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Research Article

EFFECT OF ACRYLAMIDE ON TESTIS OF ALBINO MICE

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ABSTRACT
Acrylamide is a common chemical which is used in both industrial and laboratory processes.It is formed in heated starchy foods specially potato products.Aim of the present work was to explore the harmful effects of acrylamide on the histological changes in the testis of albino mice, in an attempt to clarify its potential risks on human health. Qualitative changes were studied in testis. Exposure to acrylamide produced degenerating changes in the testis which were more prominent with a longer period of exposure. Degeneration of germ cells, numerous multinucleated giant cells with sloughed seminiferous epithelium and vacuolation in between the germ cells was observed.

Keywords*: Acrylamide, Testis, Albino Mice, Toxicity*

INTRODUCTION
Acrylamide (ACR) is an important industrial chemical that is neurotoxic, mutagenic to somatic and germ cells, and carcinogenic in chronic rodent bioassays. Recent findings of ACR in many common starchy foods have sparked renewed interest in determining toxic mechanisms and in understanding the cancer, neurotoxicity, and reproductive risks from typical human exposures (Doerge *et al.*, 2005)
Acrylamide is a white crystalline odourless compound soluble in alchohol and water, but insoluble in heptane and benzene. It is formed of Acrylic-amide with its formula C_3H_5NO.Acrylamide exists in two forms, highly toxic monomer and non toxic polymer. Solid form is stable at room temperature but may polymerize aggressively when melted or exposed to oxidizing agents (Schuur, 2008). Average daily adult intake of Acrylamide in most populations was estimated to be approximately 0.5 microg/kg body weight (BW) (Rice, 2005). However, intake may vary widely from 0.3- 2 microg/kg body weight/day or may reach even 5 microg/kg/day. Certain carbohydrate rich foods, particularly Asparagines when reacting with sugar in high temperature more than 200 degree centigrade during cooking, it is where Acrlyamide is formed and the reaction is named – Millard Reaction (Yang *et al.*, 2005). The early findings tended to focus on starch rich foods such as fried potatoes, French fries, and crisp bread, all of which showed relatively high levels of Acrylamide. Besides potatoes, coffee and crisp bread were considered as relevant sources of human exposure, since they are consumed on a regular basis by a broad group of consumers (Stadler & Scholz, 2004).
Based on positive bioassay results in mice and rats, supported by evidence that Acrylamide is biotransformed in mammalian tissues to Genotoxic metabolite. The biotransformation process by which Acrylamide is converted to glycidiamide is possible in humans, and can be demonstrated to occur efficiently in both human and rodent tissues. Effect of Acrylamide on reproductive system of mice have included decreased sperm count, increased abnormal sperm morphology, severe testicular damages, such as vacuolation and swelling of the round spermatid, and break of DNA during specific germ cell stages (Weiss, 2002; Richmond & Borrow, 2003). In addition male mice administered with acrylamide exhibited significant reduction in mating, fertility as well as transport of sperms in uterus.

MATERIALS AND METHODS
Thirty male albino Mice were used in this study and were classified into 3 groups. First group was treated as a control group; second group received the dose of ACR 10 milligram per kg body weight orally for 10 days. The third group received the dose of ACR of 20 milligram per kg body weight orally for 10days. The first group was administered distilled water and kept as control.

They were acclimatized to laboratory conditions for 15 days prior to the commencement of the treatment. Mice were kept in open air cages at room temperature and were fed standard rodent palate diet (Hindustan Lever ltd) and water was allowed ad.libitun.

Thereafter they were sacrificed by cervical dislocation. Testis was taken out for histological studies. Animals of experimental and control group were sacrificed on tenth day of treatment by cervical dislocation. The testis of experimental and control group of mice were fixed in formalin for 4 hrs. They were dehydrated, in graded EtOH series, cleared in xylene, infilterated with and embedded in pure filtered paraffin wax (M.P.58 degree centigrade). Deparaffinised sections (5-7 microns) were stained by haematoxylin and eosin to monitor the extent of changes in the testis histoarchitechture. Every alternate section of the testis was microscopically examined and appropriate areas were microphotographed and enlarged. Testis of the mice receiving ACR showed changes in the seminiferous tubules.

RESULTS AND DISCUSSION

Histological observations*:*

The testis of the control group showed sections of the seminiferous tubules containing numerous spermatozoa. These tubules were separated by intervening connective tissue containing leydig cells. Close examination of the wall of the seminiferous tubules showed that it consisted of germinal epithelium supported by sertoli cells. The germinal epithelium comprised different stages of the spermatogenic series namely spermatogonia, spermatocides, spermatids and spermatozoa.

The testis of the mice receiving 10mg/Kg body weight for 10 days showed some seminiferous tubules with normal appearance of the spermatogenic series and characteristic whorly appearance of the sperms inside the lumen. Some of the seminiferous tubules had an irregular outline. Other seminiferous tubules showed depletion of germ cells and congested blood vessels.

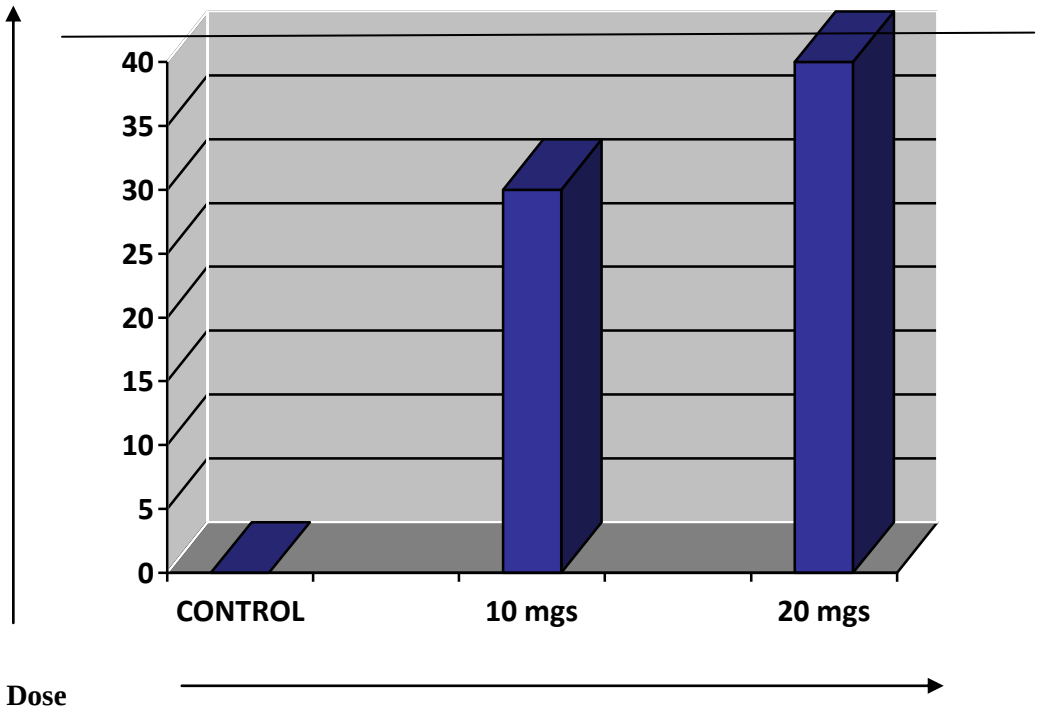

Fig.-1 Histogram of spermatocyte disintegration perccentage v/s dosage in mice

Histopathological changes of ACR treated male albino mice with a dose of 20mg/kg body weight for 10 days showed depletion of germ cells. Some of the seminiferous tubules showed irregular outline. Seminiferous tubules suffered from marked depletion of spermatogenic cells, many of which were seen sloughed in the lumen. Moreover, the striking histological change was the appearance of numerous multinucleated giant cells, these cells were large rounded cells with abundant cytoplasm and multiple peripherally arranged nuclei. They were located free in the lumen of the seminiferous tubules. Some seminiferous tubules revealed formation of vacuoles of different sizes between spermatogenic cells that were resting over a wavy limiting membrane.

Table 1: The Contamination Data of Acrylamide in Various Food Products

	Contamination data (µg/kg food stuff)	
	Number of samples	Median (Min–Max)
Baby's biscuits	5	324 (225–1217)
Bread	6	30 (27–36)
Small bread type	4	38 (29–51)
Crisps	29	676 (38–1612)
Chocolate	3	108 (104–109)
Choco-spread	2	88.5 (65–112)
French fries	33	254 (56–729)
Biscuits	6	143 (20–1514)
Coffee	11	114 (11–1291)
Gingerbread	5	1403 (108–1697)
Breakfast cereals	20	135 (37–623)
Popcorn	5	160 (129–216)
Sweet spiced biscuit	5	204 (160–677)

Table 1: Variation in simulated daily acrylamide exposure in the adolescent population via French fries, Bread and in total

Percentile	Via French fries (µgAA/kgbw/day)[a]	Via crisps (µgAA/kgbw/day)[a]	Via bread (µgAA/kgbw/day)[a]	Via biscuit (µgAA/kgbw/day)[a]	Total exposure (µgAA/kgbw/day)[a]
1	0	0	0.002	0	0.108
5	0	0	0.013	0	0.185
20	0	0	0.034	0.004	0.327
50	0.155	0	0.057	0.055	0.513
55	0.171	0	0.063	0.064	0.545
75	0.276	0.087	0.081	0.111	0.702
95	0.521	0.298	0.127	0.260	1.089
99	0.725	0.596	0.159	0.459	1.411

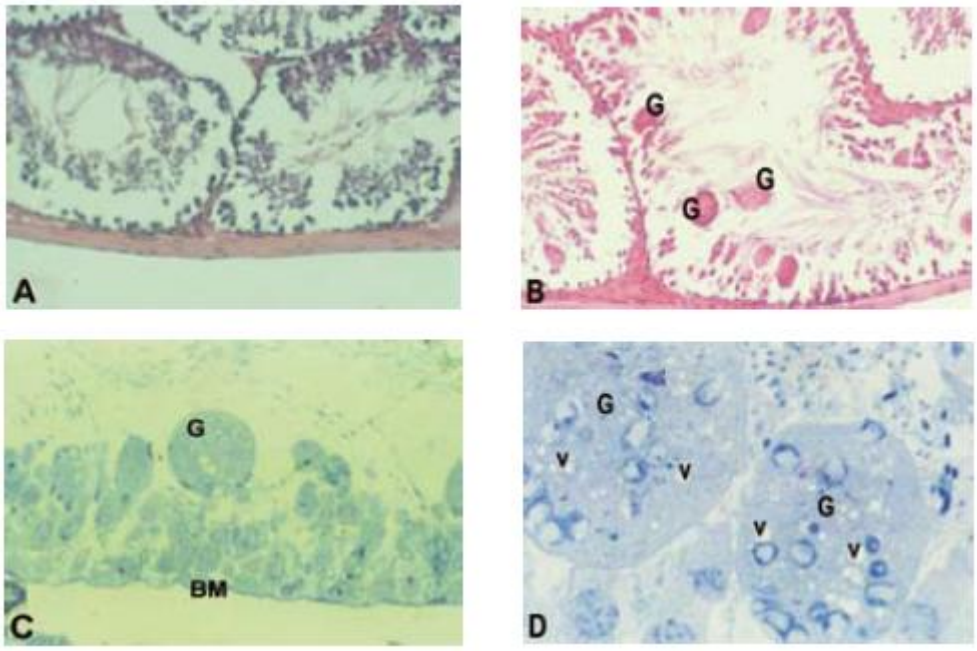

Figure 2: Photomicrograph of mice testis. A group 10mg/Kg /10days showing depletion of germ cells in seminiferous tubules. B group 20mg/kg/10 days showing depletion of spermatogenic cells numerous multinucleated giant cells (G) between the sloughed spermatogenic cells. C 20mg/kg /10 days showing depletion and separation of spermatogenic cells, multinucleated giant cells (G). D showing multinucleated giant cells (G) with large cytoplasmic masses and many vacuoles (V).

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