



For spirit of my father,
my mother, and my
dear brother and
sisters
a token of
gratitude
and love

Pathological effect of acrylamide on rabbits

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للحصول على درجة
ماجستير العلوم الطبية البيطرية

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الباثولوجيا

مقدمة إلى
قسم الباثولوجيا والطفيليات
كلية الطب البيطري
جامعة الإسكندرية
٢٠٠٨

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Introduction

Acrylamide is a water soluble, vinyl monomer used mainly in chemical industries and in the molecular laboratories for the production of polyAcrylamide gel electrophoresis beside the use of polyAcrylamide in water purification ,cosmetics and as a soil stabilizer (**Gold and Schaumberg, 2000**). Recently, acrylamide has been found as a contaminant in certain potato and grain based food which were cooked at high temperature (**Tareke *et al.* 2000 and 2002**).

Acrylamide is used as a binding, thickening or flocculating agent in grout, cement, sewage, waste, water treatment, pesticide formulations, cosmetics, sugar manufacturing and soil erosion prevention. The polymers of the compound are used in food packaging and in plastic products (**WHO, 1985; IARC, 1994 and EU, 2002**).

The main route of exposure to acrylamide can occur in workplaces (occupational exposure) or in the environment through air, water, land and ground water during its production or use (**EIMS, 2002**), exposure also occurs from cigarette smoke (**Exon, 2006**).

Occupational or environmental exposure to acrylamide resulted in neurotoxicity (**Gold and Schaumberg, 2000**). The conversion of acrylamide to glycamide considered as an important step in the mechanism of neurotoxicity (**Abou-Donia *et al.*, 1993**), acrylamide also, exerts a destructive and significant carcinogenic effect on the reproductive and nervous system of the laboratory animals (**Friedman *et al.*, 1995; Tyl *et al.*, 2000 and Lopachin *et al.*, 2003**). Moreover, acrylamide toxicity extended to produce hepatotoxicity and genotoxicity (**Mukul *et al.*, 1982; Cihak and Vontorkova, 1998**).

In Egypt, polyacrylamide used in agriculture for giving the soil a hydrophilic nature in order to increase water retention (**El-Hady and Wahba, 2003**). In addition, polyacrylamides are used as flocculants in treatment of waste water and removal of heavy metals (**EL-Hamshary *et al.*, 2003**).

the aim of the present study is to evaluate the histopathologic adverse effects of toxicity of acrylamide on the different organs with special reference to semen quality throughout the whole length of the spermatogenic cycle in rabbits .

Introduction

Material and Methods

Material and Methods

Material:

Animals:

The present study was carried out on 20 mature male New Zealand rabbits (7 months old, weighed about 1700 g) purchased from farms of the ministry of agriculture. They were given water *adlibitum*. All rabbits were housed in metal cages and received diet (balanced ration) for two weeks before the start of the experiment for acclimatization and to ensure the normal growth and behavior of the animals until the end of experiment.

Acrylamide:

-Structural formula: Acrylamide ($\text{CH}_2\text{CHCONH}_2$)
It was obtained from Sigma Co. (lot.No.494537)
in a package 1000 gm as white crystalline granules.

Methods:

Experimental design:

Animals were classified into 2 groups. Group I consisted of sixteen rabbits which were daily intubated orally with acrylamide soluble in water, given stomach tube until the end of the experiment. Group II consisted of 4 animals and they were kept as controls. The daily dose was 63 mg acrylamide/Kg body weight according to *McCollister et al., (1964)*. up to 8 weeks –the whole length of spermatogenic cycle in rabbit (*Steger., 2005*). 4 rabbits were sacrificed each two weeks, from intoxicated group. However, 2 rabbits were sacrificed on the 2nd week and at 8th weeks.

Semen collection and evaluation:

Semen was collected using a teaser and an artificial vagina. The following parameters were evaluated immediately after collection:

- 1-Sperm individual motility using a light microscope at 100X magnification after dilution of the semen with 2.9 % sodium citrate dihydrate solution.
- 2-Assessment of abnormal sperms using Eosine and Nigrosin (E&N) stain.
- 3-Sperm concentration using the improved Neubauer hemocytometer slide after staining with eosin (*Smith and Mayer, 1955*).

Material and Methods

4-Acrosome integrity according to the method of (*Woston and Martin (1972)*).

Statistical analysis:

Data of semen analysis were statistically analyzed for the effects of intoxication using the GLM procedure of the SAS computer program (*SAS, 1987*).

Pathological examination:

The necropsy protocol was designed to study the alteration associated with acrylamide toxicity. The necropsy protocol began with observing of the external appearance of the carcass. The carcass was then dissected, all gross lesions were recorded. At the time of necropsy, selected tissues were collected for ancillary laboratory tests. specimens of liver, kidney, spleen, cerebrum, , spinal cord, heart, tests , sciatic never and lungs were collected from each carcass and fixed in 10% buffered neutral formalin. Then, the samples were dehydrated in ascending grades of alcohol and embedded in paraffin wax, mounted, sectioned at 5µm and stained with hematoxylin and eosin (H&E) and then examined by the light microscope. special stain (Prussian blue stain) which used for detection of iron were processed after examination of slides stained with (H&E)

(Bancroft and stevens, 1996).

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