IN VIVO HEPATOPROTECTIVE EFFECT OF CAFFEIC ACID ON MERCURIC CHLORIDE-INDUCED BIOCHEMICAL CHANGES IN ALBINO WISTAR RATS

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ABSTRACT

Objective: Mercury (Hg) is a highly dangerous and also one of the harmful heavy metals which induces oxidative stress in the animal body. The present study is planned to examine the possible defensive result of caffeic acid (CA) against mercury chloride ($HgCl_2$)-induced hepatotoxicity in male albino Wistar rats, *Rattus norvegicus*.

Methods: Sublethal dose of $HgCl_2$ (1.29 mg/kg body weight) was administrated in rats for 15 days through oral dose and the CA was administrated for another 15 days on mercuric-intoxicated rats. After completing the scheduled exposure time, the rats were sacrificed and the whole liver organ was removed immediately from the animal, and it was used to carry out for biochemical and bioenzymological studies to observe.

Results: The level of lipid peroxidation (LPO) content reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the liver tissue. CA is an energetic component in the phenolic propolis extract and also in a wide variety of plants, and a strong antioxidant helps to prevent oxidative damage and to reduce oxidative stress. The antioxidants such as GPx, CAT, and SOD and non-antioxidant GSH were significantly decreased, and also, the LPO level was increased in mercury toxicity rats. The treatment of CA (5 mg/kg body weight) in the liver tissue shows considerable declining in the level of oxidant content and along with an increase in the level of antioxidant properties by the way of improvement in liver tissues. Antioxidant and non-antioxidant enzyme (LOP, GSH, GPx, SOD, and CAT) activities were also significantly decreased to near untreated control level when compared to Hg-treated group. The CA acid alone treatment showed the enhanced antioxidant levels and not any alteration in the levels of biochemical parameters when compared with control.

Conclusion: These observations of the present experimental study clearly explained the detoxify effects and protective effect of CA against $HgCl_2$ toxicity in liver tissue.

Keywords: Mercuric chloride, Hepatotoxicity, Caffeic acid, Lipid peroxidation, Superoxide dismutase, Catalase, Glutathione and glutathione peroxidase.

INTRODUCTION

Mercury (Hg) and its compounds are called as worldwide pollutant because it easily distributed in air, water, and soil and possess high risk to global health. Nowadays, most of the researcher's attention has been refocused on the toxicity of the heavy metal which is present in various forms of mercury compounds. Mostly, inorganic forms of mercury compounds have used in the production of various products and agriculture medicines, etc. [1]. Normally, heavy metals are having high affinity with thiol groups (−SH). If the sulfhydryl group is available at the enzyme active site, the heavy metals immediately react with it and decrease the availability of total sulfhydryl groups in cell to get oxidative stress [2,3]. Mercury (Hg) and its compounds will bind to any free thiol available in the system, and the heavy metals are most frequently bound with the thiols due to its high concentrations [4]. Once the reaction rate was initiated, the stable mercury-sulfhydryl bond was formed because it is liable in the presence of other free total sulfhydryl groups [5]. Chen [6] have also suggested that mercury and its compounds are biochemically toxic as it binds to sulfhydryl groups (−SH) and leads to disruption of protein structures and functions. In the present experimental study, the decreasing level of total protein content presents in the liver tissues of rats when treated with mercury toxicity also supporting the above findings. Proteins are common fundamental biomolecules to all living organisms, and they are responsible for most of the biocomplex functions that make life in a possible way. and proteins also act as major structural ingredients of living beings. Proteins play a vital role to carry out various types of physiological functions of an organism with the help of the enzymes or subunits of enzymes [7]. Naturally, formation of free radical process causes oxidative decoration of lipid leading to promote the production of lipid peroxidation (LPO) content and it also used as an indicator of oxidative stress. Promoting the imbalance between the production of reactive oxygen species (ROS) and free radicals (FR) in the organs due to the accumulation of LPO content caused the pathogenesis of different types of diseases, damages, and defects [8-10]. During the accumulation of LPO content such as aldehydes, inter alia, and malondialdehyde (MDA) leads to cellular membrane damages by oxidative destruction of polyunsaturated fatty acids [11,12]. Total amino acids are the building blocks of the protein in animal tissues. Most of the amino acids can easily interact with one another and with other molecules to form different complex molecules and also useful in the synthesis of cellular, subcellular, and other organic substances. The formations of different types of proteins mainly depend on the arrangement of amino acid sequences and in varying numbers. Different types of amino acid pool and their arrangement in the tissues may get vary in their chemical composition [13]. During the stressful condition, total free amino acids would also serve as energy precursor and also utilized for the synthesis of required proteins to overcome the stress. A number of experimental works have been conducted and explained the biological toxicity of $HgCl_2$ in an organism by carrying out the biochemical fate of mercury toxicity [14]. Nowadays, an emerging interest has been focused on the use of phytochelating plant products with ameliorative potentials and its antioxidant activity in heavy metal intoxication therapy. Caffeic acid (CA) is one of the non-flavonoid catecholic compounds (the most common phenolic acids). It is easily available in several species of plants, and it also occurs in parts of fruits, vegetables, tea, coffee, and wine [15] and is regularly consumed in human diet [16,17]. In the past

three decades, CA and its derivatives have a protective role that is clearly demonstrated by number of experimental works, showing that these chemicals have a variety of functions including acting as antioxidant and suppressing LPO [18-20]. It is a natural phenolic compound found in many plants. CA is a phenylpropanoid compound which is available in plant tissue metabolites [21]. This phenolic compound is mostly occurred in coffee, blueberries, apples, and ciders [22]. Sato [23] have reported that this compound is having high antioxidant property; due to this property, it is useful in neutralizing or nullifying the harmful effects of FR on cells. In the present experimental work, an increased level of protein content and simultaneously decreased level of total amino acid content were noticed in liver tissues of Hg-intoxicated rat when treated again with CA for another 15 days. This result suggested that the intoxicated cells of the respective organs are getting recovery from the toxicity effect of mercury compounds with the help of CA. An enhanced level of protein content in the examined tissues suggested that there is initiation of protein synthesis and simultaneously proteolytic conditions are stopped. The role of CA is mainly in protecting the cells from the adverse effect of $HgCl_2$-induced toxicity.

METHODS

Chemicals
In the present experimental work, mercuric chloride ($HgCl_2$), CA, and all other necessary reagents of analytical grade were used and it was purchased from Hi-Media Laboratories Ltd., Mumbai, India.

Animals
Healthy male albino rats, *Rattus norvegicus* (180–200 g), were obtained from the Central Animal House, Department of Experimental Medicine, Raja Muthiah Medical College and Hospital Annamalai University, and maintained in an air condition room (25±3°C) with a 12-h light/12-h dark cycle. Feed, water, and *ad libitum* were provided to all the animals. The study protocols were approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No: 160/1999/CPCSEA, proposal Number: 1156), Annamalai University, Annamalainagar.

Experimental design
A total of 24 animals were acclimatized in the animal cages for 15 days. The animals were randomized and divided into four groups, each group containing six rats. The toxic dosage of mercuric chloride has been obtained from our previous study as sufficient to elicit mild or moderate oxidative stress for mercuric chloride.

  Group I: Untreated control – the animals were received standard diet and clean water *ad labitum* and observed for 15 days.
  Group II: Mercury treatment – the animals were received 1.29 mg of mercuric chloride/kg body weight orally every day up to 15 days.
  Group III: Mercuric chloride followed CA treatment – the animals were received mercuric chloride followed by caffeic acid (1.29 mg $HgCl_2$/kg body weight of animal for 15 days followed by caffeic acid 5 mg/kg body weight of animal 15 days administrated) treatment group.
  Group IV: CA alone treatment – the animals were received CA (5mg/kg body weight of animal for 15 days administrated) alone treated.

At the end of the experimental duration, rats were sacrificed by cervical dislocation. The whole liver tissues were isolated immediately from the animals and kept in an ice-cold saline and then used for the estimation of oxidant and antioxidant property studies.

Estimation of LPO (TBARS)
The concentration of LPO/TBARS in the selected tissues was determined by adopting the method of Nichens and Samuelson [24]. The known weight of selected organs (liver) was isolated from the animal in a cold room. The isolated organs were immediately homogenized in Tris-HCL buffer (pH 7.5) for 5 min. From the homogenized solution, 1 ml of the organ (Liver) homogenate was taken in a clean dry test tube, and then, 2.0 ml of TBA-TCA-HCL reagent was added to this content.

The contents were mixed thoroughly with lateral shaking of the test tubes. The mixture content was kept in an incubation chamber (boiling water bath – 60°C) for 15 min, and then, the organ mixture content was cooled with the help of running tap water. After cooling, the mixture was read the absorbance of the chromospheres at 535 nm against the reagent blank in an UV visible spectrophotometer (Spectronic-20, Bausch and Lamb). 1, 1', 3, 3'-tetramethoxypropane was used to construct the standard graph. The values were expressed as n moles of MDA released/100 mg.

Estimation reduced glutathione (GSH) activity
The level of GSH in liver tissue was determined by the method of Beutler and Kelley [25]. The isolated organs (liver) were homogenized in 5 ml of PBS buffer with the help of mortar and pestle. After completing the homogenization, the contents were centrifuged at 2500 rpm for 5 min. After completing the centrifugation, 2 ml of clear supernatant was taken in a clean dry test tube and then mixed with 1.8 ml of EDTA solution. To this content, 3.0 ml of precipitating reagent was added and mixed thoroughly and then kept in the room temperature for 5 min before centrifugation. The mixed content was centrifuged at 3000 rpm for 5 min. After centrifugation, 2.0 ml of the supernatant was taken in a clean test tube and then add the following reagents. 4.0 ml of 0.3M Na_2HPO_4 solutions and 1.0 ml of DTNB reagent were added to the supernatant; the color developed was read at 412 nm in an UV spectrophotometer. A set of standard solutions containing 20–100 μg of GSH was treated similarly. The values were expressed as μg/100 mg protein for tissues.

Estimation of superoxide dismutase (SOD) activity
SOD in the liver tissue was assayed by adopting the method of Kakkar *et al.* [26]. The known weight of selected organs (liver) was isolated from the animal immediately and then homogenized in 2 ml of 0.25M sucrose solution and then centrifuged at 10,000 rpm for 30 min in cold condition. After completing the centrifugation, the supernatant was taken in a test tube and it was dialyzed with Tris-HCL buffer (0.0025M, pH 7.4) and it was used for enzyme assay. In a clean test tube, 1.2 ml of dialyzed supernatant was taken and 1.2 ml of $Na_4P_2O_7$ buffer, 0.1 ml of PMS, and 0.3 ml of NBT reagents were added. The mixture contents were kept in a water bath at 30°C for 90 s for the purpose of incubation. After completing the incubation, the contents were made up to 3.0 ml by adding glass distilled water, and then, 0.2 ml of NADH reagents were added for initiating the reaction. The reaction was stopped by the addition of 1.0 ml of glacial acetic acid and then added with 4.0 ml of n-butanol. The reaction mixture was stirred vigorously and shaken well. The mixture was allowed to stand for 10 min and then centrifuged for 15 min at 3000 rpm. After centrifugation, the butanol layer was separated and the color intensity of the chromogen was measured at 560 nm in UV spectrophotometer against butanol as blank and the system devoid of enzyme served as control. One unit of enzyme activity is defined as the enzyme reaction which generates 50% inhibition of NBT reduction in 1 min under the assay condition and is expressed as specific activity in units/mg protein. The specific activity of the enzyme was expressed as unit/min/mg of protein for tissues.

Estimation of catalase (CAT) activity
The activity of CAT in the liver tissue was determined by the method of Sinha [27]. The isolated known weight selected organs (Liver) were homogenized in 2.0 ml of PBS solution. After completing the homogenization, the contents were centrifuged for 10 min at 2000 rpm. After centrifugation, 0.1 ml organ sample (tissue homogenate) was taken in a clean dry test tube and the 0.9 ml of PBS and 0.4 ml of H_2O_2 were added in contents and kept in room temperature for 30–60 s, and then, 2 ml of dichromate acetic acid mixture was added in the contents and then kept in an incubation chamber at 37°C for 10 min. After completing the incubation period, the contents were allowed to cool in the room temperature. The color developed was read at 620 nm in an UV spectrophotometer. H_2O_2 was used to construct the standard graph. The values are expressed as μ moles H_2O_2 consumed/min/mg protein.

Estimation of glutathione peroxidase (GPx) activity

The activity of GPX in the liver tissue was measured by the method of Rotruck *et al.* [28]. The known weight of selected organs (liver) was homogenized in 2.0 ml of PBS and then centrifuged at 2500 rpm for 5 min. After completing the centrifugation, 0.2 ml of clear supernatant was taken in a clean test tube, and then, the following enzyme mixture was added. The enzyme mixture contained 0.2 ml of 0.4 mM of EDTA and 0.1 ml of 10 mM of sodium azide. The reaction mixture was thoroughly mixed well and kept for 2 min at 37°C in an incubator. After completing the incubation period, 0.2 ml of reduced GSH and 0.1 ml of H_2O_2 were again added to the above mixture and then again incubated at 37°C exactly for 10 min. The reaction was arrested by the addition of 0.5 ml of 10% TCA. The color was developed and then read at 412 nm in an UV spectrophotometer. Reduced GSH was used to construct the standard graph. The values are expressed as μ moles of GSH utilized/min/mg protein tissues.

Histology and histopathological study

For the qualitative analysis of liver tissue histoarchitecture, the liver tissue sample was fixed in 10% buffered formaldehyde for 48 h and dehydrated by passing successfully in different concentrations of ethyl alcohol and cleaned in xylene and embedded in paraffin. Sections of tissue (5–6 μm think) were prepared using a rotary microtome and rehydrated and then stained with hematoxylin and eosin dye, which was mounted in DPX medium for microscopic observations.

Statistical analysis

Values are given as mean±SD. for six rats in each group. The data for various biochemical parameters were analyzed using analysis of t-test and the group means were compared by Duncan's multiple range test [29] Values were considered statistically significant when p<0.05 and the values sharing a common superscript did not differ significantly.

RESULTS

Table 1 indicates that the level of LPO was drastically increased in mercuric chloride intoxicated rat and simultaneously there was decreased in the level of total reduced GSH content in the liver tissue of the mercuric chloride intoxicated rat when compared with control animals. During the recovery period, CA treatment followed by mercuric chloride-intoxicated rat liver tissue, the restoration of LPO and GSH level was noticed near to normal level.

Table 2 shows that the antioxidant enzyme (SOD, CAT, and GPx) activities were significantly decreased (p<0.05) in mercuric chloride-intoxicated rat liver tissue when compared to the control. During the recovery period, the treatment of CA reversed the activities of all these antioxidant enzymes toward (p<0.05) near control level [Fig. 1].

Histological and histopathological study

Histological and histopathological observations in the liver tissue of rats

In the present experimental work, the whole liver organ was isolated from the normal, untreated control rats. In normal condition, liver organ consists of numerous numbers of hepatic cells which were uniformly arranged. The hepatocyte is the cell, which is constructing the cell plates (liver plates) of the lobule. The histoarchitecture of the liver tissue consists of polygonal lobules, which contain an intralobular

vein in the central and portal triad at the periphery. The classical, structural, and functional unit of the liver is the liver lobule. The shape of the liver lobule is cylindrical in nature with a venous channel. At the same time, and the central vein travels through its axis. The hepatic veins are surrounded by a connective tissue. The sinusoids are lined by phagocytic cells called Kupffer cells.

Under the influence of $HgCl_2$ treatment, the intoxicated liver shows serious pathological modifications occurred in its cellular architecture when compared to the control group. Dissimilarity in the size and shape and the irregular arrangement of hepatocytes were also present. The hepatocyte cell boundaries were completely damaged in many areas and this also disintegrated completely, and this condition leads to the formation of multinucleated cells. The cytoplasm appears to be vacuolated and the cells are filled with pyknotic nuclei. The cell boundaries show rupture in many places. These changes are associated with the hypertrophy of hepatocytes.

Mercury-intoxicated rats were again dosed with CA; the liver tissue shows the regular arrangement of hepatocytes. There are no enlargement and puzzlement of the liver cords, and there was no focal necrosis in the liver. The disintegrated cell boundaries were restored in certain areas. Rat fed with CA alone, the histoarchitecture of liver organ shows a remarkable increase in the size, and the shape of the hepatocytes was uniformly arranged. The complete histoarchitecture of the liver is maintained as like that of control.

DISCUSSION

In animals, the defense mechanism of antioxidant enzymes might be enhanced due to the accumulation of ROS in cells [30]. Both types of non-enzymatic and enzymatic antioxidants provide very good defense
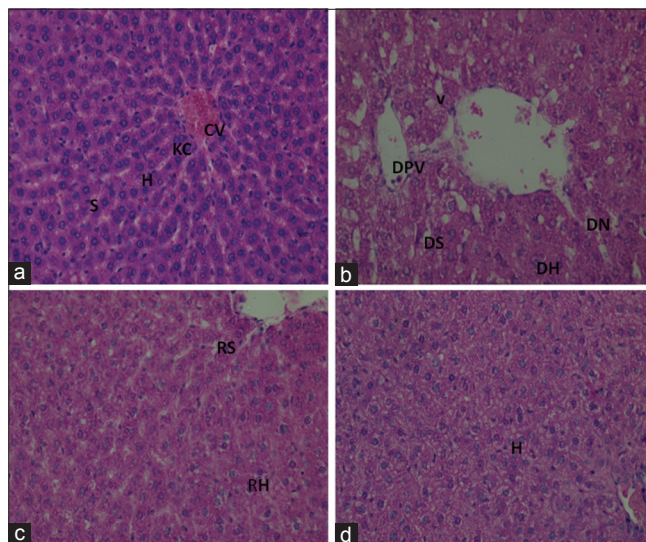

Fig. 1: Histology of experimental rat liver tissue in light microscope with hematoxylin and eosin staining at ×40 magnification. (a) Control, (b) Mercuric chloride, (c) Mercury chloride followed by caffeic acid, (d) Caffeic acid

Table 1: The level of LPO and reduced GSH content of caffeic acid on mercuric chloride-intoxicated rat liver tissue

Groups	Protein	Amino acid	LOP	GSH
Control	182.25±0.46	352.13±1.42	1.941±0.03	63.95±1.62
HgCl$_2$	119.34±0.55	467.18±0.86	3.918±0.02	32.61±0.36
HgCl$_2$+Caffeic acid	170.95±0.58	412.55±0.92	2.943±0.03	52.83±1.08
Caffeic acid	195.33±1.02	358.15±1.29	1.832±0.02	64.56±1.58

(Values expressed as μg/g wet weight of tissue) Each value is mean±SD for 6 rats in each group. The data for various biochemical parameters were analyzed using analysis of t-test and the group means were compared by Duncan's multiple range test. Values were considered statistically significant at P<0.05. LPO: Lipid peroxidation, GSH: Glutathione

Table 2: The level of SOD, CAT, and GPx activity of caffeic acid on mercuric chloride-intoxicated rat liver tissue

Groups	SOD	CAT	GPx
Control	6.125±0.12	70.38±3.23	11.513±0.54
HgCl$_2$	3.152±1.023	38.59±2.52	8.632±1.56
HgCl$_2$+Caffeic acid	5.113±0.02	60.36±2.28	10.627±0.52
Caffeic acid	6.682±0.058	71.86±1.23	12.037±0.24

(Values expressed as μg/g wet weight of tissue) Each value is mean±SD for 6 rats in each group. The data for various biochemical parameters were analyzed using analysis of t-test and the group means was compared by Duncan's multiple range test. Values were considered statistically significant at P<0.05. SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase

to the cells against ROS-induced cellular damages in the internal organs. This defense mechanism was mainly carried out by low molecular weight compounds (e.g., GSH) and antioxidant enzymes (e.g., SOD, CAT, and GSH -dependent antioxidant enzymes) [31]. GSH plays a vital role to neutralize ROS through the GSH-dependent antioxidant enzymes, and it acts as cofactor [32]. Promotion of antioxidant enzymes is one of the important factors of defense mechanism against oxidative stress in animals [30,33]. During the stress condition, the first line of defense mechanism consisting of the following antioxidant enzymes SOD, CAT, and GPx should be promoted against oxidative stress which converts superoxide FR into hydrogen peroxide and then into water and molecular oxygen [34]. ROS is a term that covers all types of elevated reactive, O_2 holding molecules comprising FR. All are competent to react with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, causing the cellular damage. ROS is produced by a number of pathways [35]. Continuous synthesis of ROS promotes major alterations in subcellular structural molecules such as proteins and DNA, LPO of polyunsaturated fatty acids (PUFA), and changes of cellular antioxidant system. In tissue level, the measurement of MDA is an major indicator of LPO because it is the end product of LPO [36-38]. In the present experimental study, the CAT enzyme activity was found to be decreased because it plays a vital role to maintain the balance of the production of H_2O_2 and superoxide radicals. The declining in the level of SOD in the respective tissue also indicated that an enhanced level of superoxide radical production and consequently higher hydroxyl radical formation might be promoted. The continuous enhancement of hydroxyl radical also initiates the production of LPO content to rise in the internal organs. This is also confirmed in the current experimental work when the animals were treated with Hg poison. An enhanced level of LPO content and simultaneously decreased level of CAT enzyme activities were noticed in liver tissues of mercury-intoxicated rat as justified the above fact. A similar type of result was also observed in experimental animals by a number of research workers [39,40]. These experimental works have been reported that the exposure of Hg poison decreases the antioxidant systems and produces oxidative damages through H_2O_2 generation, thereby leading to enhancement of LPO content. Therefore, an increase in LPO by HgCl$_2$ might provoke membrane biochemical and functional alterations that leads to the decrease in activities of SOD and CAT in liver tissues of HgCl$_2$-treated rats that may be due to the slowdown of their process by the production of H_2O_2 because it is known that H_2O_2 involved in Hg-induced cellular injury [41]. Manickam *et al.* also observed a similar type of result in CCL$_4$-treated rats, and they recommend due to injury in liver damage. CAT and GPx are very important enzyme defense against dangerous reactive oxidizing molecules converted to H_2O, thereby providing protection to the cells from FR, and also GPx reduces the lipid hydroperoxide and H_2O_2 non-toxic product [42]. During the stressful condition, CAT enzyme plays an important role to reduce the H_2O_2 produced from the metabolism of long chain fatty acids in peroxisomes. However, the role of GPx is responsible to catalyze the reduction of both H_2O_2 and lipoperoxides. Due to this, it is considered as a one of the crucial defense antioxidant enzymes against LPO [43]. In all animal tissues, GPx is evenly distributed and it is also known as H_2O_2 degrading enzyme [44]. It is present in cytosol and mitochondrial matrix. GPx is one of the

defense enzymes which scavenges harmful ROS. The main role of GPx enzyme is the removal and detoxification of hydrogen peroxide and lipid hydroperoxides in the presence of oxidized GSH [45-48]. During this reaction, the GSH is rapidly converted into oxidized glutathione (GSSG). In normal condition, the ratio of GSH/GSSG plays a vital role in adjusting the cellular redox status in animals. The disturbance occurred in the ratio of GSH/GSSG has promoting an impact on normal cellular functions [49]. Daniel *et al.* also observed comparable type of result in methotrexate-treated rats, they suggested that this is due to hepatotoxic damage. Reduction in cellular GSH, in turn, reduces the effectiveness of antioxidant defense of the liver against the increased ROS and thereby leads to hepatotoxicity [50]. In fact, GSH metabolism is one of the most essential anti-oxidative protection mechanisms in animals. During the recovery period, post-treatment of CA (mercuric chloride treatment followed by CA treatment), the decreased level of GSH content was significantly increased to reach near normal level in liver tissues of mercury-intoxicated rat. This result suggested that an administration of CA can promote the non-enzymatic antioxidant in the respective tissues leading to maintain adequate amount of GSH content in tissues. An enhanced level of GSH content has the function of protection that could provide the first line of defense against the influence of toxic heavy metals [39,51]. Moreover, an enhanced level of GSH content may be related to stop or control the oxidative challenge [52]. In the present experimental study, the decreased level of LPO content was noticed in liver tissues of mercury-intoxicated rats when treated with CA support our results. Deshmukh [53] have also been reported that the administration of CA reduces the oxidative stress in liver tissues of ICV-STZ-intoxicated rats. They suggested that the role of CA attenuates ICV-STZ-induced learning and memory deficit, cholinergic hypo-function, and oxidative-nutritive stress in rats. Yang *et al.* [54] have also examined the control of oxidative stress *in vitro* and *in vivo* condition by CA and RA combined treatment and estimated their potential in liver protection. CA also had a power of hepatic protection and also enhanced defensive effect of hepatocytes against t-BHP induced oxidative injure in rat liver cells. This could be attributed to the outstanding antioxidant properties (ability to scavenge FR) of CA. Soumya *et al.* [55] have also observed a similar type of result in liver tissues of mercury-intoxicated rat when treated with *Aerva lanata* plant extract. They observed that the decreased level of GSH was found in liver tissues due to intoxication with mercuric chloride. This reduced level of GSH content in the cells resulted from the depletion of GSH during oxidative injury of the hepatocytes [56]. During the administration of ethanolic extract of *A. lanata* on the hepatotoxic rats, the decreased level of GSH has been reverted back to normal due to an increased synthesis of GSH under the antioxidant effect of the extract [57]. They are suggested that an administration of *A. lanata* plant extract has possessing a high potential of several bioactive compounds which is responsible for promoting antioxidant properties in intoxicated animals.

CONCLUSION

CA can be used as an ameliorative agent against Hg toxicity-related disabilities in the future. Moreover, it saves the animals from HgCl$_2$-induced hepatotoxicity and carries out its transforming the biochemical and bioenzymological changes during the formation of free radical in animals. The present experimental work recommended that administration of CA can act as a preventive and protective effect of the animal from HgCl$_2$ intoxication.

AUTHORS' CONTRIBUTIONS

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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