



## Alterations of blood chemistry, hepatic and renal function, and blood cytometry in acrylamide-treated rats

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### ABSTRACT

Acrylamide is a vinyl monomer that is widely used for the synthesis of polyacrylamides, the treatment of drinking water, and as an additive in cosmetics. Acrylamide is also produced during the thermal processing of carbohydrate-rich foods. Although the potential toxic effects of acrylamide have been reported, few studies have evaluated biochemical parameters in blood. The present study investigated alterations of blood chemistry, hepatic function, and blood cytometry in acrylamide-treated rats. Thirty-two male Wistar rats were assigned to four experimental groups ( $n = 8/\text{group}$ ): one control group received 0.3 ml of vehicle (saline solution), and the other three groups received acrylamide (25, 50, and 75 mg/kg, i.p., for 14 days). At the end of treatment, blood samples were collected to obtain serum, which was then processed using a Vitros250 device. For blood cytometry, the samples were processed in a Sysmex analyzer. The blood chemistry results showed that urea nitrogen, urea, and creatinine were elevated in the acrylamide-treated groups. Tests of hepatic function showed that total and direct bilirubins, transaminases, and alkaline phosphatase were also elevated compared with vehicle, whereas the levels of total proteins and albumin decreased. Blood cytometry showed that the levels of erythrocytes, hemoglobin, hematocrit, leukocytes, and platelets and mean cell volume decreased in the acrylamide-treated groups compared with vehicle. Overall, the present findings indicate that acrylamide causes deleterious effects on renal and hepatic physiology, producing dose-dependent alterations of blood chemistry and cytometry parameters in male Wistar rats.

### 1. Introduction

Acrylamide (2-propenamide) is widely used in industry for the synthesis of polyacrylamides. It is used for the treatment of drinking water, processing pulp for paper production, and removing suspended solids from wastewater. It is also used as an additive in cosmetics, mining, and the formulation of sealing agents for dams, tunnels, and sewers [1]. Acrylamide is toxic to humans and classified as a pro-cancer agent by the International Agency for Research on Cancer [2,3].

Acrylamide can be produced naturally during the thermal processing ( $> 120^\circ\text{C}$ ) of carbohydrate-rich foods during the Maillard reaction, which provides desirable flavor, color, and aroma for the consumer [4]. In baked or fried potatoes, acrylamide concentrations of 1000 and 500  $\mu\text{g}/\text{kg}$ , respectively, have been reported [5]. This substance can produce peripheral neuropathies and neurotoxicity in experimental animals and humans, and some of its analogues can cause testicular damage [6,7]. Despite these negative effects of acrylamide on health, few studies have evaluated other

effects of acrylamide that could endanger the health of consumers. Metabolic studies that focus on interactions between acrylamide and cytochrome P<sub>450</sub> and glutathione-S-transferase (GST) in rats and mice indicate that GST in the liver, kidneys, brain, and erythrocytes can significantly bind acrylamide, and GST in the liver is three-times more efficient in the conjugation process [8].

The present study evaluated alterations of blood chemistry, hepatic function, and blood cytometry in acrylamide-treated rats to elucidate its negative effects on health.

### 2. Methods

#### 2.1. Ethics

The experimental protocols were performed according to the International Guide for the Care and Use of Laboratory Animals [9] and Official Mexican Standard NOM-062-ZOO-1999 Technical Specifications for

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the Production, Care, and Use of Laboratory Animals [10]. All efforts were made to minimize animal discomfort during the course of this investigation.

## 2.2. Animals

Thirty-two adult male Wistar rats, weighing 250–300 g at the beginning of the experiments, were included in the study. The rats were housed five per cage in Plexiglas cages under a 12 h/12 h light/dark cycle (lights on at 7:00 AM) and average room temperature of  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The animals had ad libitum access to water and food.

## 2.3. Groups and treatments

The doses of acrylamide (PubChem CID: 6579;  $\text{CH}_2\text{CHCONH}_2$ ; catalog no. A909, Sigma-Aldrich, St. Louis, MO, USA) were based on previous studies that found that 25 and 75 mg/kg caused damage to the brain and spinal cord tissue [11]. Therefore, we used these two doses. To generate a dose-response curve, we included an intermediate dose (50 mg/kg) to explore dose-dependent effects on hepatic function, kidney function, and blood cytometry. The experimental design included a vehicle group (0.9% NaCl) and three groups that received acrylamide (25, 50, and 75 mg/kg, i.p.). We selected the i.p. route of administration because the toxic effects of acrylamide can damage the esophagus when administered orally. Considering that the treatment schedule in the present study was 14 days, oral acrylamide administration may harm the esophagus and limit food and water consumption, thus influencing parameters that can be affected by food and not only by the compound.

The treatments were performed for 14 consecutive days, with one injection every 24 h in a volume of 0.3 ml/rat according to Machholz et al. [12–14]. After 14 days of treatment, blood samples were obtained 2 h after the last injection for biochemical and cytometric analyses.

## 2.4. Blood samples

This procedure utilized 5 ml syringes with a needle size of 22 mm. The rats were deeply anesthetized with an overdose of sodium pentobarbital (120 mg/kg) and then placed in the supine position. The syringe was inserted through the lateral chest wall and the intercostal space in the maximum region of the heartbeat at an angle of 20–30°. The needle was then moved slowly, thus making slight negative pressure in the cylinder of the syringe, and then carefully drawn until blood flow stopped. The blood sample was deposited into two types of Vacutainer tubes: without anticoagulant (dry) and with anticoagulant. The blood samples remained in the Vacutainer tubes until the biochemical and cytometric analyses [14]. The blood samples in the dry tubes were allowed to coagulate and then centrifuged at 3500 rotations per minute for 5 min to obtain serum, which was immediately transferred with a Pasteur pipette to corresponding containers for dry chemistry analysis using a Vitros250 device (Johnson & Johnson, Ramsey, MN, USA). For blood cytometry, the blood samples were transferred to Vacutainer tubes with ethylenediaminetetraacetic acid anticoagulant and homogenized by immersion so that all of the samples

could then be processed automatically in a Sysmex analyzer (Sysmex Diagnósticos México, S. de R.L. de C.V.). The results of the different variables were compared with normal reference values to verify possible alterations of renal and hepatic function.

## 2.5. Statistical analysis

The blood cytometry and renal and hepatic function parameters were analyzed using one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls *post hoc* test. Values of  $p \leq 0.05$  were considered statistically significant. The data are expressed as the mean  $\pm$  standard error of the mean (SEM). The statistical analysis was performed using SigmaStat 3.5 software.

## 3. Results

Only the results of the groups that were treated with vehicle and acrylamide at doses of 25 and 50 mg/kg are presented because the rats that received 75 mg/kg acrylamide were euthanized before the end of the 14 days of treatment in an effort to avoid unnecessary suffering because of their physical deterioration.

### 3.1. Tests of blood chemistry, liver function, and blood cytometry

The glucose analysis showed a significant effect of treatment ( $F_{2,18} = 10.186$ ,  $p < 0.05$ ). Acrylamide (50 mg/kg) significantly decreased glucose levels compared with the vehicle-treated group. The analysis of blood urea nitrogen (BUN;  $F_{2,18} = 38.617$ ,  $p < 0.050$ ) and urea ( $F_{2,18} = 8.648$ ,  $p < 0.001$ ) also revealed significant effects of treatment. Acrylamide (50 mg/kg) increased the values of both analytes compared with the vehicle-treated group. The creatinine analysis also showed a significant effect of treatment ( $F_{2,18} = 23.146$ ,  $p < 0.001$ ) at acrylamide doses of 25 and 50 mg/kg compared with the vehicle-treated group (Table 1).

The analysis of hepatic function showed significant effects of treatment on total ( $F_{2,18} = 336.874$ ,  $p < 0.001$ ) and direct ( $F_{2,18} = 1850.952$ ,  $p < 0.001$ ) bilirubin. Acrylamide (25 and 50 mg/kg) increased bilirubin compared with the vehicle-treated group. The analysis of indirect bilirubin also showed a significant effect of treatment ( $F_{2,18} = 2953.055$ ,  $p < 0.001$ ), in which the 50 mg/kg dose increased this variable compared with the vehicle-treated group and group that received 25 mg/kg acrylamide. The analysis of aspartate aminotransferase (AST;  $F_{2,18} = 21.917$ ,  $p < 0.001$ ) and alanine aminotransferase (ALT;  $F_{2,18} = 59.475$ ,  $p < 0.001$ ) showed significant effects of treatment. Acrylamide (25 and 50 mg/kg) significantly increased both AST and ALT. The analysis of alkaline phosphatase also showed a significant effect of treatment ( $F_{2,18} = 15.036$ ,  $p < 0.001$ ). Acrylamide (25 and 50 mg/kg) significantly increased this variable compared with the vehicle-treated group. The analysis of total proteins also showed significant effects of treatment ( $F_{2,18} = 8.190$ ,  $p < 0.05$ ). Acrylamide (50 mg/kg) decreased total proteins compared with the vehicle-treated group. The analysis of albumin showed significant effects of treatment ( $F_{2,18} = 6.901$ ,  $p < 0.050$ ). Acrylamide (25 and 50

**Table 1**  
Blood chemistry in rats after 14 days of treatment.

Analyte	Vehicle	Acrylamide 25 mg/kg	Acrylamide 50 mg/kg	Reference intervals <sup>+</sup>
Glucose	5.31 $\pm$ 0.44	4.60 $\pm$ 0.55	2.57 $\pm$ 0.29*	6.00–10.00 mmol/L
BUN	6.71 $\pm$ 0.15	11.14 $\pm$ 1.19	24.60 $\pm$ 2.29*	3.00–7.00 mmol/L
Urea	21.78 $\pm$ 3.32	32.92 $\pm$ 1.50	68.91 $\pm$ 14.03*	10.70–20.00 mmol/L
Creatinine	18.43 $\pm$ 6.90	53.61 $\pm$ 5.84*	70.02 $\pm$ 2.86*	11.00–28.00 $\mu\text{mol/L}$

The data are expressed as mean  $\pm$  SEM.

\*  $p < 0.05$ , vs. vehicle group (one-way ANOVA for independent groups followed by Student-Newman-Keuls *post hoc* test).

<sup>+</sup> Source: Suckow et al. [29], Giknis and Clifford [30], Sharp and Villano [31].

**Table 2**  
Hepatic function tests at the end of treatment.

Analyte	Vehicle	Acrylamide 25 mg/kg	Acrylamide 50 mg/kg	Reference intervals <sup>+</sup>
Total bilirubins	0.22 ± 0.01	0.98 ± 0.05 <sup>*</sup>	1.70 ± 0.03 <sup>*</sup>	0.04-0.20 mg/dl
Direct bilirubin	0.04 ± 0.01	0.28 ± 0.01 <sup>*</sup>	1.05 ± 0.01 <sup>*</sup>	0.03-0.06 mg/dl
Indirect bilirubin	0.02 ± 0.0009	0.08 ± 0.01	1.03 ± 0.01 <sup>*</sup>	0-0.10 mg/dl
AST	94.75 ± 1.84	164.75 ± 19.27 <sup>*</sup>	203.75 ± 6.53 <sup>*</sup>	63.00-157.00 UI/L
ALT	44.00 ± 1.87	70.25 ± 3.63 <sup>*</sup>	89.50 ± 3.09 <sup>*</sup>	19.00-53.00 UI/L
Alkaline phosphatase	216.75 ± 33.52	329.00 ± 3.34 <sup>*</sup>	401.00 ± 24.18 <sup>*</sup>	36.00-312.00 UI/L
Total proteins	5.83 ± 0.32	5.01 ± 0.32	4.24 ± 0.13 <sup>*</sup>	5.60-7.60 g/dl
Albumin	4.26 ± 0.16	3.21 ± 0.33 <sup>*</sup>	3.21 ± 0.14 <sup>*</sup>	4.00-5.00 g/dl

The data are expressed as mean ± SEM.

\*  $p < 0.05$ , vs. vehicle group (one-way ANOVA for independent groups followed by Student-Newman-Keuls *post hoc* test).

<sup>+</sup> Source: Suckow et al. [29], Giknis and Clifford [30], Sharp and Villano [31].

mg/kg) significantly decreased albumin concentrations compared with the vehicle-treated group (Table 2).

The blood cytometry analysis showed significant effects of treatment on the different variables, including erythrocytes ( $F_{2,18} = 38.674$ ,  $p < 0.001$ ), hemoglobin ( $F_{2,18} = 22.791$ ,  $p < 0.001$ ), hematocrit ( $F_{2,18} = 13.665$ ,  $p < 0.001$ ), mean cell volume (MCV;  $F_{2,18} = 58.999$ ,  $p < 0.001$ ), leukocytes ( $F_{2,18} = 9.179$ ,  $p = 0.002$ ), and platelets ( $F_{2,18} = 20.155$ ,  $p < 0.001$ ). Acrylamide (25 and 50 mg/kg) significantly decreased the values of these variables compared with vehicle-treated rats (Table 3).

#### 4. Discussion

The parameters that were evaluated in the present study allowed an indirect assessment of hepatic and renal function. Blood chemistry analyses that measure glucose, urea, creatinine, and urea nitrogen (BUN) concentrations are the most commonly used to evaluate renal function. Blood glucose is an indicator of the healthy state of the organism, and its gradual decrease is indicative of a condition that is either just appearing or already present but at a lower stage (i.e., its level reflects the nutritional, emotional, and endocrine conditions of the subject) [15]. Hypoglycemia occurs when blood glucose levels are below normal, usually during fasting, and is regulated by gluconeogenic or glycogenolytic processes. This condition is considered more dangerous than hyperglycemia because it can readily cause hypoglycemic shock, with seizures and coma that are caused by a lack of glucose in the brain [15]. The main causes of hypoglycemia are enzymatic alterations of hydrocarbon or amino acid metabolism, prolonged fasting, hyperinsulinism, Dumping syndrome,  $\beta$ -prostatic cell tumors, chitin or pentamidine drug treatment, Addison's disease, abundant alcohol intake, alterations of growth hormones, malnutrition, vomiting, diarrhea, and poisoning [16]. In the present study, blood glucose levels decreased in acrylamide-treated rats (25 and 50 mg/kg), suggesting pancreatic damage. Urea is the final metabolite of protein nitrogen balance, the

measurement of which allows the evaluation of the generic metabolism of proteins and amino acids through the urea cycle, which is exclusively hepatic [17–19]. Once in blood, urea is excreted primarily by the kidneys. After this glomerular filtration, between 40% and 60% is reabsorbed at the tubular level, constituting a marker of renal function [17–19]. In the present study, blood urea was elevated in acrylamide-treated rats (50 mg/kg), suggesting kidney damage [20–23]. Another metabolite that was analyzed in the present study was creatinine. Creatinine is an excretion product of muscle activity, which circulates in blood. Its elimination is exclusively renal, so there is a correlation between creatinine levels and renal function. Most creatinine that is eliminated by the kidneys is freely filtered in renal glomeruli, and a small fraction is filtered by the tubular component, which is a good indicator of renal-glomerular function [17,21–23]. Similar to urea, creatinine and BUN significantly increased in rats that received acrylamide (25 and 50 mg/kg), indicating imminent kidney damage. BUN is the amount of nitrogen that circulates in the form of urea through the bloodstream. In healthy animals, urea is filtered from plasma by the renal glomerulus. It returns to the blood through renal tubules, but most of it is excreted through urine. However, if the kidney is not functioning properly, then sufficient urea cannot be removed from plasma, leading to higher BUN levels that fluctuate under several physiological conditions, such as high protein intake, intestinal bleeding, infection, fever, dehydration, medications, burns, and poisoning. BUN should be considered together with serum creatinine to demonstrate its origin of renal type [20–25].

The pathophysiological factors that are associated with renal failure may include direct nephrotoxic agents [26], disseminated vascular coagulation [27], and proteolytic enzymes and vasoactive substances that act on the kidneys [28]. The increase in analyte concentrations corresponded to the increase in the dose of the neurotoxin. The rats that received only vehicle had values that were within the reference range, indicating that the toxicity of acrylamide is evident at the renal level and dose-dependent. At the hepatic level, acrylamide is metabolized to

**Table 3**  
Blood counts in rats at the end of 14 days of treatment.

Analyte	Vehicle	Acrylamide 25 mg/kg	Acrylamide 50 mg/kg	Reference intervals <sup>+</sup>
Erythrocytes	8.00 ± 0.09	4.79 ± 0.63 <sup>*</sup>	3.44 ± 0.11 <sup>*</sup>	7.80- 8.65 10 <sup>3</sup> /mm <sup>3</sup>
Hb	14.34 ± 0.12	9.35 ± 1.04 <sup>*</sup>	9.23 ± 0.10 <sup>*</sup>	13.20-17.10 g/dl
Hto	43.35 ± 0.68	28.51 ± 3.67 <sup>*</sup>	30.70 ± 0.32 <sup>*</sup>	35-45%
MCV	53.60 ± 0.76	41.28 ± 2.11 <sup>*</sup>	32.33 ± 0.86 <sup>*</sup>	45.00- 65.00 fl
MCH	17.70 ± 0.27	18.25 ± 0.73	18.03 ± 0.41	15.53-20.05 pg
MCHC	32.81 ± 0.34	32.52 ± 0.63	32.33 ± 0.62	25.88-32.88 g/dl
Leukocytes	6.71 ± 0.52	3.93 ± 0.71 <sup>*</sup>	3.75 ± 0.23 <sup>*</sup>	4.00-17.00 10 <sup>3</sup> /mm <sup>3</sup>
Platelets	736.14 ± 49.53	297.14 ± 94.23 <sup>*</sup>	226.66 ± 3.05 <sup>*</sup>	300.00-1500.00 10 <sup>3</sup> /mm <sup>3</sup>

The data are expressed as mean ± SEM.

\*  $p < 0.05$ , vs. vehicle group (one-way ANOVA for independent groups followed by Student-Newman-Keuls *post hoc* test).

<sup>+</sup> Source: Vaquero [32], Arcila et al. [33].

glycidamide epoxide in mice, rats, and humans [29]. The liver is susceptible to damage from direct exposure to toxic products because it participates in the metabolic detoxification of products and xenobiotics [30]. Benítez et al. [31] reported that acrylamide (25 and 100 mg/kg) produced significant changes in AST, ALT, and alkaline phosphatase (ALP) in rats. We observed elevated levels of AST, ALT, and ALP. The evidence of hepatic damage and levels of serum enzymes (e.g., AST, ALT, and ALP) were reported by other authors [20–22,32–35].

Acrylamide also impaired the viability of hepatic tissue, damaging its architecture through the loss of phenotypic characteristics. Acrylamide at doses of 10, 30, and 60 mg/kg produced an acute time-dependent toxic response in hepatic tissue [36]. Similarly, the increase in direct bilirubin, which is highly specific to liver disease [37], and the decrease in albumin, which is the most abundant plasma protein that is produced by hepatocytes [20–22,33,38], also reflected acrylamide-induced damage. In the present study, the tests of hepatic function showed that transferase and alkaline phosphatase values were elevated in the acrylamide-treated groups (25 and 50 mg/kg), thus indicating hepatic damage as reported by Benítez [31]. Such damage may be related to the permeability of hepatocyte membranes as a consequence of the generation of certain lesions following the binding of glycidamide with functional groups of membrane proteins [31].

Acrylamide, glycidamide, and ethylene oxide have electrophilic characteristics and the ability to form adducts (i.e., complexes between the hemoglobin molecule and a chemical compound that is foreign to the structure of the molecule) [39]. Some substances, such as ethylene oxide, to which workers in the sterilization area of hospitals are exposed, are capable of altering hematological parameters, including decreasing hemoglobin and hematocrit [40], and causing hematological damage, mainly anemia, leukopenia, and leukemia. Anemia is characterized by a hemoglobin concentration that is below normal levels that are established by the World Health Organization [41]. The analysis of blood cytometry in the present study showed that the levels of erythrocytes, hemoglobin, hematocrit, MCV, and leukocytes decreased in rats that were treated with 25 and 50 mg/kg acrylamide, indicating the occurrence of microcytic anemia in a manner similar to previous reports [20–22,42].

Platelets decreased only in rats that were treated with 50 mg/kg acrylamide. Based on the similar characteristics of acrylamide and ethylene oxide, we suggest that acrylamide administration decreased these blood parameters possibly through the formation of adducts with hemoglobin.

The present study has some limitations. One limitation was that we euthanized rats that were treated with 75 mg/kg acrylamide before the end of the 14-day treatment period, thus limiting the amount of data that could be generated. Another limitation was that more specific hepatic enzymes (e.g.,  $\gamma$ -glutamyl transpeptidase) that could confirm liver damage should have been measured, and histopathological analysis should have been performed.

In addition to the toxic and carcinogenic effects of acrylamide that have been reported in both humans and laboratory animals, the present study contributes to knowledge of the toxicological effects of acrylamide at the metabolic level, including its hematological, renal, and hepatic effects. Although these data were obtained in rats that were injected with pure acrylamide at doses that are unconventional in human exposure, rodents have a specific metabolic rate that is 15–20 times higher than the specific metabolic level of a resting human [48]. Therefore, lower concentrations of acrylamide in food or the environment to which humans are exposed could produce negative effects on health. However, this hypothesis requires further testing.

## 5. Conclusion

The present study found that systemic acrylamide administration for 14 days caused negative effects on renal and hepatic function, producing dose-dependent alterations of blood chemistry and cytometry parameters in male Wistar rats.

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## Conflicts of interest

The authors declare that there are no conflicts of interest.

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