Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2011 Vol. 1 (1) Jan – Mar, pp. 16-20/Arora and Gupta

Anatomical and Morphological Abnormalities Produced By Dermal Application of Acrylamide in Male and Female Swiss Albino Mice

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ABSTRACT

Acrylamide monomer (Acrl) is a well known synthetic compound used in industries, research laboratories. It has also been noticed as a by-product in starchy food processed at high temperatures. It is a potent carcinogen and has also been reported as neurotoxic, mutagenic substance in many studies. The main objective of present study was to compare morphological and anatomical effects produced by Acrl in both the sexes of Swiss albino mice. Adult, virgin male and female mice were treated with 1% solution of Acrl via dermal application at dose levels 25, 50 and 75 mg/kg/day for a period of 91 days (6 days / week). Males were found more sensitive than females. Erectness in tail, hind limb weakness, increased hair loss and swelling in external genitalia were observed comparatively more in males than in females. The difference between the skin colors was found at higher dose levels, blueness in males and redness in females were observed. However, muscular weakness, patchy hair growth and hind limb splaying were observed in both the sexes. All the symptoms observed were time and dose dependent. Tumors were also observed in the sexes but the number, size and location was different in two sexes.

Key Words: Acrylamide monomer, Neurotoxicity, Mutagenicity, Swiss albino mice

INTRODUCTION

Acrylamide monomer is a white crystalline solid that is highly reactive and water-soluble. It is commonly used in manufacturing poly-acrylamide, which is commonly used in industries, laboratories and cosmetics (Bergmark *et al.*, 1991; Bikales, 1973; Bhumenthal *et al.*, 1995). Acrylamide polymers do not degrade to monomer and have low toxicity in comparison to monomer (Bikales, 1973). Individual can be exposed to acrylamide monomer either in their work places or in environment via different routes such as, inhalation, skin absorption, drinking water and ingestion of food products treated at high temperature (Dearfield *et al.*, 1995, EIMS 2002). Acrylamide absorption through skin is believed to account for the most severe case of occupational poisoning (Auld *et al.*, 1967; Davenport *et al.*, 1976). Acrylamide monomer causes peripheral neurotoxicity, mutagenicity and carcinogenicity in experimental animals (Dearfield *et al.*, 1988; Fridman *et al*., 1995). Studies related to toxicity of acrylamide in human are not sufficient to conclude any result therefore IARC 1994 has categorized it as probable human carcinogen.

Despite of numerous studies available on the toxicity of acrylamide on animals, the comparative studies of both the sexes in any species are insufficient. Therefore, the comparative toxicity evaluation of male and female Swiss albino mice was performed in our laboratory to evaluate the comparative morphological changes when same doses were applied on the clipped skin.

MATERIALS AND METHODS

Experimental Animal

Healthy, adult Swiss Albino Mice (males and females) of 30 ± 5 grams were chosen for present experimental work. Animals were kept separately in clean polypropylene cages 25 × 21 × 18 cms and covered with chrome plated grills. They were fed with standard mice feed from Hindustan Lever Ltd. Delhi and water *ad libitum.*

Test Compound

Acrylamide monomer (99% purity) was obtained from Central Drug House (p) Ltd. Bombay-Delhi. 1% solution of acrylamide, (prepared in double distilled water) was used as the test compound.

Experimental Protocol

Males and females were kept separately and were divided into experimental and control group. The experimental group divided into 3 groups. Each group consisted of ten males and ten females. Animals of experimental groups were treated via dermal application on the clipped skin applied. The prepared solution of acrylamide was applied 6 days per week for the period of 91 days. The groups formed for the work were as follows:-

Control Group: Double distilled water equivalent to the highest dose level group was applied on the clipped skin.

Experimental Group: Three groups were made with different dose levels-

Experimental Group I	: 25 mg/Kg body weight/day
Experimental Group II	: 50 mg/Kg body weight/day
Experimental Group III	: 75 mg/Kg body weight/day

Parameters Selected

Morphological observations: Changes in fur, skin, eyes, external genitalia and animal behaviour were observed throughout the experimental period. Animal weight, mortality, appearance of tumors and other abnormal characters like bleeding, salivation, tremors, food intake, body postures and irritation were also recorded

Anatomical observations: Anatomical abnormalities were observed in all the experimental animals at the time of autopsy.

RESULTS

Body Weight

In group I the reduction in body weight gain was significant in females where as in males it was highly significant (Table.1). Highly significant reduction in body weight gain was also observed in both males and females of group II and group III, but weight gain was higher in females than in males of both the groups.

Mortality Rate

There was no mortality in animals of experimental group I and II. The animals of group III showed mortality. Males of this group showed higher mortality (22.22%) than females (11.11%).

Morphological Abnormalities

The toxicity symptoms which appeared in group I include slight reduction in the activity of animal, with the colour difference in the chemical applied area – blueness in the males and redness in the females**.** Tail erectness, irritation on skin, weakness in hind limb with splaying, patchy hair growth, redness in external genitalia with sluggish appearance were the common toxicity symptoms which appeared in both males and females of group II (Fig. 1). The abdominal skin of males of this group was also found blue with some scars; where as the abdominal skin of females were red. Tumors and bleeding of tumors were also found only in the chemical applied area of males whereas swelling in external genitalia and limbs were observed only in females of group II. All the animals of group III were sluggish in appearance and showed increased rate in the symptoms appeared in earlier groups. Black scars near external genital regions and tumors on skin (including the area other than the application area, Fig. 1) were observed only in the males, whereas, only one tumor was found in the acrylamide applied area of one female of this group. In all the experimental groups the onset of symptoms were found earlier in males than in females.

Anatomical Abnormalities

In males it was observed that the liver stomach and spleen became abnormally enlarged. Reproductive tract and kidney became degenerated in almost all experimental males. In experimental females fluid filled uterine horns and ovaries were observed in some animals with darkened liver kidney and spleen. Splenomegaly and enlarged liver was also observed in animals (Fig.1).

DISCUSSION

The onset of toxicity is a series of morphological and behavioral changes in an individual. But there is little information available in literature regarding the difference in response of male and female animals to the acrylamide exposure. Therefore, the study was carried out to evaluate the comparative morphological toxicity symptoms, which appeared as a result of long term (91 days) dermal exposure to Swiss Albino Mice. Our results show various symptoms of acrylamide toxicity in both the sexes. Highly significant reductions in the body weight gain have been observed in most of the animals of all the experimental groups. Similar results have also been reported by Yang *et al.,* 2005. Various clinical symptoms of neurotoxicity have been observed in males and females exposed to different dose levels of acrylamide such as, muscular weakness, hindlimb splaying, crossing of hind limbs when held up by tail, and tremors. Many scientists while working on acrylamide toxicity in different animal species (e.g. cats, dogs, monkeys) have also reported similar symptoms (Fullerton and Barnes, 1966; IPCS, 1985; European-Union 2002; JIFSAN/NCFST, 2002). Although the toxicity symptom in both males and females were similar but males were observed more severely affected than females at the same dose level. These result correlated with the study which indicates that incidence of neoplasia and degrees of tibial nerve degeneration were significant in males as compared to females (US EPA, 1982b). Blueness appeared in the experimental males might have appeared due to the poisoning effects produced by acrylamide as also reported by Auld and Bedwell in 1967.

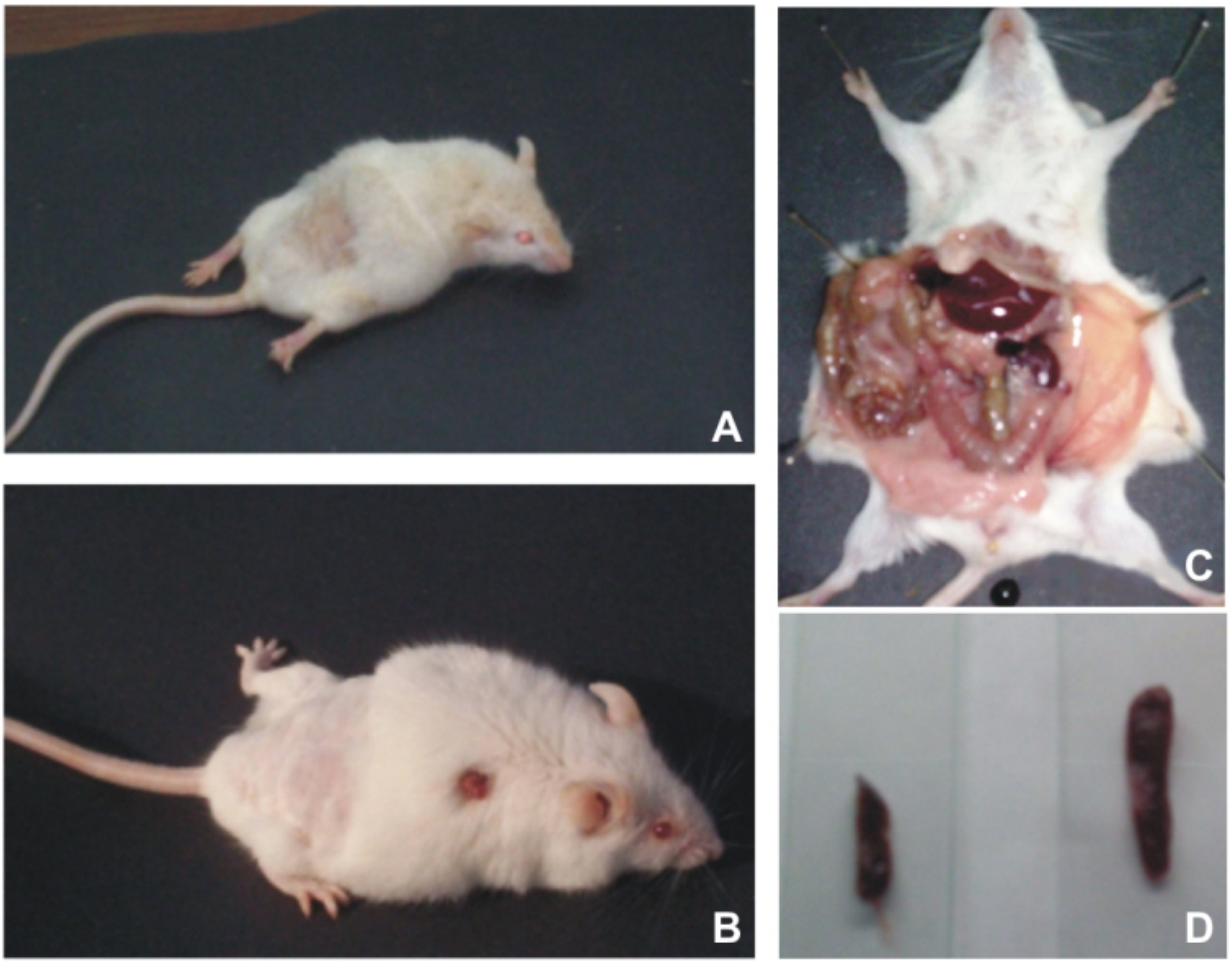

Fig. 1: Effect of acrylamide on mouse. A. Hind limb splaying in animal (female). B. Skin tumor in animal (male). C. Photograph of animal of group A2 showing fluid filled reproductive tract with enlarged liver and spleen and darkened kidney. D. Spleanomegaly smaller spleen of control animal, larger spleen of experimental animals.

Table 1: Body weight of the animals.

Experimental group	Sex of animal	Initial body weight (grams)	Final body weight (grams)	Difference in body weight
CONTROL	**Males**	30.5 ± 0.718	42.5 ± 0.477	+11.9 ± 0.766
	Females	30.0 ± 0.666	41.6 ± 0.933	+11.6± 0.561
GROUP I	**Males**	30.0 ± 0.683	39.0 ±0.333	+9.2 ± 0.442**
	Females	30.3 ± 0.978	40.1 ± 0.525	+9.8 ± 0.769*
GROUP II	**Males**	30.6 ± 0.718	34.6 ± 0.426	+ 4± 0.577**
	Females	30.2 ± 0.757	37.2 ± 0.679	+6.9 ± 0.546**
GROUP III	**Males**	30.1 ± 0.888	31.7 ± 0.856	+2 ± 0.557**
	Females	30.0 ± 0.745	33.1 ± 0.674	+ 3.1 ± 0.433**

Mean ± S.E. Student't' test was performed for calculation. Number of animals per sex = 10
P value: P<0.02 Significant * P<0.001 Highly significant**

Table 2: Morphological effects produced in mice when acrylamide was administered on clipped skin.

Experimental groups	Common toxicity symptoms	Symptoms seen only in males	Symptoms seen only in females	Onset days	
				M	F
GROUP-I	-Slight reduction in the activity of animal	-Area of application turned blue	-Area of application turned red	78-85	80-85
GROUP- II	-Erectness in tail -Irritation on skin -Hind limb weakness -Patchy hair growth -Redness in external genitalia -Sluggish in appearance	-Blueness in skin -Black scares seen on skin of few animals -Tumor on drug applied area observed in two males -Bleeding in tumors	-Increased redness on abdomen and external genitalia. -Swelling in limbs and external genitalia	69-75	73-80
GROUP-III	-Increased tail erectness -Swelling in external genitalia -Hair loss -Increased redness with swelling on mouth, palms and soles -Increased irritation on mouth and abdominal skin -Hind limb splaying -Dragging in of back legs -Sluggish in appearance	-Hardness and blueness in skin -Dark blueness in abdomen -Black scars near external genitalia -Skin tumors observed in few animals	-Dark redness in abdomen -Tumor was observed only in 1 animal on clipped area -Tumor only in application area observed in 1 female.	45-55	54-66

Any abnormality in the internal organ is an indication of illness and abnormal physiological functioning of body. Male or female experimental animals showed similar observations like splenomegaly enlarged liver darkness in internal organs and abnormal reproductive tract. These symptoms suggests abnormalities in hematology of animals as such symptoms have also been reported in similar studies (Hashimoto and Aldrige, 1970; Edwards *et al*., 1978; Burek *et al.*, 1980)

The toxicity differences in the two sexes are dependent on the xenobiotic metabolism. Generally male rats have a higher capacity to metabolize xenobiotics than the females. Mice generally do not show much gender difference but slight gender difference is found which is dependent on the strain of mice. As the mice are commonly used in toxicological studies it is important to focus on the gender difference and to extrapolate mice data to humans. In the present investigation gender dependent toxicity was found as the female mice have the higher capacity to metabolize the xenobiotics. Therefore our results show that the toxicity symptoms which appeared in males at lower dose were similar to $_{that}$ found in females at higher dose level.

ACKNOWLEDGEMENT

Authors express sincere thanks to the Head, Department of Zoology, and University of Rajasthan for providing the facilities to carryout the present work.

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