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Research Article

ACRYLAMIDE CAUSED HEMATOTOXICITY ON *MUS MUSCULUS* THROUGH GAVAGE

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ABSTRACT
Acrylamide monomer is a known carcinogen and genotoxic compound which is released into the environment during its use as polymer. This compound can be absorbed readily through skin. The objective of this study was to evaluate the hematotoxicity produced by acrylamide given in different dose levels (10 mg/kg, 15 mg/kg and 25 mg/kg/day) through gavage to Swiss albino mice. The various toxicity symptoms were observed which include significant reduction of body weight, hair loss, hindlimb splaying, dragging of back legs and irritation on skin. Hematotoxicity symptoms include significant reduction in levels of hemoglobin, TLC, DLC and total protein and highly significant increase in SGOT SGPT activity as compared to control group. Prolonged oral exposure of acrylamide also induces skin tumors. This elementary study concludes that this compound is disturbing the equilibrium of blood composition as well as affecting some of the vital organs like liver and spleen.

Key Words*: Acrylamide, Gavage, Hemoglobin, TLC, DLC, SGOT, SGPT*

INTRODUCTION
Acrylamide (CH_2=CH-$CONH_2$) is a highly reactive and water soluble compound, which is commonly used in industries and laboratories (Nordian *et al.*, 2003). Polymers of acrylamide have wide range of applications e.g. waste water treatment, paper and pulp processing, mineral processing, cosmetics and other industrial works. Acrylamide is also used in scientific laboratory for the separation of macro molecules. All acrylamide in the environment is man made (Friedman, 2003). Although polymer is non toxic but monomer is toxic, as it is readily is absorbed by skin, ingestion and inhalation (Dearfield *et al.*, 1995). Occupational exposure of acrylamide has been reported in many studies (EIMS, 2002). Recently it has also been reported in fried and baked starchy foods (Motteram *et al.*, 2002; Konings *et al.*, 2003; Nan *et al.*, 2010).
Acrylamide when absorbed binds to hemoglobin and then it is distributed to different organs by body fluid (Hashimoto and Aldridge, 1970; IPCS, 1985). Now a day's acrylamide exposure is a renewed growing concern because of its toxicity in human beings and rodents (Bolt, 2003; Klaunig, 2008). Acrylamide is a mutagen and rodent carcinogen. Its genotoxicity has also been reported, which is comprised of chromosomal aberrations, sister chromatid exchanges, unscheduled DNA synthesis (Paulsson *et al.*, 2003; Ghanayem *et al.*, 2005). It is also a neurotoxic substance for central and peripheral nervous system (Gold *et al.*, 2004). Its reproductive toxicity has also been reported by Chaplin *et al.,* (1995).The present study was performed to evaluate the hematotoxic effects of acrylamide.

MATERIALS AND METHODS
Drug Used**:** Acrylamide (99% purity) was obtained from Central Drug House (P).Ltd. Delhi.
Animals**:** Healthy adult Swiss albino mice (*Mus musculus*) aged 45-50 days and weighing 25-32 gm was chosen for the present experimental work. The animals were housed in polypropylene cages and provided standard mice feed and water *ad-libitum* with natural light dark cycle (12:12 hrs) throughout the experiment. The experimental animals were divided into two groups. Control and experimental groups having 6 animals per group:

1. Control Group: Double distilled water equivalent to the volume of drug provided to experimental animals were given by gavage per day.
2. Experimental Group: Animals were divided into three groups depending on the acrylamide concentration.
   Group A – 10 mg/kg/day
   Group B – 15 mg/kg/day
   Group C - 25 mg/kg/day

Drug was given with the gap of 24 hours for 90 days. The experiment was terminated on 91st day and the animals were sacrificed via cervical dislocation. The blood was drawn immediately from the heart with the help of sterile syringe. The blood was collected in different vials coated with anticoagulant and without anticoagulant according to the selected parameters. The parameters include body weight, mortality, hemoglobin concentration, serum bilirubin level, TLC, DLC, Total protein, SGOT and SGPT activities. Morphological and behavioral changes were also recorded during the experimental period.
The hemoglobin (Crosby *et al*., 1954), TLC and DLC (Kolmer *et al*., 1969), serum bilirubin (Jendrassik and Grof's method 1938), serum protein (Lowry *et al.,* 1951) and SGOT and SGPT (Reitman and Frankel 1957) were determined by using standard methods.
***Statistical Analysis*:** Student't' test was performed to evaluate the significance level of observed results

RESULTS
The animals of experimental group and control group were regularly observed for any morphological and behavioural abnormalities. No abnormality was observed in control group animals whereas, some prominent symptoms were observed in all experimental group animals.
Redness on mouth and ear pinna with irritation was observed in animals of group A after 60 days of experiment. The intensity of symptoms increased with progressed days. Hair loss, swelling on fore limbs, hind limb splaying and dragging of backlegs with the abnormalities reported in group A were the symptoms observed in all the animals of group B and C but the onset of symptoms was on 42nd day of the experiment. The intensity of abnormalities increased with experimental days.
No mortality was observed in control as well as experimental group animals. The animals of experimental group were found weak due to highly significant reduction in body weight in group B and C whereas significant reduction was observed in animals of group A. This indicates that animals were greatly affected with high dose levels of acrylamide (Table 1).

Table 1: Changes in body weight (gm) after acrylamide exposure through gavage

Dose levels	Initial	Final	Difference
Control	29.47 ± 0.94	38.0 ± 1.40	+8.53 ± 0.46
Group A (10 mg / kg)	29.25 ± 0.85	28.25 ± 1.1*	–1.0 ± 0.25*
Group B (15 mg/kg)	29.75 ± 0.85	25.25 ± 1.1***	–4.50 ± 0.25***
Group C (25 mg/kg)	30.0 ± 1.29	22.75 ± 1.85***	–7.25 ± 0.44***

Values are depicted as Mean ± SEM.
*NS – Non significant; * - Significant; ** - Medium Significant; *** - Highly Significant*

Hematological evaluation showed highly significant decrease in hemoglobin content in group B and C whereas no significant reduction was observed in Group A animals. All the experimental animals showed

highly significant reduction in total leucocytes count. A significant reduction was observed in neutrophyl, lymphocytes and monocytes counts. Serum analysis in present study indicates a reduction in total protein which was significant in all experimental groups, whereas significant increase in serum bilirubin content was observed in experimental animals. The activity of SGOT and SGPT increased significantly and highly significantly in all the experimental groups (Table 2).

Table 2: Hematological changes after acrylamide exposure through gavage

Parameters	Control	Experimental Group		
		Group A	Group B	Group C
Hb (%)	13.25±0.10	12.6±0.16 ^{NS}	7.7±0.21 ***	6.78±0.19 ***
TLC (cells/mm^3)	8706±31.17	5262.5±26.02 ***	4775±32.27 ***	4581±34.42 ***
DLC Neutrophils (%)	52.25±0.62	42.0±1.6 **	37.5±1.7 **	30.25±2.0 ***
Lymphocytes (%)	64.0±0.91	57.75±1.30 **	42.25±2.25 ***	35.0±1.50 ***
Monocytes (%)	1.07±0.08	0.825±0.08 ^{NS}	0.575±0.06 **	0.525±0.08 **
Serum Bilirubin Indirect (%)	0.29±0.00	0.34±0.01 **	0.42±0.01 **	0.53±0.01 ***
Direct (%)	0.41±0.12	0.52±0.01 ***	0.64±0.01 ***	0.72±0.01 ***
Total (%)	0.49±0.01	0.56±0.01 ***	0.65±0.01 ***	0.73±0.01 ***
Protein (gm/dl)	7.7±0.09	7.25±0.10 **	6.4±0.19 **	5.5±0.21 **
SGOT (IU/L)	78.0±0.91	90.5±2.10 **	95.75±2.17 ***	135.0±2.48 ***
SGPT (IU/L)	72.25±0.85	105.75±1.05 **	111.25±1.41 ***	195.50±2.38 ***

Values are depicted as Mean ± SEM.
*NS – Non significant; * - Significant; ** - Medium Significant; *** - Highly Significant*

DISCUSSION

The results of present study indicate that acrylamide is a toxic compound as observed by many other workers (Sumner, 2003; Arora and Gupta, 2011). The morphological symptoms like redness of mouth, ear pinna, hair loss and swelling in fore limb might have occurred due to the excessive irritation and rubbing done by experimental animals. Limb weakness and irritation have also been reported in experimental animals when acrylamide was administered via different routes (Chaplin *et al.,* 1995). Dragging of back legs, behind limb splaying symptoms have also been reported while evaluating the neurotoxic effects of acrylamide (Crofton *et al.,* 1996).

The decrease in hemoglobin content might be either due to decrease in hemoglobin synthesis or due to an increase in hemoglobin destruction. Decrease in hemoglobin content indicates that animals were suffering from anemia. Low hemoglobin concentration reduces the oxygen carrying capacity of blood, which may result in capacity of blood, which may result in clinical sign such as weakness in limbs and body.

Barber *et al.,* (2001) reported that acrylamide and its reactive metabolite glycidamide are electrophilic in nature and form adducts with sulphydril group of hemoglobin resulting degradation of haem part of hemoglobin, this might have been the cause of reduction in hemoglobin content. Reduction in total leucocyle count and differential leucocyte count indicates that acrylamide is also a cytotoxic compound and might have some effect on hematopoiotic system.

The decreased protein levels with higher doses of acrylamide can be attributed to retarded protein synthesis, or to some change in protein metabolism. Abramsson-Zetterberg *et al.,* (2003) have shown that acrylamide and its metabolite glycidamide have an affinity to bind with DNA and can cause chromosomal aberration. Thus, any abnormality in DNA structure can affect transcription and ultimately protein synthesis. Acrylamide can bind with protein that also can make them undetectable (Friedman, 2003).

Increased levels of SGOT, SGPT and serum bilirubin indicate that the experimental animals were suffering from liver malfunctioning. Overall results of this study suggest bone marrow depression in experimental animals.

Conclusions

In the present study significant reduction in hematological parameters were observed. The study concludes that acrylamide is a potent hematotoxin and causes disturbance in different body functioning. Further detailed investigations regarding its effects on hematopoiotic system is required.

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