Caffeic Acid attenuates acrylamide induced biochemical, hematological and histological alterations in rats

Divya Gupta

divy121gupta@gmail.com

Jiwaji University

Sadhana Shrivastava
Jiwaji University

Shamli S. Gupte
Jiwaji University

Sangeeta Shukla
Jiwaji University

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Abstract

Acrylamide is formed during processing of food at high temperature and is documented as a well-known neurotoxic, carcinogenic and has many adverse effects on human health. Therefore, it is the need of hour to reduce the detrimental effect of acrylamide. In the present sub-chronic study 18 adult Wistar rats were randomly divided into three groups. These groups were normal saline, toxicant, and toxicant (19.13 mg/kg) + therapy (20 mg/kg). Our result showed all the sign and symptoms of acrylamide toxicity which include reduction in the body weight, hind limb splaying, hair loss and skin irritation. A significant alteration in the hematological parameters and sharp enhancement in AST, ALT, ALP, LDH, GGT, urea, uric acid and triglycerides was recorded. Activity of acetylcholinesterase significantly reduced in brain after acrylamide administration. Acrylamide exposure resulted into increased mean DNA damage and tail length in liver, kidney and brain as compared to normal evident by COMET assay. Our biochemical findings were reinforced by histological observation. Treatment with caffeic acid restored tissue and serological indices accompanying towards normal.

1. Introduction

Acrylamide (AA) (2- propionamide) is a colorless, crystalline, non-volatile, vinyl monomer having a high solubility in water. Its presence had been recorded in various carbohydrate rich food items, treated at high temperature (Baking, grilling, roasting or frying) through Maillard Reaction. It is also detected in tobacco smoke, coffee and green [1]. Industries such as cosmetic, mining, paper, water purification, oil field industries, etc. uses AA, because of its polymerizing nature and to form poly acrylamide. Therefore, every one of us exposed to it directly or indirectly. In human body it enters by different routes; digestive tract, respiratory tract and skin. AA is documented as a potent neurotoxic, genotoxic, hepatotoxic, nephrotoxic, mutagenic, developmental and reproductive toxic [2] and highly carcinogenic in humans and many rodents, it is classified as a group 2A carcinogen.

Therefore, it is need of the hour to develop therapeutic agent that could protect the tissues from severe effect and could further be used clinically. Thus, the biological antioxidant could be a better option to combat the toxicity caused by AA. Phenolic acids are the compound derived from plants and have many health benefits, including anti-inflammatory, anticancer, and antiviral abilities. These compounds are extensively used as nutrients due to their antioxidant activity and should be incorporated in diet [3].

Caffeic Acid (CA) which is a di-hydroxy cinnamic acid synthesized via secondary metabolism of plants and present in variety of foods such as olives, coffee, beans, fruits, potatoes, carrots and propolis. CA is found as organic esters, sugar esters, amides, glycosides as a very simple and complex forms like dimers, trimers and phenolic acid derivatives [4].

Previous investigations reported that CA has innumerable physiological effects for instance antimicrobial activity, anti-inflammatory activity, immunostimulatory activity, anti-atherosclerotic activity, antiproliferative activity, hepatoprotective activity, cardioprotective activity, antidiabetic activity,
anticancer activity, anti-hepatocellular carcinoma activity [3]. Among phenolic acids, CA bioavailability and its free radical scavenging activity generally makes it an excellent antioxidant and a potential therapeutic agent.

Various pharmacological beneficial effect of CA has been reported, but less is known about its protective efficacy against AA induced damage in liver, kidney and brain of rats. Hence, this study is design to investigate the protective role of CA on biochemical and histological integrity in sub-chronic intoxication of AA in rats.

2. Material And Method

2.1 Animals and chemicals

Albino rats of Wistar strain (160 ± 20 g b.w.) were used in this study. All the experimental animals were housed under standard husbandry conditions (25 ± 2°C temp, 60–70% relative humidity, 12 hours’ photoperiod). Standard pellet diet and water *ad libitum* were used to fed the rats. Experimental animals were treated and cared according to the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Animal experimentation was approved by Institutional Animal Ethical Committee of Jiwaji University (1854/GO/Re/S/16/CPCSEA).

All the chemicals used in this study were of analytical grade and all the reagents were prepared with ultrapure water. AA and trans caffeic acid were obtained from Sigma Aldrich Company and other chemicals were obtained from Sigma Aldrich Company, E-Merck, Ranbaxy and BDH etc. All the diagnostic kits used in the experiment were obtained from E-Merck.

2.2 Experimental protocol

18 adult Wistar rats were randomly divided into three groups (six animals in each group). All the groups were given treatment regimen by post orally.

Group I: Normal saline

Group II: AA at a dose of 19.13 mg/kg, *p.o.* (1/6th of LD$_{50}$) [5] for 28 days

Group III: AA (as in group II) + CA (20 mg/kg, *p.o.* for 7 days)

All the animals were sacrificed after 24 hours of last treatment, blood was collected, serum was separated and tissue was collected for further experiments to be done.

2.3 Hematological assays

The blood samples collected during the euthanasia by pricking up the retro- orbital venous sinus, was used to estimate following hematological parameters RBC count, hemoglobin, hematocrit, MCV, MCH,
MCHC, platelet count and total WBC count by using automated blood analyzer (HORIBA ABX Diagnostics).

2.4 Serological assay

Blood samples was collected by pricking up retro-orbital venous sinus, then the samples were allowed to stand for 30 min at 37°C followed by centrifugation at 3000 rpm for 10 min, serum was isolated and stored at -20°C. aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alanine aminotransferase (ALP), glutamyl transferase (GGT), lactate dehydrogenase (LDH), triglycerides, cholesterol, creatinine, urea, uric acid, amino levulinate dehydrogenase (ALAD), aminolaevulinic acid synthase (ALAS) and butyl cholinesterase (BuChE) activity were measured as instruction given in kit.

2.5 Assessment of oxidative stress biomarkers

After necroscopy, liver, kidney and brain were excised out immediately, rinse in cold saline and kept in -20°C for biochemical estimation. Subsequently brain homogenates were processed to determine acetyl cholinesterase (AChE) in different parts of brain using dithionitrobenzoic (DTNB) acid [6] and absorbance was recorded at λ 412 nm. Hepatic, renal and cerebral homogenate were used to estimate lipid peroxidation (LPO) [7].

2.6 Assessment of antioxidant defense enzyme

Homogenates were prepared in 1.15% KCl then centrifuged at 10000 rpm for 10 minutes. Activity of glutathione reductase (GR) is measured [8]. Activity of Glutathione-S-Transferase (GST) was measured [9]. Activity of Glutathione Peroxidase (GPx) was measured [10]. Glucose-6-Phosphate Dehydrogenase (G6PDH) activity was determined [11].

2.7 Assessment of drug metabolism enzymatic activity

Microsomes were prepared by CaCl$_2$ precipitation method [12]. The CYP2E1 activity was determined in terms of aniline hydroxylase (AH) [13] and Amidopyrine-N-demethylase (AND) [14] activity along with level of microsomal lipid peroxidation (MLPO).

2.8 Assessment of DNA damage

Single cell gel electrophoresis, comet assay was used to evaluate DNA damage, using method with slight refinement [15]. Lysis buffer was prepared using 2.50 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10, 1% Triton X-100, 1% sodium sarcosine, and 10% dimethyl sulfoxide was prepared. 1 ml chilled mincing solution (HBSS with 20 mM EDTA and 10% DMSO) was taken in Petri dish then 0.2 g of liver, kidney and brain was chopped into pieces and place into mincing solution and leave the pieces to settle and centrifuge for 400 rpm for 10 min. Supernatant having single cells were taken out for comet assay. Frosted glass microscopic slides were coated with 1% normal melting agarose (PBS, pH 7.4), on them single cells suspended in low-melting point agarose (LMPA) (0.65% LMPA, w/v in PBS, pH 7.4) on each slide was pipetted out. Then leave the slides to set for 10 min at 4°C, following this the slides were exposed for 24 hours to lysis solution. At last, the slides were treated with distilled water and
Electrophoretic buffer (pH 13) for 40 min to remove salts, thereafter, subjected these slides to electrophoresis for 20 min (300 mA, 25 mV). Subsequently Tris–HCl buffer neutralized alkali and then after rinsed the slides with distilled water and methanol and then stained the slides with ethidium bromide. Images of 50 randomly selected cells were analyzed from each sample. The comet moment was calculated from 50 counted cells and was expressed in micrometer.

2.9 Optical microscopy histopathological assays

After necroscopy liver, kidney and brain were immediately dissect out for histopathological studies. Each of the three tissues were washed in saline following by fixing in bouin's fluid, embedded in paraffin and then sections of 5 µm thickness were cut, stained in hematoxylin and eosin for further examination under light microscope.

2.10 Statistical analysis

P values at the level of ≤ 0.05 was evaluated by student’s t test. Significance of the difference among various groups was evaluated by one way analysis of variance (ANOVA) considering significantly at 5%.

3. Results

3.1 Symptoms noted during experiment

During the whole experimental duration, control group seemed to be clinically normal. Some morphological symptoms have been noted in AA treated animals such as redness around mouth and ear pinna, swelling in the limbs, hind limb splaying and dragging, hair loss and weight loss had also been reported in experimental animals.

3.2 Hematological analysis

Upon AA exposure in group II there was a significant alteration in the hematological parameters (Hb%, Ht%, RBC, WBC, MCV, MCH, MCHC, PLT) when compared with group I. CA treatment for 7 days recovered all the altered parameter toward normal (Table 1).

3.3 Serological analysis

In serological analysis marker enzymes of liver, kidney and brain function test markers AST, ALT, ALP, GGT, LDH, cholesterol, creatinine, bilirubin, albumin, urea, uric acid, ALAD, ALAS and BuChE (P ≤ 0.05) were recorded in AA treated group when compared with control. CA treatment significantly restored these altered markers enzymes (Table 2).

3.4 Oxidative stress marker:

Activity of AChE significantly reduced in the brain of AA treated group, treatment with CA restores the level of AChE towards control at 5% level of significance. Alterations in oxidative stress marker in different
tissues in terms of LPO was found to be increased in liver, kidney and brains tissues, subsequently treatment with CA restores level of LPO towards control.

3.5 **Assessment of antioxidant defense system enzymes:**

Activity of antioxidant defense system enzymes such as glutathione reductase (GR), glutathione peroxidase (GPx) (Table 6A), Glutathione-S-transferase (GST) were decreased in all the organs whereas activity of glucose-6-phosphate dehydrogenase (G-6-PDH) was increased after AA intoxication (Table 6B). Treatment with CA for 7 days ameliorate these antioxidant defense system enzymes.

3.6 **Assessment of drug metabolism enzymatic activity**

Activities of drug metabolizing enzymes (aniline hydroxylase and amidopyrine-N-demethylase) and MLPO was found to be altered. Therapy for seven days after AA administration, significantly recovered activity of these drug metabolizing enzymes (Table).

3.7 **Histological observations**

Histology of control rat liver discloses the normal hepatocytes, with compact and well- maintained cord arrangement, normal structure of central vein and sinusoidal spaces (Fig. 1A). Upon AA administration for 28 days, microscopic examination of experimental rat liver revealed that there was alteration in the histological structure of liver, disruption of hepatic cords and sinusoidal spaces, degeneration of hepatocytes with cytoplasmic vacuolization (Fig. 1B). Therapy with CA showed well-arranged cuboidal hepatocytes with well-maintained nuclei and prominent recovery. Clear sinusoidal spaces along with well-formed portal triad, well preserved central vein was observed in CA treated group (Fig. 1C).

Kidney of the control rat shows normal histological structure of glomeruli and surrounding tubules (Fig. 2A). Sub-chronic AA administration showed congestion in the cortical blood vessels, swallowed glomeruli, distortion in endothelial lining, hypertrophy in epithelial cells of convoluted tubule with reduced lumen (Fig. 2B). Treatment with CA revealed compact rounded glomerulus with well-formed Bowman’s capsule and intact endothelial lining along with reduced vacuolation in epithelial cells of proximal tubules (Fig. 2C).

Brain tissue of control rats exhibit normal cerebral hemisphere with well-arranged neurons having a well-maintained central nucleus and granular cytoplasm (Fig. 3A). Upon AA exposure cerebral atrophy with degenerated neural cells and reduced nerve fibers and cytoplasmic vacuolization in the brain. large number of glial cells could be seen and darkly stained degenerated pyramidal cells were observed (Fig. 3B). Treatment of CA showed improvement in cerebral cortex showing well-formed pyramidal cells with loss of vacuolization. Neurons were also seemed to appear normal and well arranged with loss of pericellular space (Fig. 3C).

3.8 **Measurement of DNA damage**

AA exposure increased the mean DNA damage and tail length in liver, kidney and brain as compared to normal. The damage was expressed as percent DNA migration in the tail and 20–25 nuclei were counted
in each slide. Therapy with CA for 7 days in AA treated rats showed recovery in DNA damage significantly as evident from the decrement of mean tail length (Fig. 4).

4 Discussion

In the modern society, our dependency on ready to eat food increases due to lifestyle we have. These processed foods are very harmful for mankind upon their consumption and they could even adversely affect the quality of food [16]. AA is one of those harmful processed chemicals. AA is formed when carbohydrate rich food is processed at high temperature (> 120 °C). There are other modes of exposure, from where AA can enter in human body such as its use in industrial products like water purification, cosmetics, laboratories and even present in cigarette smoke. Its presence in coffee, green tea and confectionaries had also been detected [1]. Hence, toxicity caused due to AA is one of the major health problems.

AA is electrophilic in nature and when it enters in body, it can react with nucleophilic compounds containing SH, NH$_2$ and OH groups and form Hb-AA adducts. AA is a small organic molecule, hence is readily absorbed and thereafter distribute to all the tissues in a rapid manner. AA get converted to its genotoxic form, glycadiamide by epoxidation reaction through CYP2E1 enzyme system which are aniline hydroxylase and amidopyrine-N-demethylase. Glycadiamide is more reactive toward protein and form both GA-Hb and GA-DNA adducts [17]. During AA metabolism it get conjugated with GSH and form GSH adduct, which rapidly transformed to mercapturic acid and eliminated out from the body. Almost every organ of the body is a target of AA toxicity. Long term AA exposure can cause paresthesia, muscle weakness, muscular atrophy, absence of tendon reflex, hind limb splaying, tremor, illusion or cognitive dysfunction [2].

Use of plant product as medicinal purpose had always gain interest of researchers because of their high bioavailability and non- toxic nature. There are increasing evident that most of the disorders results from oxidative imbalance caused by ROS. To prevent the oxidative stress generated by toxic chemicals, antioxidant role of bioactive phytochemicals has been reported.

To reduce the risk of AA toxicity phytochemicals like phenolic acids are used. CA (3,4-dihydroxy cinnamic acid) a plant flavonoid produced through the secondary metabolism, is most abundant bioactive compound. The radical scavenging properties associated with the structure of CA defend against oxidative stress preventing the production of ROS (reactive oxygen species) and in doing so it has many other biological properties such as anti- inflammatory, antimutagenic, cardioprotective, reno-protective, reduce blood sugar level as well as prevent cancer and slow the aging processes in cells responsible for degenerative diseases [18].

Result of this study shows that in comparison with control there is a significant reduction in the percentage of hemoglobin upon AA intoxication. Hemoglobin is dependent on red blood cells population and the number of red blood cells had also been decrease significantly. This may be due to hindrance in
the synthesis of hemoglobin or hemoglobin destruction. Activity of ALAD had also significantly decreased in AA administered rats. ALAD is -SH containing enzyme involved in the synthesis of heme [19], AA had high affinity to conjugate with -SH group. This leads to decrease in the activity of ALAD and ultimately results in decrease hemoglobin content, which leads to anemia. Its inactivation also induces the accumulation of ALAS, which accelerate the production of ROS and ultimately induce oxidative stress.

Sub-chronic exposure of AA accelerates ROS generation process which disturbed the oxidant/ antioxidant level in the cell. This resulted into decline activity of antioxidant enzymes (GR, GPx, GST, G-6-PDH) in liver, kidney and brain tissue. This may be due to cellular injury or death of healthy cells, which are responsible to counter the oxidative stress. Therapy of CA maintained antioxidant enzymes toward normal due to its radical scavenging property.

Lipid peroxidation is the metabolic process in which ROS results in peroxidation of membranous lipids. Our study demonstrated that the level of TBARS significantly increased in the AA intoxicated rats, might be because of the failure in detoxification of AA. Treatment with CA subsequently reduced the level of LPO toward normal. This may be because of antioxidant nature of CA, it is characterized by the presence of a benzene ring, a carboxylic acid group and hydroxyl groups.

Aniline hydroxylase (AH) and aminopyridine-N-demethylase (AND) are CYP 450 enzymes of CYP-450 enzymatic system and responsible for drug metabolism. Activities of these enzymes was determined in liver microsomes, originated from smooth endoplasmic reticulum (SER), a major site of lipid biosynthesis. In this present study activities of these drug metabolizing enzymes were significantly declined in AA intoxicated rats, suggesting SER damage. Therapy of CA restored the activities of these enzymes toward control.

In the present study, elevated level of AST, ALT, ALP, GGT, LDH, albumin and bilirubin in serum has been recorded after sub-chronic exposure of AA. These enzymes are cytoplasmic in origin and may release into the circulation due to the damage in cell membrane integrity or due to exposure of toxicant and are sign of liver damage induced by AA toxicity. This increase activity of serum enzymes may be due to the bipolar nature of AA and it can conjugate with -SH and α-NH₂ group of amino acids. Treatment of animals with CA showed protection against AA induced hepatic damage. CA treated animals showed the restored activity of AST, ALT, ALP, GGT, LDH, albumin and bilirubin which may be due to its ability to repair liver damage and maintain the integrity of plasma membrane. Increased bilirubin is due to increase in the production of bilirubin more than the normal or due to damage or blockage of excretory duct of liver. Our results are in agreement with the result of Uthra et al. 2022 [20]. Elevated level of cholesterol and triglycerides in AA administered group also demonstrate the liver damage. These results indicated that changes in plasma lipoproteins can serve as sensitive and simple markers for rat’s liver disorders caused by AA. Histopathological data of this study clearly signify the liver damage characterized by development of cytoplasmic vacuolation, hepatocyte necrosis and lymphocytic infiltration upon AA intoxication [21]. Treatment of CA help in the restoration of altered parameter toward normal values.
Level of serum urea, uric acid and creatinine, main clinical parameters for assessing renal damage altered upon AA administration. Level of urea, uric acid and creatinine may be elevated because AA intoxication can impair brush border epithelium and make them impermeable to urea, uric acid and creatinine [22], or it may be due to excessive production or degradation of purine and pyrimidine. Treatment with CA restored the altered parameters toward normal. It could be possible by the antioxidant property of CA which ultimately modulates the key enzymes and recover the renal damage, which is also evident by our histological analysis of kidney.

Neurotoxicity of AA was reported by many researchers [23]. These researchers reported that AA produces central and peripheral distal axonopathy. AA exposure also leads to decrease the level of norepinephrine in the hippocampus and noradrenergic axons in the prefrontal cortex. This study demonstrate that AA intoxication caused inhibition in the activity of AChE and BuChE in brain. Administration of CA significantly recouped the AChE and BuChE activity towards normal highlighting its neuroprotective property. After the therapy of CA, improvement in the histoarchitecture of brain tissue also supported these results.

Genotoxicity of AA is associated with its biotransformation to a highly reactive glycidamide. Glycidamide can directly conjugate with DNA and form glycidamide-DNA adducts [24]. Genotoxic assessment by comet assay shows a significant increase in the tail length and tail DNA in the liver, kidney and brain tissue of AA administered rats. CA has an anti-genotoxic effect, due to its antioxidant property. The antioxidant property of caffeic acid is due to the presence of -OH group in its structure. Not only the OH group present on para position makes it a potent antioxidant but, the presence of second hydroxyl group in the ortho position increases its antioxidant property due to an additional resonance stabilization and form o-quinone.

5 Conclusion
In conclusion, our experimental findings demonstrate that, the histopathological changes in liver, kidney, and brain as well as alteration in biochemical parameters and DNA damage were recovered toward normal after administration of CA. This ultimately proves that CA exerts a protective affect against AA induce toxicity. We consider that these effects of CA were due to its strong antioxidant property. This investigation provides a useful information for future deep research on the molecular mechanism of pharmaceutical potential of CA against AA induced toxicity.

Declarations
Competing interests: Authors declare no competing interests

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glycidamide guanine adducts in human blood DNA following exposure to dietary acrylamide using
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rutin against acrylamide-induced oxidative stress, biochemical alterations and histopathological
García, M., et al.: Curcumin reverses glomerular hemodynamic alterations and oxidant stress in 5/6

Tables

Table 1 Effect of caffeic acid on hematological variables test against acrylamide induced
toxicity
<p>| Abbreviations: AA=Acrylamide; CA=Caffeic acid; ANOVA*=Significant at 5%. Values are Mean ± SE; N=6; #P≤0.05 VS Control; *P≤0.05 VS AA. | Table 2 Effect of caffeic acid on serological tests against acrylamide induced toxicity |</p>
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>AA</th>
<th>AA+CA20</th>
<th>Anova (F Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU /L)</td>
<td>60.00 ± 3.31</td>
<td>23.60 ± 1.30#</td>
<td>50.25 ± 2.77*</td>
<td>(73.21%)</td>
</tr>
<tr>
<td>ALT (IU /L)</td>
<td>42.00 ± 2.32</td>
<td>24.93 ± 1.37#</td>
<td>39.29 ± 2.17*</td>
<td>(84.12%)</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>210 ± 11.60</td>
<td>280.5 ± 15.50#</td>
<td>233.75 ±12.92*</td>
<td>(66.31%)</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>2.5 ± 0.13</td>
<td>6.63 ± 0.36</td>
<td>2.8 ± 0.15*</td>
<td>(92.73%)</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>480 ± 26.5</td>
<td>837 ± 46.2#</td>
<td>542 ± 29.9*</td>
<td>(82.63%)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>66.00 ± 3.64</td>
<td>237.8 ± 13.14#</td>
<td>81 ± 4.47*</td>
<td>(91.26%)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.3 ± 0.016</td>
<td>0.4 ± 0.022#</td>
<td>0.3 ± 0.016*</td>
<td>(100%)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.0 ± 0.16</td>
<td>3.90± 0.21#</td>
<td>3.10 ± 0.17*</td>
<td>(88.88%)</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>3.2 ± 0.17</td>
<td>1.3± 0.07#</td>
<td>2.2 ± 0.12*</td>
<td>(90%)</td>
</tr>
<tr>
<td>Urea(mg/dl)</td>
<td>30 ± 1.65</td>
<td>80.2± 4.43#</td>
<td>37.5 ± 2.07*</td>
<td>(85.05%)</td>
</tr>
<tr>
<td>Uric acid(mg/dl)</td>
<td>3.4 ± 0.18</td>
<td>5.9 ± 0.32#</td>
<td>3.7 ± .20*</td>
<td>(88%)</td>
</tr>
<tr>
<td>ALAD (µmole/min/erythrocytes)</td>
<td>9.20 ± 0.50</td>
<td>3.81 ± 0.21#</td>
<td>6.67 ± 0.36*</td>
<td>(53.06%)</td>
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<tr>
<td>AIAS</td>
<td>11.00 ± 0.60</td>
<td>14.66 ± 0.81#</td>
<td>11.63 ± 0.64*</td>
<td>(82.78%)</td>
</tr>
<tr>
<td>BuChe (µmole/min/mg protein)</td>
<td>36.00 ±1.99</td>
<td>6.050 ± 0.33#</td>
<td>27.61 ± 1.52*</td>
<td>(71.98%)</td>
</tr>
</tbody>
</table>

# significant at p < 0.05
* significant at p < 0.01
@ significant at p < 0.001
Abbreviations: AA=Acrylamide; CA=Caffeic acid; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; ALP=alanine phosphatase; GGT=glutamyl transferase; ALAD=δ-aminolaevulinic acid dehydratase; ALAS=δ-aminolaevulinic acid synthetase; BuChe=butyl cholin esterase.

ANOVA®=Significant at 5%. Values are Mean ± SE.

Table 3 Effect of caffeic acid on oxidative stress against acrylamide induced toxicity

<table>
<thead>
<tr>
<th></th>
<th>Acetylcholinesterase (μmole/min/mg protein)</th>
<th>Lipid peroxidation (n mole TBARS/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Forebrain</td>
<td>midbrain</td>
</tr>
<tr>
<td></td>
<td>36.00 ± 1.99</td>
<td>25.00 ± 1.38</td>
</tr>
<tr>
<td></td>
<td>15.55 ± 0.85*</td>
<td>8.940 ± 0.49*</td>
</tr>
<tr>
<td></td>
<td>26.10 ± 1.44*</td>
<td>18.21 ± 1.00*</td>
</tr>
<tr>
<td></td>
<td>(51.58%)</td>
<td>(57.82%)</td>
</tr>
<tr>
<td></td>
<td>33.41®</td>
<td>35.59®</td>
</tr>
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</table>

Abbreviations: AA=Acrylamide; CA=Caffeic acid; LPO= lipid peroxidation; AChE= acetyl choline esterase; ANOVA®=Significant at 5%. Values are Mean ± SE; N=6; #P≤0.05 VS Control; *P≤0.05 VS AA.

Table 4 Effect of caffeic acid on GSH cycle enzymes against acrylamide induced toxicity

A; GR and GPx, B; GST and G6PDH

Table 4A
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Glutathione Reductase (µmole/min/mg protein)</th>
<th>Glutathione Peroxidase (µmole/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Control</td>
<td>7.40 ± 0.40</td>
<td>4.60 ± 0.25</td>
</tr>
<tr>
<td>AA</td>
<td>0.40</td>
<td>0.25</td>
</tr>
<tr>
<td>AA + CA₂₀</td>
<td>3.02 ± 0.16</td>
<td>2.20 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>±0.09#</td>
<td>±0.14#</td>
</tr>
<tr>
<td>Anova</td>
<td>(81.7%)</td>
<td>(79.2%)</td>
</tr>
<tr>
<td>(F Value)</td>
<td>6.60 ± 0.36</td>
<td>4.10 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>±0.18*</td>
<td>±1.40*</td>
</tr>
<tr>
<td></td>
<td>(81.7%)</td>
<td>(79.2%)</td>
</tr>
<tr>
<td></td>
<td>30.078@</td>
<td>24.796@</td>
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<table>
<thead>
<tr>
<th>Treatments</th>
<th>Glutathione-S-Transferase (Unit/min/mg protein)</th>
<th>Glucose-6-Phosphate Dehydrogenase (µmole/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Control</td>
<td>4.00 ± 0.22</td>
<td>4.60 ± 0.25</td>
</tr>
<tr>
<td>AA</td>
<td>2.01 ± 0.11</td>
<td>1.76 ± 0.09</td>
</tr>
<tr>
<td>AA + CA₂₀</td>
<td>4.43 ± 0.24</td>
<td>3.68 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>(52.8%)</td>
<td>(67.6%)</td>
</tr>
<tr>
<td></td>
<td>24.370@</td>
<td>30.885@</td>
</tr>
</tbody>
</table>

**Abbreviations:** AA=Acrylamide; CA=Caffeic acid; GR= glutathione reductase; GST=Glutathione-S-Transferase; GPx= Glutathione Peroxidase; G6PDH= Glucose-6-Phosphate Dehydrogenase; ANOVA@=Significant at 5%. Values are Mean ± SE; N=6; #P≤0.05 VS Control; *P≤0.05 VS AA.

**Table 5** Effect of caffeic acid on CYP2E1 enzymes against acrylamide induced toxicity
<table>
<thead>
<tr>
<th>Treatments</th>
<th>AH (U/g liver/mg protein)</th>
<th>AND (U/g liver/mg protein)</th>
<th>MLPO (n mole TBARS/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32 ± 1.76</td>
<td>6.20 ± 0.34</td>
<td>0.40 ± 0.02</td>
</tr>
<tr>
<td>AA</td>
<td>12.14 ± 0.67*#</td>
<td>3.87 ± 0.21*#</td>
<td>1.37 ± 0.07*#</td>
</tr>
<tr>
<td>AA + CA₂₀</td>
<td>18.46 ± 1.02*(31.8%)</td>
<td>5.1 ± 0.28*(52.7%)</td>
<td>0.787 ± 0.04*</td>
</tr>
<tr>
<td>Anova (F Value)</td>
<td>49.39®</td>
<td>9.861®</td>
<td>46.519®</td>
</tr>
</tbody>
</table>

Abbreviations: AA=Acrylamide; CA=Caffeic acid; AH= aniline hydroxylase; AND= Amidopyrine-N-demethylase; MLPO= microsomal lipid peroxidation; ANOVA ®=Significant at 5%. Values are Mean ± SE; N=6; #P≤0.05 VS Control; *P≤0.05 VS AA.

Figures
Figure 1

Histopathological observations of liver (X400).

Abbreviations: Liver of control rats showed normal hepatocytes, with well-formed cord arrangement, central vein and sinusoidal spaces (A); Sub chronic exposure of AA, disruption in hepatic cords, heterochromatic nuclei were detected in between the hypertrophied and vacuolated cytoplasm of hepatocyte, Congestion was seen in the central vein (B); Therapy with CA group showed improvement in hepatic cords with well-maintained nuclei, clear central vein with hexagonal hepatocytes (C).

Figure 2

Histopathological observations of kidney (X200).

Abbreviations: Control rats showed normal histological structure of glomeruli and renal tubules (2A); most remarkable effects of administration of AA showed congestion in the cortical blood vessels, swallowed glomeruli, distortion in endothelial lining, hypertrophy in epithelial cells of convoluted tubule
with reduced lumen (2B); Treatment with CA revealed compact rounded glomerulus with well-formed Bowman’s capsule and intact endothelial lining along with reduced vacuolation in epithelial cells of proximal tubules (2C).

**Figure 3**

**Histological observation of brain (X400).**

Abbreviations: Control rats (3A); AA intoxicated group (3B); CA treated group (3C).
Figure 4

4A. Comet observation of liver and kidney tissue

4B. Comet observation of brain tissue