 mg/kg. bwt. S/C). Seventy rats (10 rat/group) were used and administered by curcumin, broccoli, ginger, garlic and mint extracts for seven days followed by injection of CdCl₂, also positive group injected by CdCl₂. After twenty four hours, all the animals were sacrificed and the testes were removed. The extent of haemorrhage was estimated by determining the absorbance of haemoglobin at 414 nm in the soluble fraction of organ homogenates. Exposure to CdCl₂ increased the haemoglobin absorbance of the testes from 0.533 ± 0.015 (control group) to 1.51 ± 0.24 (CdCl₂ group). Oral pre-treatment with several extracts of antioxidant plants seven successive doses for seven days greatly provoked reduced the cadmium-induced testicular haemorrhage. It is concluded that all the vegetable extracts had gradual protective effects against cadmium induced testicular damage in rats according the presence of antioxidant and phenolic contents.

Keywords: Antioxidants; Cadmium toxicity; Garlic; Broccoli; Curcumin; Menth; Ginger

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Introduction

Food and food products may be pollute by some metals and this cause serious problems. The source of these metals comes from food plants and cereals [1,2]. Cadmium content in food plants do not exceed the permissible limits but the cadmium has accumulating in tissues and has long half-life time [3], so low level of cadmium is very dangerous. About 2.5 mg/kg b.wt./week is a tolerable intake level of cadmium [1]. There are no methods used to minimizing the amount of Cd⁺⁺ in foods. Chelating agents are using to reduce Cd⁺⁺ through prevention the absorption, increasing oxidative capacity of the body. Some antioxidants such as curcumin, vitamin C, vitamin E, rutin have been antioxidant effects [4-6]. From view of nutritional point it is very great to examine the eatable food for its antioxidant content to be using them in daily diet to reduce the dangerous effects of toxic metals. Some available literature has considerable amounts of antioxidants and recorded [7-10]. The half-live time of Cd⁺⁺ is 7-15 years, so it excreted very slowly and induced serious effect to the body [11, 12].

One important of food spices is ginger (*Zingiber officinale*). It's spread all over many countries around the world. It contains several active principles include trace elements, vitamin C,

resins, vitamin E, gingerol, volatile oil and vitamin B. It is used to detoxycating the metals effects of body tissues. It is suitable to use therapeutically instead of prophylactically [13]. It is also used an antitoxic and antitumor [14-18].

The active principle of *Curcuma longa* (*Zingiberaceae*) is Curcumin, it is used in India as a coloring agent. It has a fretfully effect as antioxidant [18,19]. It is playing an important role as a therapeutic agent and has no dangerous effects [20]. Sharma and Ahuja recorded that curcumin play a benefit effect against cadmium toxicity.

Brassica oleracea var. *capitata* (cabbage) rich of antioxidant and uses against metal toxicity and prevent its side effects [21]. It is playing an important role for treatment of some human and animal diseases. It was used as antitumor, antithrombotic, hepato-protective effects [22,23].

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In experimental study [24] reported that garlic protect and weaken the hepatotoxic and teratogenicity effects of cadmium on egg embryo. Garlic help in treatment of cancer and some other diseases [25], its used as a protective and therapeutic agent against metal toxicity [26]. Garlic used for cancer treatment because it rich in an antioxidant content and contains S-allylcysteine, alliin, allicin and alliinase [27,28].

This study was performed to further explore the effects of extracts of some antioxidant plants on the haemorrhages of testicular blood vessels in male rats.

Literature Review

Chemicals: Cadmium chloride (CdCl₂), tris-HCl, MgCl₂ and CaCl₂.

Animals: Seventy apparently healthy male albino rats weighing 150-175 g. were kept in fibre glass cages and were allowed to clean food and water ad-libitum. They were acclimatized for 2 weeks.

Experimental design

The rats were randomly divided into seven groups of ten animals each.

Group 1: were administered with 1 ml saline orally and one hour later injected with 1 ml saline subcutaneously (control).

Group 2: were administered with 1 ml saline orally and one hour later injected with 1 ml CdCl₂ S/C at a dose of 7 mg/kg.b.wt. [29].

Group 3: were administered with 1 ml ethanolic ginger extract (500 mg/kg.bwt.) orally for seven days and at the eighth day rats injected S/C with CdCl₂ at the same dose.

Group 4: were administered with 0.5 ml pure garlic juice orally for seven days and at the eighth day rats injected S/C with CdCl₂ at the same dose.

Group 5: were administered with 1 ml *Brassica oleracea* pure juice orally for seven days and at the eighth day rats injected S/C with CdCl₂ at the same dose.

Group 6: were administered with 1 ml menth pure juice orally for seven days and at the eighth day rats injected S/C with CdCl₂ at the same dose.

Group 7: were administered curcumin 15 mg/kg orally for seven days and at the eighth day rats injected S/C with CdCl₂ at the same dose.

After twenty four hours, rats of all groups were I/P injected with pentobarbital sodium (50 mg/kg) and rats were sacrificed and the testes were removed.

To determine the extent of Cd²⁺ induced haemorrhaging, both testes, from individual rats were homogenized in a mortem in 10 ml of 5 mM (tris-HCl buffer, pH, 7.0) containing 1 ml mM MgCl₂ and 100 μm CaCl₂. The homogenate was centrifuged at 5000 rpm for 30 min. A 1 ml aliquot of the supernatant fluid was diluted in 5 ml of buffer. The absorbance of haemoglobin at 414 nm was determined using spectronic 21 spectrophotometer (Busch and Lomb) as adopted by [29].

Statistical analysis was performed by SPSS software.

Results

The administration of cadmium chloride induced a characteristic haemorrhages and testicular edema. The testes from cadmium-treated rats were swollen and dark violet in color. Cadmium treatment significantly increased ($P \leq 0.05$) the haemoglobin absorbance of the testes (**Tables 1 and 2**). Testes from rats that were pre-treated with different plant extracts for seven days showed different low level visible damage. Furthermore, the haemoglobin absorbance were significantly ($P \leq 0.05$) reduced when compared with the CdCl₂ treated group. There were different significant between all groups compared with CdCl₂ treated group. The optical density of curcumin was significantly different with optic density of other extracts.

Discussion

Cadmium toxicity has been inducing cardiovascular diseases such as atherosclerosis and hypertension [30-32]. Hypertension in animals due to chronic cadmium toxicity was observed [33-35].

Cadmium toxicity has been increasing oxidative stress, leading to damage and dysfunction to vascular cells [36-38]. Cadmium induced vasodilator of arterioles and arteries leading to hypertension [39]. The testicular toxicity of cadmium mechanism is poorly understood. The histopathological damages of blood vessels were caused by cadmium as a principle cause of toxicity [40].

Our results were agreement with that mentioned by several authors who were studied the effect of several antioxidant extracts, curcumin, garlic, ginger, broccoli, and green tea, respectively.

Groups	Testes O.D. Mean ± SE
Control	0.533 ± 0.015 ^c
CdCl ₂	1.51 ± 0.24 ^a
CdCl ₂ +Ginger for 7 days	0.743 ± 0.162 ^c
CdCl ₂ +Garlic pure juice for 7 days	0.632 ± 0.079 ^c
CdCl ₂ +broccoli pure juice for 7 days	0.465 ± 0.062 ^c
CdCl ₂ +mint for 7 days	0.956 ± 0.117 ^{ab}
CdCl ₂ +curcumin for 7 days	0.427 ± 0.064 ^d
Mean ± Standard error of the mean	
A,b,c,d=Means with the same letters in the same column are not significantly different.	

Table 1: Testes optical density of the different experimental groups.

Parameter	Sum of squares	Degress of freedom	Mean squares	F-ratio	Significant result
Between groups	5.569	6	0.928	7.286	0.000
Within groups	11.465	70	0.127		
Total	17.034	76			

Table 2: It represents Analysis of variance (ANOVA).

The extract of the rhizome of turmeric is curcumin, it is a phenolic compound. Curcumin has a strong power of free radical scavenging and antioxidant [41, 42]. It has also anti-inflammatory, antimicrobial, anti-carcinogenic, antiviral and anti-apoptotic properties [43]. The unique structure of curcumin has a protective effect against cadmium damages of the testicular tissue. Switch off the lipid oxidation is achieved by the action of the active structure of curcumin (β -diketone moiety and orthomethoxylated phenols) before they strike to lipid membrane, and safeguard the testicular tissue from Reactive Oxygen Species (ROS) [44,45]. Manikandan et al. studied the mode of action of curcumin they were decided that curcumin decrease significantly the free radical's generation and the activities of antioxidant enzymes increased thereby manifested refreshment. Curcumin play an important role for preventing the damage of testicular membrane [46-49].

Cadmium administration induced mild congestion of seminiferous blood vessels. Garlic treatment prevented the cadmium damages on seminiferous blood vessels of the testes [50]. Our results were agreement with these results and also with the results obtained by [51]. Platelet aggregations were inhibited by garlic, this action was related to garlic active principles and their antioxidant effects [52,53]. In experimental study, administration of aged garlic extract to mice was prevented the first stage of Apo lipoprotein and atherosclerosis, so inhibited lipid deposition and vascular inflammation [54,55]. In previous studies Ponnusamy and Pari were indicated that the active principle, Daily Tetrasulfide (DTS) of garlic has the antioxidant and cytoprotective activity against Cd-induced toxicity *in vivo* and *in vitro*. Lipid peroxidation and oxidative damage in tissues caused by cadmium were prevented and reduced by DTS [56,57].

Ginger is stimulating heart muscles and enhances diluting blood circulation [58]. The ameliorate of circulation is considered to rise the cellular metabolic activity, thus engaging to reduction of tension and cramps. The inhibitory effects of platelet aggregation of ginger were the similar effects of garlic [59].

Cadmium was increased malondialdehyde (MDA), lipid peroxidation (LPO) and reduced protein content in testicular and prostatic tissues compared to control. Broccoli has a useful effect on reduction of lipid peroxidation (LPO) and (MDA) [60,61].

Cabbage has treated haematological indicator and pathological of cadmium damages. The increases of the activity of antioxidant enzymes have been protecting the tissues against cadmium damages. Vitamin E has been renewing the antioxidant enzymes of the tissues. Cadmium has been reducing vitamin E and C as non-enzymatic antioxidants [62]. Broccoli has a natural enzymatic antioxidant, sulforaphane enzyme [63, 64]. Sulforaphane acts as a hunter of free radicles [65].

The certain vegetables used in our experiment are commonly consumed by our populations. They had little to high effects on the protection against cadmium toxicity, the descending gradient of potent effects were curcumin, broccoli, garlic, ginger, and mint, respectively.

Our results of evaluation of antioxidant activity of the extracts were used in our experiments, parallel with studied by [66-69]. Also our results were agreement with that authors mentioned in our discussion. Some extracts had high antioxidant contents, also had high total phenolic content. On the other hand some extracts like broccoli and turmeric had low phenolic content but had high antioxidant activities. This phenomena was due to some phenolic units had high antioxidant activates. While, other authors decided that there were no correlation between antioxidant contents and phenolic contents of plant extracts [70-72]. The presence of total phenolic compounds of the herbs and spices were correlated with the antioxidant activity [73].

Conclusion

Some extracts had high antioxidant contents, also had high total phenolic content. On the other hand some extracts like broccoli and turmeric had low phenolic content but had high antioxidant activities. This phenomena was due to some phenolic units had high antioxidant activates. All the vegetable extracts had gradual protective effects against cadmium induced testicular damage in rats according the presence of antioxidant and phenolic contents.

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