

## Research Article

### Effects of Acrylamide Toxicity on Growth Performance and Serobiochemistry of Wistar Rats

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**Abstract:** The present study was conducted to obtain information on the effects of various dietary doses of the Acrylamide on Wistar rats. Emphasis was put on changes on growth and serobiochemical constituents of treated rats. Extra pure Acrylamide was fed to Wistar rats at 10, 30, 60 and 90 mg/kg, respectively of the standard diet for 6 weeks. Acrylamide was then withdrawn from the diet for four weeks. Incorporation in diet of the doses 10, 30, 60 and 90 mg/kg acrylamide was toxic to Wistar rats, but fatal only to those of group 5 fed on 90 mg/kg, where five rats (62.5%) died on day 18 of the experimental period. Depression in growth was observed in rats that had been fed on the experimental doses for 6 weeks. Neurotoxicity was observed only in the rats fed on acrylamide at 10 (Group 2) and 60 mg/kg (Group 4). These findings were accompanied by alterations in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities and albumin, globulin and cholesterol concentrations. The alteration on enzymes activities, urea and cholesterol remained even after the 4 weeks withdrawal of acrylamide, whereas, total protein, albumin, globulins and electrolytes concentration returned to their normal values. Acrylamide is considered neurotoxic at dietary levels of 10 and 60 mg/kg and enterohepatonephrotoxic to Wistar rats at dietary level of 10, 30, 60 and 90 mg/kg and fatal at the treatment dose of 90 mg/kg.

**Keywords:** Acrylamide, body weight, electrolyte, metabolites, neurotoxicity, serum enzymes

## INTRODUCTION

Thermal cooking is an important method of food processing as it increases the palatability (flavor, appearance, texture) and stability of foods and also improves the digestibility of foods. Moreover, it kills toxic microorganisms and deactivates such toxic substances as enzyme inhibitors. It also induces the formation of health promoting components, such as antioxidants and antimicrobial agents in food (Lingnert and Wailer, 1983).

The chemical changes in food components caused by high-heat treatment result in reducing nutritive values and even the formation of some toxic chemicals such as Polycyclic Aromatic Hydrocarbons (PAHs), amino acid or protein pyrolysates and N-nitrosamines. Among the many reactions occurring in processed foods, the Maillard Reaction plays the most important role in the formation of various chemicals (including toxic acrylamide) (Ames, 2009).

Acrylamide is an odorless, white crystalline solid at room temperature, with a molecular formula of C<sub>3</sub>H<sub>5</sub>NO. It is an industrial toxic and cancer-causing

chemical. Environmental exposure to acrylamide may occur from polymer manufacture and polymer use as flocculent to separate solids from aqueous solution in the disposal of industrial wastes and purification of water supplies (NICNAS, 2002). It is also used for producing grouts and soil stabilizers (Blackford, 1974). Recently, it has been reported that acrylamide monomer may form in certain foods cooked at high temperatures, the highest concentrations of which have been identified in potato and grain-based (e.g., frying, grilling or baking) principally from the interaction of the amino acid asparagine with glucose or other carbohydrates (Tareke *et al.*, 2002).

Acrylamide is readily absorbed into the circulation and thereafter distributed to various organs, reacts with cellular DNA, hemoglobin, nerve cells and enzymes (Rayburn and Friedman, 2010) and acts as animal carcinogen and germ cell mutagen (Ghanayem *et al.*, 2005a) and as human neurotoxicant and suspected carcinogen (Klaunig and Kamendulis, 2005; Nuno *et al.*, 2008). Acrylamide is genotoxic through its metabolite glycidamide formed via epoxidation by CYP2E1 and leads to the formation of glycidamide-

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Table 1: Percent inclusion rates (fresh basis) of ingredients of the basal diet fed to experimental rats

Ingredients	%
Meat meal	42.5
Grain starch	39.2
Granulated sugar	05.0
Cellulose powder	03.0
Corn oil	05.0
Super concentrate	05.0
DL-methionine	00.3

DNA and hemoglobin adducts (Ghanayem *et al.*, 2005b).

This experiment was design to obtain information on the effect of various dietary doses (10, 30, 60 and 90 mg/kg) of extra pure acrylamide on Wistar rats for 6 weeks and the effect of 4 weeks withdrawal (recovery period). Emphasis was put on changes in growth, lesions and alteration in serobiochemical constituents of treated rats.

### MATERIALS AND METHODS

**Animals and experimental design:** Forty, two weeks old male Wistar rats were obtained from the Aromatic Herbs and Medicinal Plants Research Institute (A.H.M.P.R.I), National Centre for Research, Khartoum, reared within the premises of the institute under 12 h photoperiod with starter feed (Table 1) and drinking water provided *ad libitum* before the commencement of experimental feeding. Room temperature was maintained at 25±2°C at adequate house ventilation. Then the animals were randomly allotted to five groups 1, 2, 3, 4 and 5 each of eight rats.

Extra pure Acrylamide powder (Promega Co.Ltd, Iran) was thoroughly mixed with the basal diet and fed to rats at 10 mg/kg (Group 2), 30 mg/kg (Group 3), 60 mg/kg (Group 4) and 90 mg/kg (Group 5) whereas Group 1 was fed the basal diet and served as control. Experimental feeding was continued for 6 weeks. Recovery period was adopted thereafter for 4 weeks.

#### Data collected:

**Body weight changes:** Average body weight and body weight gain were estimated weekly for each group and the clinical signs were recorded. Lots of four rats from each group were anaesthetized with diethyl ether and humanely slaughter at the end of week 6 and 4 weeks after acrylamide withdrawal.

**Serobiochemical parameters:** Blood samples were collected at slaughter in dry test tubes, serum was separated and stored at -20°C until analyzed for the activities of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline phosphatase (ALP) and for the concentrations of total protein, albumin, globulin, cholesterol and urea and for the electrolytes, calcium, phosphorous, potassium and sodium using the commercial kits (Biosystem Chemicals, Barcelona, Spain) according to the manufacturer instructions.

**Statistical analysis:** Mean values in body weight and serum data were compared using student's t-test after (Snedecor and Cochran, 1989).

### RESULTS

**Clinical observations:** The control Group 1 remained clinically normal throughout the experimental period. At the end of week two (day 13), overt signs of neurotoxicity were observed in Group 2 rats receiving diet containing acrylamide at 10 mg/kg particularly of the hind limbs showing grip strength or leg splay (Fig. 1). The same symptoms were observed in rats of Group 4 receiving dietary acrylamide at 60 mg/kg but during the last week of the treatment period (38-42 days). Five rats (62.5%) of Group 5 receiving the highest dose 90 mg/kg died 16-17 days and the remaining rats were slaughter at extremis on day 18 post experimental feeding. Group 3 receiving 30 mg/kg acrylamide showed no clinical abnormalities and half the number of rats (4 rats) was slaughtered at the end of the experimental period (6 weeks). The nervous symptoms were less pronounced or absent during the recovery period.

**Growth performance:** The effects of feeding diets containing 10, 30, 60 and 90 mg/kg extra pure acrylamide on growth performance of rats were shown in Table 2. At the end of the experimental period (6 weeks), the rats on diet containing 10 mg/kg pure acrylamide had the significant lowest ( $p<0.01-0.001$ ) body weight and growth rate than the controls Group 1 and the other groups. The growth rate of Groups 3 and 4 was also lower ( $p<0.01$ ) than the controls, however the growth rate of group 4 is the highest ( $p<0.05$ )



Fig. 1: A rat of group 2 (10 mg/kg acryl amide) showing hind legs play

Table 2: Average (mean±S.E.) performance value (g) of rats fed on Acryl amide during treatment (6 weeks) and recovery (4 weeks) with dietary acryl amide

Groups	Initial Weight	Body weight	Weight gain
Treatment (6 week)			
1 (control)	72.91±0.58	81.66±0.57	8.75±0.01
2 (10 mg/kg)	65.00±0.00	68.96±0.35***	3.96±0.35**
3 (30 mg/kg)	70.00±0.00	74.69±2.69**	4.69±0.69**
4 (60 mg/kg)	73.38±0.32	78.30±0.78*	4.92±0.46**
5 (90 mg/kg)	65.00±0.00		
Recovery (4 week)			
1 (control)	81.66±0.57	109.00±1.29	27.34±0.72
2 (10 mg/kg)	68.96±0.35	90.15±0.41***	21.19±0.06**
3 (30 mg/kg)	74.69±0.69	95.73±0.41***	25.04±0.19*
4 (60 mg/kg)	78.30±0.78	91.80±0.75***	13.50±0.03***
5 (90 mg/kg)	-----	-----	-----

NS: Not Significant; \*: Denotes mean values significant at ( $p<0.05$ ); \*\*: Significant at ( $p<0.01$ );\*\*\*: Significant at ( $p<0.001$ )

amongst the test group. After the four weeks recovery period the test groups had significant lower ( $p < 0.001$ ) body weight and weight gain ( $p < 0.05-0.001$ ) than the control animals of Group 1.

**Changes in serum constituents:**

**Serum enzymes:** The effects of various doses of dietary acrylamide on the activities of serum enzymes AST, ALT and ALP are shown in Table 3. At the end of the treatment period (6 weeks), serum AST activity of Groups 3 and 5 were significantly lower ( $p < 0.05-0.001$ ) than the control rats of Group 1, where as that of Group 4 receiving dietary acrylamide was significantly higher ( $p < 0.05$ ). Decreased activity ( $p < 0.001$ ) of serum ALT of groups 3 and 4 and increase activity ( $p < 0.01$ ) of the same enzyme in Group 5 was also observed. There were significant decreases ( $p < 0.05-0.01$ ) in ALP activity in the test groups 2, 4 and 5 with an increased activity ( $p < 0.01$ ) of the enzyme in Group 3 receiving acrylamide at 60mg/kg. AST and ALT activities of Group 2 receiving dietary acrylamide at 10 mg/kg remained unchanged. At the end of the recovery period, significant increases ( $p < 0.05$ ) of AST activity in Groups 2 and 4 and ALT of Groups 3 than the controls were observed. Decreased ( $p < 0.05$ ) activity of AST in Group 3 receiving 30 mg/kg dietary acrylamide and ALP ( $p < 0.01$ ) in groups 2 and 3 were also seen.

**Serum metabolites:** Changes in the concentration of the total proteins, albumin, globulins, urea and cholesterol are presented in Table 4. In the test groups, the concentrations of urea were lower ( $p < 0.01$ ),

whereas cholesterol was higher ( $p < 0.05-0.01$ ) than in the control rats. The total protein in Group 2 fed on 10 mg/kg dietary acrylamide was significantly ( $p < 0.05$ ) higher, but lower ( $p < 0.05$ ) in Group 5 on 90 mg/kg than the controls. Four weeks post the treatment feeding (recovery period), there were no differences in the concentrations of the total proteins, albumin and globulins between the test and control groups. Significant increase ( $p < 0.05-0.01$ ) in the concentrations of urea and cholesterol was recorded in all the test groups.

**Serum electrolytes:** The effects of different doses of dietary acrylamide on the concentration of calcium, phosphorous, sodium and potassium are given in Table 5. No change was observed in the concentration of calcium in the test groups and the controls. There were significant increase ( $p < 0.05-0.01$ ) in phosphorous

Table 3: Average values (mean±SE) of serum enzymes in rats during treatment (6 weeks) and recovery (4 weeks) with dietary acryl amide

Groups	AST (iu)	ALT (iu)	ALP (iu)
Treatment			
Group 1 (control)	134.28±0.94	11.50±0.54	165.00±1.44
Group 2 (10 mg/kg)	133.38±1.03 <sup>NS</sup>	11.49±0.46 <sup>NS</sup>	163.00±1.29*
Group 3 (30 mg/kg)	110.90±1.57***	4.74±0.36***	175.25±2.36**
Group 4 (60 mg/kg)	137.25±0.85*	3.76±0.50***	163.75±1.03*
Group 5 (90 mg/kg)	130.03±1.41*	15.37±0.95**	153.50±0.96**
Recovery			
Group 1 (control)	129.82±0.13	20.15±0.48	181.67±1.18
Group 2 (10 mg/kg)	134.80±3.66*	20.17±0.18 <sup>NS</sup>	172.75±0.11**
Group 3 (30 mg/kg)	124.65±1.04*	24.18±1.06*	172.13±0.88**
Group 4 (60 mg/kg)	134.85±2.77*	21.29±1.00 <sup>NS</sup>	180.05±1.45 <sup>NS</sup>

NS = Not Significant; \*: Denotes mean values significant at ( $p < 0.05$ ); \*\*: Significant = ( $p < 0.01$ ); \*\*\*: Significant = ( $p < 0.001$ )

Table 4: Average (mean±SD) values of serum metabolites of rats during treatment (6 weeks) and recovery (4 weeks) with dietary acryl amide

Groups	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Urea (mg/dL)	Cholesterol (mg/dL)
Treatment					
Group 1 (control)	7.80±0.30	4.35±0.17	3.45±0.13	64.70±0.62	75.87±0.83
Group 2 (10 mg/kg)	5.08±0.28*	2.60±0.09*	2.48±0.19 <sup>NS</sup>	53.25±1.11**	79.25±0.11**
Group 3 (30 mg/kg)	5.53±0.15*	2.45±0.41*	3.08±0.26 <sup>NS</sup>	58.75±1.50**	87.25±0.96**
Group 4 (60 mg/kg)	5.58±0.44*	3.35±0.17 <sup>NS</sup>	2.23±0.2*	53.50±0.65**	82.88±0.72**
Group 5 (90 mg/kg)	6.20±0.42*	3.38±0.27 <sup>NS</sup>	2.82±0.23 <sup>NS</sup>	53.50±1.32**	76.63±0.01*
Recovery					
Group 1 (control)	6.05±0.06	3.50±0.04	2.55±0.028	33.33±1.25	65.67±1.65
Group 2 (10 mg/kg)	6.30±0.07 <sup>NS</sup>	3.65±0.13 <sup>NS</sup>	2.65±0.14 <sup>NS</sup>	53.50±1.71**	71.7±1.02*
Group 3 (30 mg/kg)	6.20±0.11 <sup>NS</sup>	3.70±0.09 <sup>NS</sup>	2.50±0.11 <sup>NS</sup>	43.73±0.74**	70.23±0.29*
Group 4 (60 mg/kg)	6.48±0.32 <sup>NS</sup>	3.78±0.19 <sup>NS</sup>	2.70±0.33 <sup>NS</sup>	42.33±1.35**	77.33±1.44**

NS = Not Significant; \*: Denotes mean values significant at ( $p < 0.05$ ); \*\*: Significant = ( $p < 0.01$ )

Table 5: Average (mean±S.D) values of rats changes in serum electrolytes during treatment (6 weeks) and recovery (4 weeks) with dietary acryl amide

Groups	Ca (mg/dL)	P (mg/dL)	Na (mg/dL)	K (mg/dL)
Treatment				
1 (Control)	10.78±0.12	5.83±0.07	151.38±2.11	7.40±0.24
2 (10 mg/kg)	10.75±0.18 <sup>NS</sup>	7.41±0.42*	152.75±0.75 <sup>NS</sup>	8.13±0.23 <sup>NS</sup>
3 (30 mg/kg)	10.70±0.28 <sup>NS</sup>	6.89±0.68*	150.50±1.29 <sup>NS</sup>	6.90±0.75*
4 (60 mg/kg)	10.67±0.41 <sup>NS</sup>	6.31±0.76 <sup>NS</sup>	142.25±0.85**	7.08±0.13 <sup>NS</sup>
5 (90 mg/kg)	9.48±0.50 <sup>NS</sup>	7.99±0.63*	140.25±0.85**	7.45±0.48 <sup>NS</sup>
Recovery				
1 (Control)	9.37±0.03	7.37±0.28	124.00±0.41	7.25±0.14
2 (10 mg/kg)	9.30±0.02 <sup>NS</sup>	6.82±0.71 <sup>NS</sup>	122.20±0.89 <sup>NS</sup>	7.45±0.10 <sup>NS</sup>
3 (30 mg/kg)	9.11±0.13 <sup>NS</sup>	6.62±0.44 <sup>NS</sup>	123.85±0.83 <sup>NS</sup>	7.02±0.25 <sup>NS</sup>
4 (60 mg/kg)	9.76±0.43 <sup>NS</sup>	5.69±0.55*	124.32±0.52 <sup>NS</sup>	7.20±0.12 <sup>NS</sup>
5 (90 mg/kg)	.....	.....	.....	.....

NS = Not Significant; \*: Denotes mean values significant at ( $p < 0.05$ ); \*\*: Significant = ( $p < 0.01$ )

concentrations in Groups 2, 3 and 4 whereas the values of sodium were lower ( $p < 0.01$ ) in Groups 4 and 5 than the controls. Potassium concentration was significantly lower ( $p < 0.05$ ) only in Group 2 fed on 30 mg/kg dietary acrylamide than the test groups and the controls.

Although the concentration of the above electrolytes turned to normal after the 4 weeks acrylamide withdrawal, only a significant ( $p < 0.05$ ) decrease was observed in phosphorous concentration of Group 4 fed on 60 mg/kg dietary acrylamide (Table 5).

## DISCUSSION

The purpose of this discussion is to take into account the most important features of the lesions and dysfunction of the liver, kidneys and other organs of Wistar rats fed with dietary acrylamide at different doses in order to provide a proper evaluation.

Food is proved to have a significant contribution to the total exposure of the general population to acrylamide; therefore, its presence in foodstuffs may be an important risk factor regarding the general population health and also a concern for authorities Grivas *et al.* (2002), Stadler *et al.* (2002) and Kelly (2003) reported that the levels of acrylamide vary considerably between single foodstuffs within food groups, but potato crisps and French fries generally contained high levels of acrylamide compared to many other food groups. The average content is approximately 1000  $\mu\text{g}/\text{kg}$  in potato crisps and in French fries is approximately 500  $\mu\text{g}/\text{kg}$ . Maillard reaction was concluded to be the main pathway of acrylamide formation on food model system (Friedman, 2003; Pedreschi *et al.*, 2006). Moreover, acrylamide is easily absorbed by the body through all possible entry points: inhalation-cigarette smoking, ingestion-drinking water from flocculent-treated water, eating (sugar containing polyacrylamides used in food refining processes or potato chips, French fries, processed cereals) and through skin absorption (cosmetics) (Shipp *et al.*, 2006).

The present study has shown that acrylamide is enterohepatonephrotoxic to rats at dietary levels of 10, 30, 60 and 90 mg/kg. The neurotoxic effect of acrylamide was observed in rats fed on dietary levels of 10 and 60 mg/kg. Acrylamide is known to inhibit the axonal energy production; change in the rate, quantity and deposition of anterograde transported materials; and disruption of cytoskeletal structure and function (LoPachin and Lehning, 1994). Based on the role of transmembrane ion shifts in cell injury (Trump *et al.*, 1979), it was reported that acrylamide might cause distal axonopathy by disrupting subaxonal distribution of  $\text{Ca}^{+2}$ ,  $\text{K}^{+}$  and  $\text{Na}^{+}$  (Lehning *et al.*, 1998; LoPachin *et al.*, 1992, 1993). LoPachin *et al.* (2000) concluded that classically defined axonopathy is expressed only during subchronic, low-dose exposure paradigms. Higher dosing rates produce either no structural changes or retrograde atrophy. The author determined the time of onset and development of neurotoxicity as

11 days post 50 mg/kg/day intra-peritoneal acrylamide intoxication. Abnormal neurobehavior including hind limb dysfunction and abnormal gait recorded in the present study was also shown by Shukla *et al.* (2002) who found that exposure of rats to acrylamide caused hind limb paralysis in 58% of the animals on day 10 post experiment commencement.

It has been found in the present study, that dietary acrylamide in different doses produce structural and functional changes in body organs e.g., liver, kidneys, spleen and intestines. However, neoplasia was not detected in the test rats fed the mentioned doses. The carcinogenic action of acrylamide has been confirmed by carrying out experimental researches on laboratory animals and by epidemiologic studies. Acrylamide was proved to be a liver cancer initiator where neoplastic structures and histopathological alteration together with atypical cell foci were detected when administered to Azaserine- rat model at 5 and 10 mg/kg/day for 16 weeks in drinking water (Kalipci *et al.*, 2012). Glycidamide, a metabolite of acrylamide formed via the cytochrome P 450 pathway, binds to DNA causing genetic damage and the prolonged exposure was found to induce tumors. But species difference in the formation of this metabolite has been observed with acrylamide converted to glycidamide to a great extent in the mouse than in the rat (Sumner *et al.*, 1997). So, acrylamide was considered to be a genotoxicant and a reproductive and developmental toxicant (Hashimoto and Tanii, 1985; Butterworth *et al.*, 1992; Generoso *et al.*, 1996 and Adler *et al.*, 2000).

In rats fed with different concentrations of acrylamide, damage to the vital organs probably contributed to significant decrease in growth rates of the test groups compared to the controls, alterations in AST, ALT and ALP activities, decreased total protein, albumin, globulin and urea concentrations and elevated cholesterol concentration. In this study the alteration of these parameters are dose independent.

Awad *et al.* (2002) evaluated the activity of ALT and AST enzymes of isolated rat hepatocytes after treatment with acrylamide at 1 mM and found a significant increase in ALT and AST leakage after 60 min. and 2 h respectively. A significant increase in serum AST and ALT activities, which was dose dependent, were recorded by Chinoy and Memon (2001) and Yousef and El-Demerdash (2006) in serum and plasma of mice and rats post acrylamide intoxication. Totani *et al.* (2007) reported that the serum levels of AST and ALT in Wistar rat were not much increased after continuous intake of trace of acrylamide in frying oils for 12 weeks. It seems that the susceptibility of an animal to feeding a toxic substance is dependent at least to the type of the toxicant as well as the rate of metabolic conversion in the liver to metabolites and consequent excretion but also sometimes to the amount added to the diet and route of administration. These marker enzymes are cytoplasmic in origin and their release into the circulation depends on the abnormal dynamic properties of the cellular

membrane following exposure to toxic substances (Lin *et al.*, 2000; Uboh *et al.*, 2005). The decreased activity of these enzymes in some of the test groups could be attributed to decreased endogenous induction or due to increased catabolism.

In the present study there were significant decreases of the total protein, albumin and globulin. The hypoproteinaemia in rats fed on different concentrations of acrylamide might have resulted from hepatocellular dysfunction. This as well as the occurrence of hypercholesterolemia is further evidence of liver damage characterized by the development of cytoplasmic fatty vacuolation and necrosis of the centrilobular hepatocytes with lymphocytic infiltration. Asha *et al.* (2008) reported a steady decrease in hepatic protein level with higher doses of acrylamide and attributed that to retarded protein synthesis; change in protein metabolism or to the leakage of protein reserves from hepatocytes. Acrylamide molecule has two reactive sites, viz, the conjugated double bond and the amide group which can conjugate with the -SH group of a sulfur containing amino acids and  $\alpha$ -NH<sub>2</sub> group of a free amino acid (Friedman, 2003). The above scenario can explain the unavailability of few amino acids for protein synthesis. Furthermore, being an electrophilic compound, acrylamide can bind with proteins and make them undetectable.

In this study acrylamide intoxication induced electrolyte imbalance which has been attributed to the derangement of renal function resulting from interference with ions transport across the renal tubules (Shirley and Unwin, 2005).

### CONCLUSION

Acrylamide is considered neurotoxic at dietary levels of 10 and 60 mg/kg and enterohepatonephrotoxic to Wistar rats at dietary level of 10, 30, 60 and 90 mg/kg and fatal at the treatment dose of 90 mg/kg.

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