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Protective effect of gallic acid on oxidative stress induced by acrylamide in isolated rat hepatocytes

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ABSTRACT

Acrylamide is one of the most widely used in the industry, which found in potatoes, breads, chips, biscuits and many carbohydrate-rich foods and processed at high temperatures. Therefore, human exposure to this compound is high. The toxicity of this compound has been proved in various studies. Acrylamide also causes genetic, neurological, and cancerous effects. The main toxicity mechanism of acrylamide is oxidative stress. Using a diet containing fruits and vegetables that contain natural antioxidant, they are recommended as the main protective strategy against oxidative stress and liver damage. In this study, the protective effect of gallic acid (10,20,50,100 µM) against oxidative damage induced by acrylamide in hepatocytes isolated from rats was studied. Hepatocytes were prepared from rat liver by collagenase perfusion and then the protective effect of gallic acid on acrylamide-induced cellular toxicity by measurement cell death, ROS production, lipid peroxidation, mitochondrial membrane potential loss and lysosomal membrane damage were investigated. To determine the toxicity of acrylamide, EC50,2h concentration of acrylamide (2 mM concentration) was used acrylamide in EC50,2h concentration caused ROS production rise within 3 hours of incubation, Extension of lipid peroxidation, mitochondrial membrane potential loss and cell death compared to control group ($p < 0/05$). Our results showed that gallic acid at concentrations of 50 and 100 µM can reduce the production of reactive oxygen species, lipid peroxidation, mitochondrial damage, and acrylamide-induced cell death.

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1.INTRODUCTION

The synthetic monomer acrylamide (AA), also known as 2-propenamide (C3H5NO), possesses a wide scope of industrial applications, from water management to paper making, cosmetics and even gel-based laboratory methods, including gel chromatography and electrophoresis (Friedman 2003). It is a biodegradable, α, ß-unsaturated reactive molecule, which can significantly disseminate in soil and groundwater (Blancher and Cormick 2012). Beside its environmental and industrial affluence, AA can also be produced in carbohydrate-rich heat-treated foods such as crisps, chips and bread, as a result of chemical reactions between reducing sugars or carbonyls with asparagines (Ubaoji and Orji 2016). This chemical compound can be encountered by both humans and animals through dermal contact, ingestion, and inhalation, with the ability to distribute to different organs and traverse placental and mammary tissues (Semla et al. 2017).

KEYWORDS:
Acrylamide,
oxidative stress,
gallic acid,
reactive oxygen species,
lipid peroxidation

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The metabolism of AA can result in the formation of a DNA-reactive metabolite, glycidamide, which occurs through the process of epoxidation via cytochrome 2E1 (CYP2E1), and both compounds can form complexes with albumin, hemoglobin, enzymes, and DNA (Pruser and Flynn 2011). Approximately half of the volume of AA is excreted mainly through urine (Bjellaas et al. 2007).

The induction of oxidative stress by AA has been highlighted in numerous studies, leading to significant cellular damage that is associated with various diseases. As a result, these findings have sparked concerns regarding the impact of AA on human health (Zhang et al. 2023). The nervous system can be significantly impacted by AA, as a potent neurotoxin, resulting in a variety of neurological symptoms such as general weakness, tremors, numbness and ataxia, depending on the duration and level of exposure (Zamani et al. 2017). Convincing evidence exists on the potential of AA to cause chromosomal disorders, serious spermatid mutations, abnormal sperm morphology in mice, birth defects, decreased weight gain in newborns, and a decline in fertility rates (Fennell et al. 2005; Friedman 2003; Jin et al. 2013). Additionally, the International Agency for Research on Cancer (IARC) has categorized AA as a potential carcinogen for humans (Rice 2005). Liver, an essential organ with diver metabolic functions, can be seriously affected by AA toxicity, primarily through induced oxidative stress. The overproduction of reactive oxygen species (ROS) and reduced cellular antioxidant capacity owing to AA can lead to the manifestation of apoptotic and necrotic characteristics in hepatic cells. AA induces an increase in malondialdhyde (MDA) enzyme activity, while simultaneously reducing the levels of several hepatic molecules such as glutathione (GSH), catalase (CAT), ATPase, lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), and superoxide dismutase (SOD). Moreover, it has been found to significantly decrease the level of high-density lipoprotein (HDL) and increase triglyceride (TG), cholesterol, glucose, urea, and creatinine levels (Zhang et al. 2023). Consequently, researchers have been investigating natural substances that possess antioxidant properties in order to counteract the negative consequences of AA.

Phenolic compounds are a class of plant-based chemicals that contain a benzene ring with one or more hydroxyl groups attached to it (Cheynier 2012). Gallic acid or 3,4,5-trihydroxybenzoic acid is a widely prevalent phenolic acid found in various plants. This water and alcohol-soluble compound, which can be either colorless or slightly yellow in its crystalline form, is extensively used in the food industry and pharmaceutical sectors due to its numerous applications (Badhani et al. 2015). It can be found in a wide range of plants, including grapes, tea leaves, oak bark, strawberries, and blueberries. Additionally, gallic acid is present in various food sources like nuts, seeds, and wine (Fernandes and Salgado 2016). In addition to its role as a flavoring agent and preservative in the food industry, gallic acid and its ester derivatives have garnered significant attention in scientific research. Numerous studies have highlighted the diverse biological and pharmacological activities exhibited by these phytochemicals, particularly their antioxidant properties (Choubey et al. 2015). Gallic acid and gallates act as free radical scavengers, which inhibit the rancidity and oxidation of oils and fats (Choubey et al. 2015). Such compounds play a crucial role in protecting the body from the harmful effects of oxidative agents like AA and maintain a balanced redox homeostasis. These findings demonstrate the potential for gallic acid to have a wide range of beneficial effects on human health.

The present study was done to reveal how gallic acid can protect isolated rat hepatocytes from oxidative stress induced by AA. The outputs of this research are expected to provide valuable insights into potential therapeutic interventions and dietary strategies that can mitigate the harmful effects of AA exposure. Ultimately, this knowledge can contribute to a healthier and safer future for individuals.

2. MATERIALS AND METHODS

2.1. Chemicals and buffers

The chemicals used in the present study were as follow: AA, gallic acid (Sigma Aldrich, USA), trypan blue due, sodium chloride, potassium chloride, hydrated and dehydrated magnesium sulfate, sodium hydrogen phosphate, potassium dihydrogen phosphate, HEPES, sodium hydrogen carbonate, dehydrated calcium chloride, magnesium phosphate, trichloroacetic acid (Merck, Germany), dimethyl sulfoxide (DMSO; Scharlau, Spain), collagenase, 2,7-dichlorofluorscein diacetate (DCFH-DA; Sigma Aldrich, USA), heparin (Daru Pakhsh, Iran), bovine serum albumin (Biowest, France), EGTA (AppliChem, Germany), ketamine hydrochloride 10% and xylazine 2% (Alfasan, Netherlands). Seven buffers were prepared and used in the current study, including, Krebs buffer, normal and 10X Hanks buffer, washing buffer (buffer I), tissue digestive buffer (buffer II), hepatocyte isolation buffer (buffer III) and dilution buffer (buffer IV).

2.2. Isolation of rat hepatocytes

Wistar rats (average weight: 250-300 gr) were used in this study, being kept under optimum temperature with free access to food and water. Liver perfusion by collagenase was done to isolate rat hepatocytes (Moldéus et al. 1978) (**Figure 1**). Briefly, rats were anesthetized using intraperitoneal (i.p.) injection of xylazine (15 mg/kg) and ketamine (75 mg/kg), then intravenous (i.v.) administration of 0.25 ml heparin (5000 U/ml) into the inferior vena cava was done through a U-shaped abdominal incision, in order to prevent blood coagulation in liver. After cannulation of the portal vein, buffer I was used to wipe out the rest of blood from liver, then the organ was taken out and tissue lysis was done using buffer II (collagenase) perfusion for 10 min. Subsequently, buffer III (washing) was added to the lysed liver tissue under agitation, then liver was sieved through a sterile gas, and liver cells were isolated through centrifugation (5500 rpm, 4 ^{o}C). Enumeration of obtained cells was done in a haemocytometer chamber by making a mixture containing 200 µl of trypan blue dye (0.2% w/v), 100 µl of isolated liver cells and 300 µl buffer IV. Trypan blue dye can permeate dead cells, hence they are stained blue, while alive cells stain yellow (**Figure 1**). A defined concentration (10^6 cells/ml) of cells were re-suspended in buffer IV, added to the 10-ml culture flasks and agitated using a bioreactor at 37 ^{o}C and 5% CO_2.

2.3. Cytotoxicity evaluation

In case of water-soluble substances, liver cell suspensions (10 ml) were mixed with 100 µl of 100X stock solution in agitating culture flasks. Moreover, in terms of DMSO-soluble compounds, the maximum DMSO volume used was 40 µl. Cytotoxicity evaluation was done after 3 h incubation of isolated rat hepatocytes with 2mM AA or different concentrations of gallic

acid (10, 20, 50 and 100 µM). The flasks were divided into three groups: control, sample, and protective compounds. The control flask only contained a suspension of liver cells and was not incubated with any substances. The sample flask contained a suspension of hepatic cells and $EC50_{2h}$ of AA (2 mM concentration), and those flasks with protective agents included liver cell suspension, 2 mM AA and different gallic acid concentrations (10, 20, 50 and 100 µM).

2.4. ROS measurement

For the purpose of ROS production measurement, 1 ml of isolated hepatocytes (10^6 cells/ml) were mixed with 3 ml of DCFH-DA (1 M in buffer IV, pH = 7.4) and incubated at 37 °C for 15 min. Next, a spectrofluorometer was used to record the fluorescence activity t 490-520 nm wavelengths. In this assay, the DCFH-DA crosses the cell membrane because of high lipophilic properties, then it is hydrolyzed into a non-fluorescent agent by hepatic esterases, being immediately oxidized the presence of ROS, resulting in the formation of highly-fluorescent dichlorofluorscein (DCF) (Pourahmad et al. 2009).

2.5. Lipid peroxidation activity

One of the potential indicators of lipid peroxidation is MDA. In the current study, in an acidic milieu, an MDA molecule reacts with two barbitoric acid molecules and the subsequent disseminated pink color can be detected at 532 nm wavelength. After preparation of tiobarbitoric acid (TBA) solution (1.5 gr trichloroacetic acid, 210 µl HCl and 0.04 gr tiobarbitoric acid, final volume 10 ml), liver cells were centrifuged at 1000×g, 1 min and mixed with 200 µl TBA solution in a sterile microtube. In addition, blank (200 µl TBA and 100 µl Ethylenediaminetetraacetic acid [EDTA]) and standard (200 µl TBA with 100 µl standard MDA) solutions were prepared. Upon sonication and cell lysis at 40 v for 20 sec, the cells were transferred to a water bath (90 °C) for 60 min to form MDA-TBA. Finally, the microtubes were centrifuged (14000×g, 10 min), and 100 µl of supernatant was used for colorimetry at 532 nm using a spectrophotometer (Sepand et al. 2013).

2.6. Mitochondrial membrane potential

In this assay, rhodamine 123 fluorogenic cation was used to measure mitochondrial membrane potential. This hydrophobic dye is accumulated within mitochondria, based on their membrane potential, so that in an intact mitochondrial membrane possessing highly-negative potential, the dye cannot be detected by fluorometric method; on the other hand, lowered membrane potential can let the cytosolic release of rhodamine 123 through mitochondrial permeability transition (MPT) pores. This assay was done by making a mixture of 2 ml of rhodamine 123 (1.5 mM) with 0.5 ml of cell suspension (1000×g, 1 min) and incubation at 37 °C for 10 min. The intensity of fluorescence due to rhodamine 123 was measured using spectrofluorometer in 490-520 nm (Andersson et al. 1987).

2.7. Lysosomal membrane damage

Another hydrophobic dye, the fluorogenic acridine orange, specifically aggregates within lysosomes and reacts with acidic hydrogen, resulting in the formation of HA^+ as a charged molecule. This charged molecule lacks fluorogenic properties and is maintained within lysosomal membrane. In cases of oxidative stress and ROS formation, the lysosome membrane destruction is done, so that the dye can be released into the cytosol, as a non-charged molecule with fluorogenic properties. In brief, 2 ml of acridine orange solution (5g/ml) was mixed with 1 ml of hepatic cell suspension (10^6 cells/ml) in incubation buffer (pH = 7.4), and incubated for 15 min at 37 °C. The intensity of diffused fluorescence was measured at 490-520 nm (Pourahmad et al. 2011).

2.8. Ethical considerations

This study was conducted on wistar rats within the framework of animal studies protocols and in full compliance with the guidelines of the World Health Organization (WHO).

2.9. Statistical analyses

One-way ANOVA was used as a statistical test, and to evaluate the variance homogeneity in a group, Leven test was used and the results were reported as a mean of triplicate experiments (mean±SD).

3. RESULTS

3.1. Cytotoxicity evaluation

A 60% cell death in rat hepatocytes (10^6 cells/ml) was determined upon 3 h incubation of the cells with 2mM AA substance. Also, different concentrations of gallic acid (50 and 100 µM/ml) could significantly alleviate the AA-induced cytotoxicity after 3 h incubation ($P < 0.05$). Of note, 10 and 20 µM/ml concentrations of gallic acid could not significantly decrease AA cytotoxicity in rat hepatocytes during 3 h incubation (Table 1).

3.2. ROS measurement

Although AA (2mM) increased the ROS levels in rat hepatocytes (10^6 cells/ml) during 30 min incubation, but all experimental concentrations of gallic acid (10, 20, 50 and 100 µM/ml) significantly decreased the level of ROS production during 30 min, in comparison to control group ($P < 0.05$) (Table 1).

3.3. Determination of lipid peroxidation

After 1 h incubation of rat hepatocytes (10^6 cells/ml) with 2 mM AA, lipid peroxidation was increased, whereas this biochemical process was significantly reversed in those hepatocytes incubated with 50, 20 and 100 µM/ml concentrations of gallic acid ($P < 0.05$) (Table 1).

3.4. Mitochondrial membrane potential measurement

The mitochondrial membrane potential of rat hepatocytes (10^6 cells/ml) in the presence of 2mM AA was significantly decreased, while 50 and 100 µM/ml concentrations of gallic acid prevented a decline in membrane potential in rat

hepatocyte mitochondria ($P < 0.05$) (Table 1).

3.5. Lysosomal membrane damage measurement

No remarkable lysosomal damage was found in those hepatocyte cells (10^6 cells/ml) incubated with AA after 1 h incubation, in comparison with control group ($P < 0.05$) (Table 1).

4. DISCUSSION

Humans are exposed to AA through a diverse range of industrial (*e.g.*, cosmetics, materials used in molecular tests), nutritional (*e.g.*, heated starchy foods) and environmental sources (*e.g.*, water), this compound may entail life-threatening consequences, including neurotoxicity, genotoxicity and hepatotoxicity (Stadler and Scholz 2004; Taeymans et al. 2004). Oxidative stress is the likely reason of AA-induced hepatotoxicity, so discovery and application of antioxidant compounds would alleviate the detrimental effects of AA in humans (Pruser and Flynn 2011). Gallic acid is a plant-derived phenolic compound with various pharmacological impacts, comprising antioxidant, antimicrobial, anti-inflammatory, anticancer, cardioprotective, gastroprotective, and neuroprotective effects (Bai et al. 2021). The present study was done to demonstrate the healing effects of gallic acid on the AA-induced oxidative stress in the hepatocytes of wistar rats.

As anticipated, AA caused an increased toxicity condition in rat hepatocytes. However, when administered in concentrations of 20, 50, and 100 µM, gallic acid mitigated these effects. In a study by Hammad *et al*. (2013), varying concentrations of AA (10, 30, and 60 mg/kg) were found to induce acute toxicity in the hepatic tissue of rats, in a time-dependent manner (Hammad et al. 2013). It has been known as a necrosis /apoptosis inducing agent in damaged cells (Elblehi et al. 2020; Yildizbayrak and Erkan 2019), as evidenced in Mehri *et al*. study (2012) on AA-induced toxicity in PC12 cells (Mehri et al. 2015). As mentioned above, AA induced the production of ROS in the isolated rat hepatocytes, comparable to control group, consistent with Chen et al. (2014) finding, on the incubation of AA with Caco-2 cells; they also showed an increase in the levels of hydrogen peroxide and oxidative molecules (Chen et al. 2014). An encouraging finding from the current research was the notable decrease in ROS production in rat hepatocytes when exposed to varying concentrations (10, 20, 50, and 100 µM) of gallic acid, as a powerful antioxidant compound. Phenolic substances such as gallic acid contain two or more hydroxyl groups in their chemical structure, which inhibit the oxidative stress conditions through deactivating free radicals like ROS (*e.g.*, superoxide anion, hydroxyl radical, peroxy nitrates, nitric oxide radical) and reactive nitrogen species (NOS) (Nabavi et al. 2013; Senevirathne et al. 2012; Yigitturk et al. 2017).

In the present study, although lipid peroxidation was dramatically increased in rat hepatocytes in the presence of AA, it was substantially declined using various concentrations (50 and 100 µM) of gallic acid. In Tanaka *et al*. studies in 1997 and 1998, it was shown that the levels of GST and quinone reductase were significantly increased in the liver and colon of examined rats in a dose-dependent manner (Tanaka et al. 1997; Tanaka et al. 1998). As a significant reducing agent, GSH preserve thiol groups on antioxidant molecules and intracellular proteins, hence a decreased GSH level would favor the subsequent production and accumulation of ROS (Wüllner et al. 1999). Higher concentrations of AA can exacerbate this situation by further depleting GSH and inducing oxidative stress. In a study by Erdemli *et al*. (2017), the hepatotoxicity of AA was demonstrated in liver of mother and fetus during pregnancy through increased MDA, xanthine oxidase, ROS and cell death (Erdemli et al. 2017). Moreover, another study by Zhang and colleagues (2009), on the protective effects of hydroxytyrosol against AA-induced cytotoxicity and DNA damage, showed that detrimental functions of AA can result in excessive oxidative stress and liver cell damage (Zhang et al. 2009). Endogenous antioxidant enzyme systems play a crucial role in reducing oxidative stress and neutralizing harmful oxidant species. Two key enzymes involved in lipid peroxidation are CAT and SOD (Torsdottir et al. 2010). In previous studies, the hepatoprotective effects of gallic acid have been reported against liver toxicity caused by carbon tetrachloride and acetaminophen. This natural compound can ROS and NOS production and decreasing MDA levels, while elevating GSH in affected cells, hence exerting its antioxidant activity and preventing lipid peroxidation.

In normal cells, the mitochondrion serves as the primary source for ROS, and about 80% of hydrogen peroxide (H2O2) free radicals being generated by this organelle. Additionally, mitochondria convert about 1-2% of consumed oxygen into superoxide anions. The production of free radicals by mitochondria is further enhanced in the presence of toxic substances and inhibitors of electron transport chain. The presence of toxic agents and electron transport chain inhibitors further enhances the formation of free radicals by mitochondria (Gonzalez-Flecha and Boveris 1995; Isahara et al. 1999). Normal mitochondrial membrane is relatively impermeable to many molecules, with the exception of some metabolites and ions (Halestrap et al. 2007). When there is an excess production of ROS, they can cause oxidation of thiol groups located near the MPT pores. This oxidation can alter the conformation of the thiol groups and ultimately result in the opening of these pores. The mitochondrial proton pump, which is crucial for ATP synthesis, relies on the continuous transport of protons from the mitochondrial matrix to the intermembrane space to generate the mitochondrial membrane potential (Ψm) (Kowaltowski et al. 2001). The opening of MPT pores disrupts the permeability of the inner mitochondrial membrane, leading to a decrease in membrane potential and triggering inflammation (Halestrap et al. 2007).

According to our findings, the incubation of AA with wistar rat hepatocytes resulted in a decrease in the mitochondrial membrane potential, which was effectively prevented when the hepatic cells were incubated with gallic acid concentrations of 50 and 100 µM. In a study conducted by Zhao *et al*. in 2015, the protective effects of blueberry anthocyanin on AA-induced antioxidant activity in the mitochondria of hepatocyte were examined, revealing that AA had a significant detrimental impact on mitochondrial functions such as SOD, ATPase, and electron chain transport, leading to a decrease in mitochondrial membrane potential (Mengyao Zhao et al. 2015). Lysosome is an acidic organelle, containing high-concentration ferrous ions, that interact with AA and destroy this compound (Pourahmad et al. 2012). In the present study, no lysosomal damage was reported, which can be a consequence of AA destruction in this organelle.

The aim of this study was to examine the potential protective role of gallic acid against AA-induced oxidative stress in isolated hepatocytes of wistar rats. The results obtained from the experiment showed that gallic acid, when administered at

concentrations of 10, 20, 50 and 100 µM demonstrated a reduction in the formation of reactive oxygen species (ROS), lipid peroxidation, decline in mitochondrial membrane potential, and cell death induced by acrylamide (AA), compared to the group solely exposed to AA. These findings support previous research indicating the protective effects of gallic acid against various oxidative toxins.

REFERENCES

Andersson B, Aw TY, Jones DP (1987) Mitochondrial transmembrane potential and pH gradient during anoxia American Journal of Physiology-Cell Physiology 252:C349-C355

Badhani B, Sharma N, Kakkar R (2015) Gallic acid: A versatile antioxidant with promising therapeutic and industrial applications Rsc Advances 5:27540-27557

Bai J et al. (2021) Gallic acid: Pharmacological activities and molecular mechanisms involved in inflammation-related diseases Biomedicine & pharmacotherapy 133:110985

Bjellaas T, Stølen LH, Haugen M, Paulsen JE, Alexander J, Lundanes E, Becher G (2007) Urinary acrylamide metabolites as biomarkers for short-term dietary exposure to acrylamide Food and Chemical Toxicology 45:1020-1026

Blancher C, Cormick RM (2012) Sodium Dodecyl Sulphate–Polyacrylamide Denaturing Gel Electrophoresis and Western Blotting Techniques Metastasis Research Protocols:89-110

Chen W, Shen Y, Su H, Zheng X (2014) Hispidin derived from Phellinus linteus affords protection against acrylamide-induced oxidative stress in Caco-2 cells Chemico-Biological Interactions 219:83-89

Cheynier V (2012) Phenolic compounds: from plants to foods Phytochemistry reviews 11:153-177

Choubey S, Varughese LR, Kumar V, Beniwal V (2015) Medicinal importance of gallic acid and its ester derivatives: a patent review Pharmaceutical patent analyst 4:305-315

Elblehi SS, El Euony OI, El-Sayed YS (2020) Apoptosis and astrogliosis perturbations and expression of regulatory inflammatory factors and neurotransmitters in acrylamide-induced neurotoxicity under ω3 fatty acids protection in rats Neurotoxicology 76:44-57

Erdemli ME, Altinoz E, Aksungur Z, Turkoz Y, Dogan Z, Gozukara Bag H (2017) Biochemical investigation of the toxic effects of acrylamide administration during pregnancy on the liver of mother and fetus and the protective role of vitamin E The Journal of Maternal-Fetal & Neonatal Medicine 30:844-848

Fennell TR, Sumner SC, Snyder RW, Burgess J, Spicer R, Bridson WE, Friedman MA (2005) Metabolism and hemoglobin adduct formation of acrylamide in humans Toxicological Sciences 85:447-459

Fernandes FHA, Salgado HRN (2016) Gallic acid: review of the methods of determination and quantification Critical reviews in analytical chemistry 46:257-265

Friedman M (2003) Chemistry, biochemistry, and safety of acrylamide. A review Journal of agricultural and food chemistry 51:4504-4526

Gonzalez-Flecha B, Boveris A (1995) Mitochondrial sites of hydrogen peroxide production in reperfused rat kidney cortex Biochimica et Biophysica Acta (BBA)-General Subjects 1243:361-366

Halestrap AP, Clarke SJ, Khaliulin I (2007) The role of mitochondria in protection of the heart by preconditioning Biochimica et biophysica acta 1767:1007-1031 doi:10.1016/j.bbabio.2007.05.008

Hammad AY, Osman ME, Abdelgadir WS (2013) Effects of acrylamide toxicity on growth performance and serobiochemisty of Wistar rats British Journal of Pharmacology and Toxicology 4:163-168

Isahara K et al. (1999) Regulation of a novel pathway for cell death by lysosomal aspartic and cysteine proteinases Neuroscience 91:233-249

Jin C, Wu X, Zhang Y (2013) Relationship between antioxidants and acrylamide formation: A review Food research international 51:611-620

Kowaltowski AJ, Castilho RF, Vercesi AE (2001) Mitochondrial permeability transition and oxidative stress FEBS letters 495:12-15 doi:10.1016/s0014-5793(01)02316-x

Mehri S, Abnous K, Khooei A, Mousavi SH, Shariaty VM, Hosseinzadeh H (2015) Crocin reduced acrylamide-induced neurotoxicity in Wistar rat through inhibition of oxidative stress Iranian journal of basic medical sciences 18:902

Mengyao Zhao P, Yuchen Z, Xin L, Xiaosong H, Fang C (2015) The chemoprotection of a blueberry anthocyanin extract against the acrylamide-induced oxidative stress in mitochondria: unequivocal evidence in mice liver 9 6:3006-3012

Moldéus P, Högberg J, Orrenius S (1978) [4] Isolation and use of liver cells. In: Methods in enzymology, vol 52. Elsevier, pp 60-71

Nabavi SF, Nabavi SM, Habtemariam S, Moghaddam AH, Sureda A, Jafari M, Latifi AM (2013) Hepatoprotective effect of gallic acid isolated from Peltiphyllum peltatum against sodium fluoride-induced oxidative stress Industrial Crops and Products 44:50-55

Pourahmad J, Amirmostofian M, Kobarfard F, Shahraki J (2009) Biological reactive intermediates that mediate dacarbazine cytotoxicity Cancer chemotherapy and pharmacology 65:89-96

Pourahmad J, Eskandari MR, Kaghazi A, Shaki F, Shahraki J, Fard JK (2012) A new approach on valproic acid induced hepatotoxicity: involvement of lysosomal membrane leakiness and cellular proteolysis Toxicology in vitro : an international journal published in association with BIBRA 26:545-551 doi:10.1016/j.tiv.2012.01.020

Pourahmad J, Mortada Y, Eskandari MR, Shahraki J (2011) Involvement of lysosomal labilisation and lysosomal/mitochondrial cross-talk in diclofenac induced hepatotoxicity Iranian journal of pharmaceutical research: IJPR 10:877

Pruser KN, Flynn NE (2011) Acrylamide in health and disease Frontiers in Bioscience-Scholar 3:41-51

Rice JM (2005) The carcinogenicity of acrylamide Mutation Research/Genetic Toxicology and Environmental Mutagenesis 580:3-20

Semla M, Goc Z, Martiniaková M, Omelka R, Formicki G (2017) Acrylamide: a common food toxin related to physiological functions and health Physiological research 66:205

Senevirathne M, Jeon Y-J, Kim Y-T, Park P-J, Jung W-K, Ahn C-B, Je J-Y (2012) Prevention of oxidative stress in Chang liver cells by gallic acid-grafted-chitosans Carbohydrate polymers 87:876-880

Sepand MR, Soodi M, Hajimehdipoor H, Soleimani M, Sahraei E (2013) Comparison of neuroprotective effects of Melissa officinalis total extract and its acidic and non-acidic fractions against a ß-induced toxicity Iranian journal of pharmaceutical research: IJPR 12:415

Stadler RH, Scholz G (2004) Acrylamide: an update on current knowledge in analysis, levels in food, mechanisms of formation, and potential strategies of control Nutrition reviews 62:449-467

Taeymans D et al. (2004) A review of acrylamide: an industry perspective on research, analysis, formation, and control Critical reviews in food science and nutrition 44:323-347

Tanaka T et al. (1997) Citrus auraptene inhibits chemically induced colonic aberrant crypt foci in male F344 rats Carcinogenesis 18:2155-2161

Tanaka T et al. (1998) Chemoprevention of 4-nitroquinoline 1-oxide-induced oral carcinogenesis by citrus auraptene in rats Carcinogenesis 19:425-431

Torsdottir G, Kristinsson J, Snaedal J, Sveinbjörnsdóttir S, Gudmundsson G, Hreidarsson S, Jóhannesson T (2010) Case–control studies on ceruloplasmin and superoxide dismutase (SOD1) in neurodegenerative diseases: a short review Journal of the neurological sciences 299:51-54

Ubaoji KI, Orji VU (2016) A review on acrylamide in foods: Sources and implications to health Mgbakoigba: Journal of African Studies 6

Wüllner U et al. (1999) Glutathione depletion and neuronal cell death: the role of reactive oxygen intermediates and mitochondrial function Brain research 826:53-62

Yigitturk G, Acara AC, Erbas O, Oltulu F, Yavasoglu NUK, Uysal A, Yavasoglu A (2017) The antioxidant role of agomelatine and gallic acid on oxidative stress in STZ induced type I diabetic rat testes Biomedicine & pharmacotherapy 87:240-246

Yildizbayrak N, Erkan M (2019) Therapeutic effect of curcumin on acrylamide-induced apoptosis mediated by MAPK signaling pathway in Leydig cells Journal of Biochemical and Molecular Toxicology 33:e22326

Zamani E, Shokrzadeh M, Fallah M, Shaki F (2017) A review of acrylamide toxicity and its mechanism Pharmaceutical and biomedical research 3:1-7

Zhang L, Yang L, Luo Y, Dong L, Chen F (2023) Acrylamide-Induced Hepatotoxicity Through Oxidative Stress: Mechanisms and Interventions Antioxidants & Redox Signaling 38:1122-1137

Zhang X, Cao J, Jiang L, Geng C, Zhong L (2009) Protective effect of hydroxytyrosol against acrylamide-induced cytotoxicity and DNA damage in HepG2 cells Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 664:64-68

Table 1: Evaluation of cytotoxicity, ROS levels, lipid peroxidation, mitochondrial membrane potential and lysosomal membrane damage in wistar rat hepatocytes incubated with AA and gallic acid.

Groups	Mean±SD				
	Cytotoxicity (3h)	ROS levels (30 min)	Lipid peroxidation (60 min)	Mitochondrial membrane potential (60 min)	Lysosomal membrane damage (60 min)
Rat hepatocytes (control)	39 ± 2	34.4 ± 2.3	0.191 ± 0.11	6.34 ± 0.45	83 ± 5
2 mM AA	57 ± 4[a]	56.4 ± 5.4[a]	0.25 ± 0.008[a]	11.45 ± 0.74a	99 ± 9
10 µM gallic acid	54 ± 4*	50.8 ± 4.2*	0.228 ± 0.013	10.33 ± 1.14	81 ± 9
20 µM gallic acid	50 ± 3*	41.1 ± 3.3*	0.210 ± 0.014*	10.34 ± 0.73	79 ± 12
50 µM gallic acid	46 ± 2	40.3 ± 3.5*	0.215 ± 0.007*	8.44 ± 0.61*	89 ± 8
100 µM gallic acid	42 ± 6	38.5 ± 4.7 *	0.203 ± 0.012*	6.93 ± 0.68*	85 ± 6

a denotes a significant difference with control group ($P < 0.05$).

* denotes significant differences with AA-exposed liver cells ($P < 0.05$).

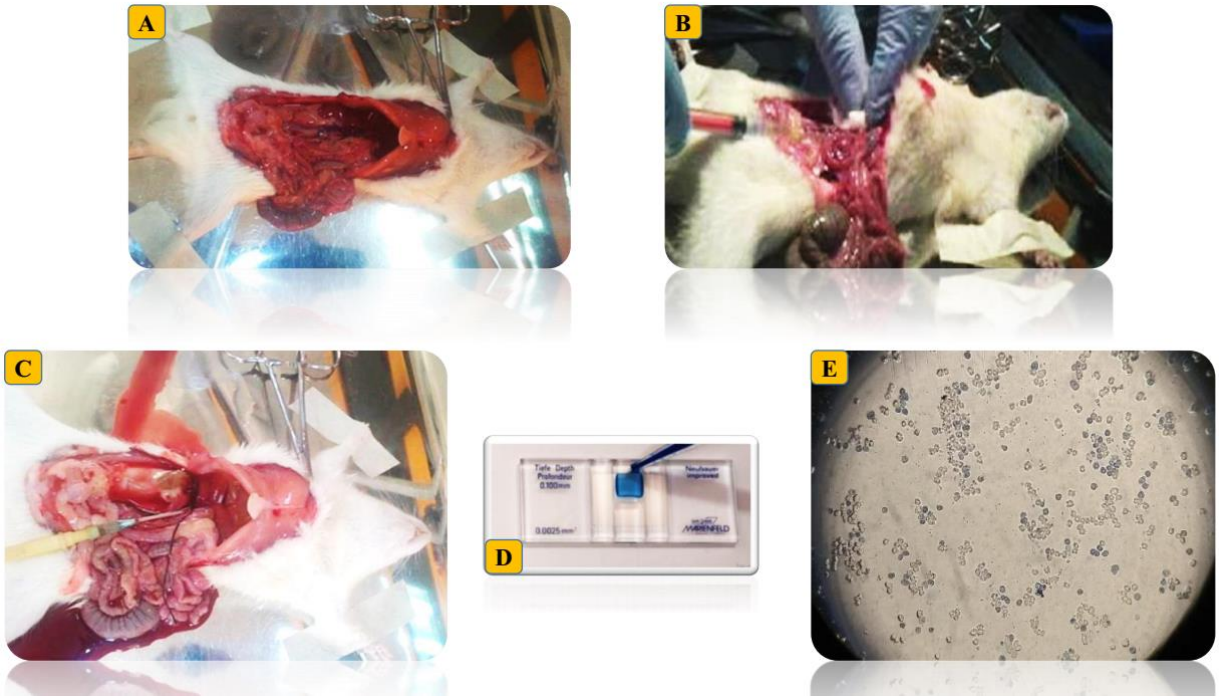

Figure 1: (A) U-shaped abdominal incision and exposing liver after pulling over the intestines; (B) Heparin injection to avoid blood coagulation (0.25 ml, 5000 U/ml); (C) liver perfusion using collagenase for the isolation of rat hepatocytes; (D) staining and enumeration of the harvested cells using trypan blue dye; and (E) dead cells stain blue, while alive ones are yellow in color.