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Alleviative Efficacy of Caffeic Acid against Cadmium provoked Oxidative Dysfunction in Rats

- Kalist Shagirtha[a*]
- Priya Nagappan[a]
- Celine Hilda Mary[a]

Abstract

Aim of the current investigation was to evaluate the attenuvative potential of caffeic acid (CA) on cadmium (Cd) induced oxidative liver ailment in rats. Intoxication of Cd (3mg/kg body weight/day) subcutaneously for 3 weeks elevated the hepatic levels of lipid peroxidative markers thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOH), conjugated dienes (CD) and protein carbonyl content (PCC) along with the significant decrease in the levels of vitamin C, vitamin E, reduced glutathione (GSH), total sulphydryl group (TSH) and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxides (GPx), glutathione-S-transferase (GST), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PD) in rats. Treatment with CA at a dose of 60mg/kg body weight/day for 3 weeks in Cd intoxicated rats significantly revert back the altered levels of serum hepatic markers, lipid peroxidative markers and antioxidant markers in the hepatic tissue back to near normal level. Accordingly our findings imply that CA acts as a potent hepatoprotective agent against Cd elicited liver dysfunction in rats.

Key words: Caffeic acid; Cadmium; Hepatotoxicity; Rat

INTRODUCTION

Cadmium (Cd) is a occupational and pollution related toxin that has been distinguished as a hazard factor for actuating various organ dysfunctions in people. Event of Cd in all parts and bundle of nature can prompt serious organ harm, even at insignificant introduction levels [1]. Already it has been found out that consistent low level of cadmium presentation incites negative wellbeing consequences for uncovered subjects. The take-up of Cd from the dirt through the foundations of the plants acquires a hoisted level vegetables, organic products, and grains, with the most elevated amounts in verdant greens and potatoes. Cd came into the body generally by inward breath or by ingestion, i.e. through GI tract. Cd is a powerful dangerous metalloid and it actuates the wounds to numerous fundamental organs, similar to liver, kidney, cerebrum, heart, lung, testis, bone and so forth.

[a] Department of Biochemistry, St. Joseph's College of Arts and Science (Autonomous), Cuddalore, Tamil Nadu, India.
*E-mail: shagirtha@gmail.com
Mobile No.: +91 9842435145

Cd has been sorted as a cancer-causing agent by IARC. The hurtful results of Cd answered to date, incorporates oxidative anxiety, consumption of cell cancer prevention agents like decreased glutathione (GSH), lifted lipid peroxidation and changed cancer prevention agent protein exercises, articulation of apoptosis and hesitance of DNA repair chemicals [2]. Caffeic corrosive (CA) (3,4-dihydroxycinnamic corrosive) is a catecholic compound broadly accessible in many plants as a feature of natural products, tea, espresso and wine [3]. It has been demonstrated as a compelling cancer prevention agent against different maladies [4]. CA decreased the levels of intracellular free radicals and oxidized bases in DNA perhaps by its broad searching movement. Likewise, its ability to keep the arrangement of ROS/RNS incited oxidative and nitrative anxiety has been accounted for in numerous in vivo contemplates [5]. Sud'ina et al (1993) likewise revealed that a convergence of 10 μM, CA totally obstructs the creation of ROS in human neutrophils and the xanthine/xanthine oxidase framework [6]. Based on its extensive health benefits against various oxidative ailments, still there is a lacuna on its protective efficacy against Cd induced oxidative liver damage. Hence the present study has been designed to establish its potential benefit against Cd intoxication in rats.

MATERIALS AND METHODS

Animals Male albino Wistar rats, body weight of 210-220g bred in central animal house, Rajah Muthiah Medical College, Annamalai University were used in this study. This study was approved (Vide No. 187, 2011) by the Institutional Animal Ethical Committee of Annamalai University and the study conducted in accordance with the "Guide for the care and use of Laboratory Animals", India. Six rats were housed in each polypropylene cage and were kept up as per the rules and regulations of the national foundation of Nutrition, Indian Council of Medical Research, Hyderabad, India. The rats were nourished on a pellet feed (Lipton India Ltd., Mumbai, India) and water ad libitum. Chemicals like Caffeic acid, cadmium chloride, 2-thiobarbituric acid (TBA). Decreased glutathione (GSH), 2,2'- Dipyridyl, xylenol orange, trichloro acidic acid (TCA) were gotten from Sigma Chemical Co. (St. Louis, MO, USA).

EXPERIMENTAL DESIGN

The animals were randomly divided into four groups of six rats in each group.

Group 1: control rats subcutaneously administrated with isotonic saline for 28 days.

Group 2: control rats received CA (60 mg/kg body weight) in aqueous solution daily using intragastric tube for 28 days.

Group 3: rats received Cd as cadmium chloride (3 mg/kg/day) subcutaneously (sc) in isotonic saline for 28 days.

Group 4: rats received Cd as cadmium chloride (3 mg/kg/day) with oral administration of CA (60 mg/kg/body weight) in aqueous solution for 28 days.

At the end of experimental period, animals in different groups were sacrificed by cervical decapitation under pentobarbitone sodium (60 mg/kg body weight) anesthesia. The blood samples were collected in tubes, without heparin for serum separation. Serum was separated by centrifugation and used for various biochemical estimations.

BIOCHEMICAL ASSAYS

Estimation of Lipid Peroxidation

Lipid peroxidation in liver was estimated spectrophotometrically by measuring thiobarbituric acid reactive substances (TBARS) and hydroperoxides by the method of Niehiaus and Samuelsson and Jiang et al [7,8] respectively. As a hallmark of protein oxidation, total protein carbonyl content was determined in the liver by a spectrophotometric method described by Levine et al. [9]. The level of conjugated dienes (CD) was assessed by the method of Rao and Racknagel [10].

Determination of Non-enzymatic Antioxidants

Vitamin C concentration was measured by Omaye et al. [11]. Vitamin E was estimatd by the method of Desai [12]. Reduced glutahione (GSH) was determined by the method of Ellman [13]. Total sulfhydryl groups (TSH) in liver homogenate were measured after the reaction with dithiobisnitro benzoic acid using the method of Sedlak and Lindsay [14].

Assay of Antioxidant Enzymes

Superoxide dismutase (SOD) activity was determined by the method of Kakkar et al[15]. The activity of catalase (CAT) was determined by the method of Sinha [16]. Glutathione peroxidase (GPx) activity was estimated by the method of Rotruck et al [17]. The glutathione S-transferase (GST) activity was determined spectrophotometrically by the method of Habig et al[18].

Glutathione reductase (GR) that utilizes NADPH to convert metabolized glutathione (GSSG) to the reduced form was assayd by the method of Horn and Burns [19]. The estimation of glucose-6-phosphate dehydrogenase (G6PD) was carried out by the method of Beutler [20].

Statistical Analysis

Data were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a statistically software package (SPSS for Windows, V. 13.0, Chicago, USA). Results were presented as mean ± S.D., $p<0.05$ were considered as statistically significant [21].

Results

Impact of CA on lipid peroxidation (LPO) and protein oxidation status in control and test rats are portrayed in Fig 1-4. The levels of TBARS, LOOH, protein carbonyl substances and conjugated dienes were significantly ($p<0.05$) inflated in Cd treated rats. Administration of CA alongside Cd fundamentally diminished the levels of lipid peroxidaion products in liver.

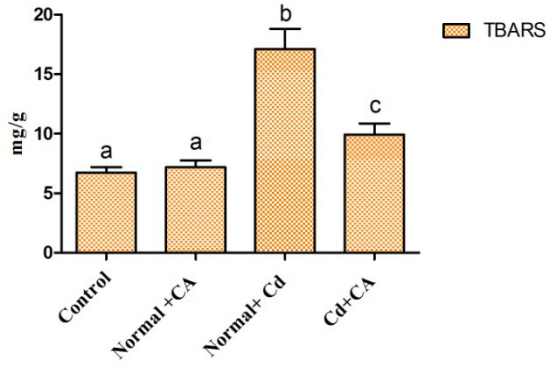
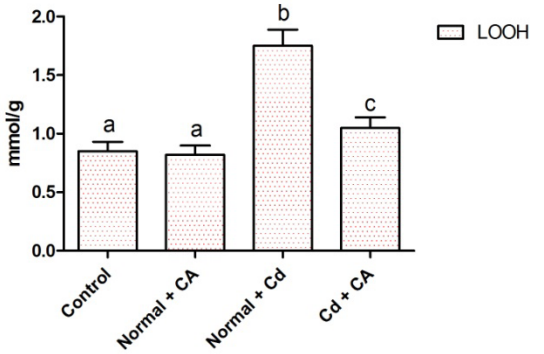


Fig.1



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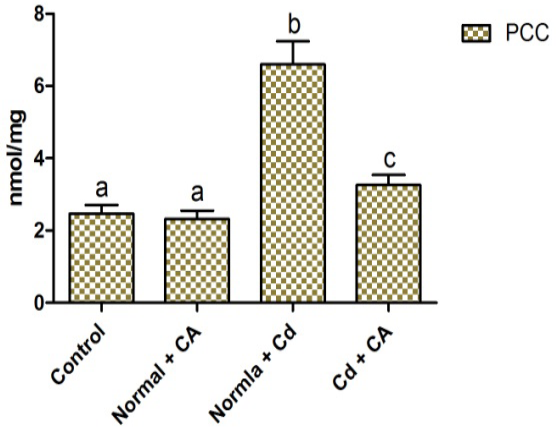

Fig.3

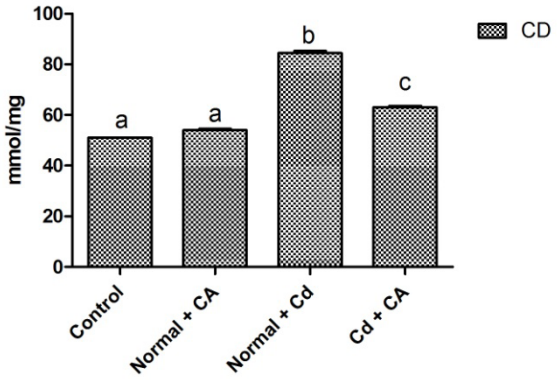

Fig.4

Fig. 1-4. Changes in the levels of lipid peroxidation, lipid hydroproxides, protein carbonyl substance and conjugated dienes of control and test rats. Values are given as mean±S.D. from six rats in each gathering. Values not sharing a typical letter (a-c) contrast altogether at $p<0.05$ (DMRT).

Impact of CA on non-enzymatic cell reinforcement's status Fig. 5 and 6 delineates the levels of non-enzymatic cell reinforcements in particular vitamin C, vitamin E, GSH and TSH in liver of control and test rats. A huge ($p<0.05$) diminish in the exercises of non-enzymatic cancer prevention agents in Cd treated rats were watched. Treatment with CA fundamentally ($p<0.05$) expanded the levels of non-enzymatic antioxidants in liver of Cd treated rats.

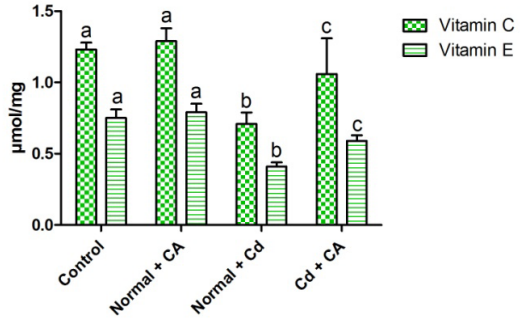

Fig.5

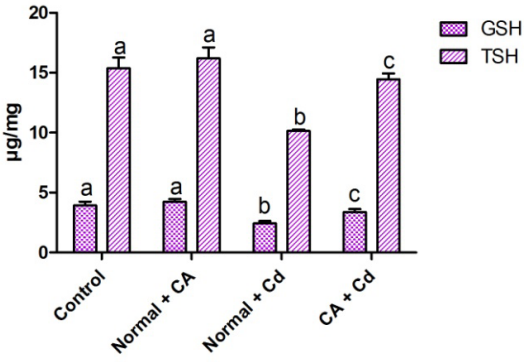

Fig.6

Fig.5 &6. Changes in the levels of non-enzymatic cell reinforcement status of control and exploratory rats. Values are given as mean ±S.D. from six rats in each gathering. Values not sharing a typical letter (a-c) contrast essentially at $p < 0.05$ (DMRT).

Impact of CA on enzymatic cell reinforcement status Fig. 7-12 shows the levels of enzymatic antioxidant agents to be specific SOD, CAT, GPx, GST, GR and G6PD in liver of control and exploratory rats. A huge ($p < 0.05$) diminish in the exercises of enzymatic cell reinforcements in Cd treated rats were watched. Treatment with CA fundamentally ($p < 0.05$) expanded the levels of enzymatic antioxidants in liver of Cd treated rats.

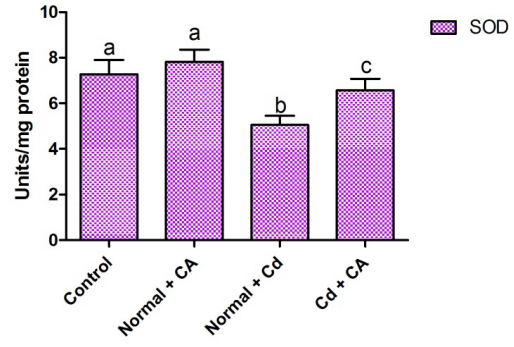

Fig.7

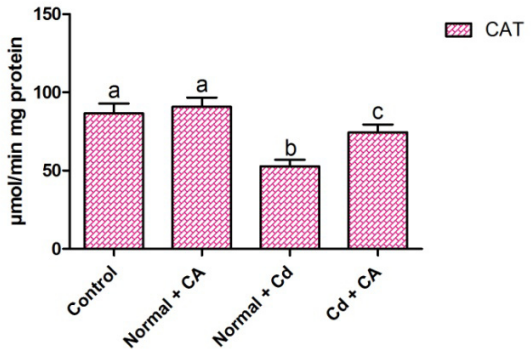

Fig.8

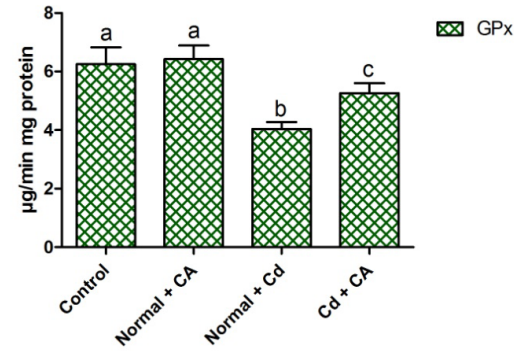

Fig.9

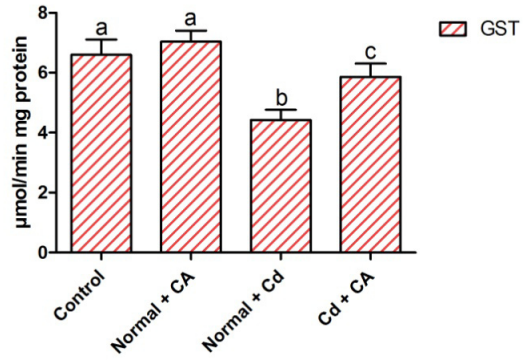

Fig.10

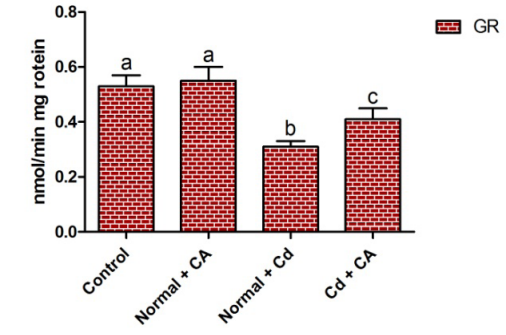

Fig.11

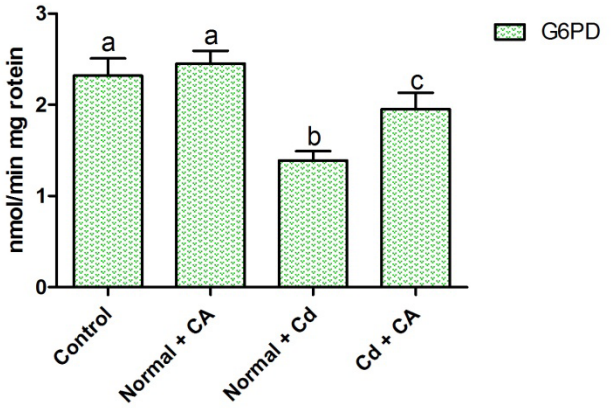

Fig.12

Fig. 7-12. Changes in the activities of enzymatic antioxidant status of control and experimental rats. Values are given as mean± S.D. from six rats in each group. Values not sharing a common letter (a-c) differ significantly at $p < 0.05$ (DMRT).

DISCUSSION

Cadmium (Cd) is a persuasive and natural harmful metal worried about an assortment of unfavorable impacts. A few components have been accounted for about the Cd intervened oxidative liver damage is primarily because of expanded lipid peroxidation, consumption of GSH or by the reticence of antioxidants and interaction with membrane components [22]. Karbownik et al, revealed that the security against Cd danger can have the capacity to accomplished by the admittance of cell reinforcements like natural antioxidants [23].

Our research outcome likewise substantiated that the supplementation of CA (60mg/kg) fundamentally secured the hepatic capacity against Cd inebriation in rats. Lipid peroxidation is a detrimental oxidative process where major responsive free radicals associate with layer PUFA to start a progression of responses that outcome in a scope of debasement items. Cd in a roundabout way creates receptive free radicals like hydroxyl, superoxide, nitric oxide and hence causes harm tried and true with oxidative anxiety [24]. The free radicals straight forwardly focus on the layer prompting peroxidative harm, in this way causes destabilization and crumple of cell membrane as a result of LPO. In our examination, the hoisted levels of TBARS, LOOH, PCC and CD following Cd inebriation confirmed with the discoveries of Renugadevi and Prabu in Cd treated rats [25].

Treatment with CA fundamentally diminished the Cd instigated peroxidative liver harm which is confirmed from the diminished levels of TBARS, LOOH, PCC and CD. This might be because of the CA organization which additionally acts as a chain breaking cell reinforcement, along these lines ending the chain response of lipid peroxidation and limiting its harmful impacts [26]. Non-enzymatic cell reinforcements, for example, Vitamin C and Vitamin E have the synergetic activity in rummaging oxygen determined free radicals and they are probably going to be most vulnerable to Cd induced free radical oxidations. In the present examination, the depressed levels of GSH and vitamins C&E in Cd treated rats further aggravate the oxidative damage to the liver. Our discoveries are in consonance with the other distributed reports which has been cited that GSH hub is diminished in the midst of Cd toxicity [27].

CA through its intense cell reinforcement and metal chelating movement, may limit the use of these antioxidants and there by reestablishing their levels. The above outcomes show the physiological significance of CA and its cancer prevention agent activity in an *in vivo* strategy. As revealed before, our investigation demonstrated that presentation to Cd prompt an expansion of LPO focus related with an unmistakable diminishing levels of SOD, CAT and GPx in liver. Cd initiated the heightening of LPO in liver may be a result of expanded development of free radicals and the decrement of SOD, CAT and GPx activities [28]. The vast majority of the cell reinforcement catalysts are being latent by Cd presentation, which is related with guide authoritative of Cd to their dynamic site, on the off chance that they contain SH gatherings or dislodging of metal cofactors from their dynamic site [29].

In the present investigation the decreased levels of antioxidant compounds mirrors the disappointment of cell reinforcement barrier movement to defeat the deluge of ROS on Cd initiated poisonous introduction. In the present examination the levels of SOD, CAT, GPx, GST, GR and G6PD were decreased fundamentally in Cd instigated rats, which is in accordance with the report of Renugadevi and Prabu (2010) [25]. Treatment with CA in Cd treated rats essentially expanded the rejuvenation of antioxidants which were modified by Cd.

CA was found to expand the levels of antioxidants, proposing that searching of ROS might be because of its powerful cell reinforcement action, thus bringing about decreased oxidative anxiety. Another conceivable instrument which may add to the defensive part of CA is that its metal chelating capacity may diminish the Cd toxicity prompting expanded movement of cell reinforcements and diminished lipid peroxidation [30]. Also, CA can twofold the oxidative prevention and limit of plasma even in its micromolar levels as revealed by Nardini et al., 1995, along these lines saving its endogenous non-enzymatic cell reinforcements in this way reestablishing their ordinary levels [31].

CONCLUSION

Taken together, the findings of our present investigation shows that CA weaken the unsafe impacts of Cd in liver by lessening the Cd aggregation and the biochemical component identified with the concealment of oxidative anxiety consequently enhanced the status of cell reinforcement framework and the capacity of liver. Our outcomes additionally uncover that the antioxidant prospective of CA may include in the constriction of Cd initiated hepatic damage and CA supplementation may be a fruitful regimen to lessen the harmful impacts of Cd on Liver. Dietary expansion with CA could be a straightforward, modest and significant strategy to safeguard the individuals who are occupationally/ naturally presented to Cd and its lethal impacts.

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