Effect of acrylamide in presence of vitamin E on sperm parameters and testosterone hormone in mice

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Abstract: Acrylamide (AA) is an important industrial chemical primarily used in the production of polyacrylamide and as a chemical intermediate in the synthesis of a variety of other chemicals. The discovery of acrylamide in a variety of human foods including heet processed starchy foods such as potato chips and bread has been reported. Acrylamide is an animal carcinogen, neurotoxin, and reproductive toxin. AA is able to induce sperm damage in male mice. The present study is carried out to investigate the protective effect of vitamin E (Vit.E) against sperm damage induced by acrylamide(viability, count , motility and morphology).32 adult male mice were divided equally into for groups each conaining 8 mice . mice of group 1 served as control fed on basal diet ,group 2 received basal diet and acrylamide (10 mg/kg, water solution) ,group 3 received basal diet and vitamin E (100 mg/kg, intraperitoneal) and group 4 received basal diet, acrylamide and vitamin E for 35 days. Blood was taken for the determination of serum testosterone. Finally, right tail of epididymis was cut in Ham's F10. Released sperm were used to analyze number, motility, viability (eosin-Y stsining) and morphology (Papanicolaou stain) of the sperm. In acrylamide mice, a significant decrease was found in sperm viability, normal morphology and sperm motility compared to control and acrylamid + vitamin E groups. A significant increase was also found in sperm viability in vitamin E group compared to both acrylamide and control groups. Vit.E not only is able to compensate the toxic effects of acrylamid on sperm viability, normal morphology and sperm motility, but also increases sperm count in mice.

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Introduction:

Infertility is one of the major health problems in life and approximately about 30% of this problem is due to male factor (Isidori AM 2006).

Acrylamide (AA; CH2 CH-CO-NH2) is an important industrial chemical primarily used in the production of polymers and copolymers. Polyacrylamide polymers and copolymers are used as binders in the paper and textile industries, as flocculants in the treatment of sewage and wastewater, as soil conditioners, in ore processing, and in cosmetics (Friedman 2003). AA is found in carbohydrate-rich foods that are prepared at high temperatures, such as French fries and potato chips and consumed by humans. Consumption of these foods may result in significant human exposure to AA (Tareke 2002). AA is neurotoxic (LoPachin 2003), clastogenic (Ghanayem 2005c), and mutagenic in somatic and germ cells (Ghanayem 2005a, b) in experimental animals and was classified as a probable human carcinogen (IARC, 1994). AA is well absorbed regardless of the route of administration, and it is metabolized by either conjugation with glutathione and/or oxidation to glycidamide (GA) (Sumner 1992). Oxidation of AA to GA is mediated primarily by cytochrome P450 2E1 (CYP2E1) (Sumner 1999; Ghanayem 2005c). Both AA and GA are capable of binding with hemoglobin and DNA to form adducts (Friedman, 2003; Gamboa da Costa 2003; Ghanayem 2005c). Recent studies in this laboratory confirmed that AA metabolism to GA is a prerequisite for AA-induced somatic and germ cell mutagenicity (Ghanayem 2005a, b). Furthermore, formation of GA-DNA adducts may be involved in AA-induced toxicity, mutagenicity, and carcinogenicity (Ghanaian 2005c; Manjanatha 2006).

The World Health Organisation estimated total daily intakes of AA from food to be in the range of 0.3-0.8 Ìg/kg of body weight (WHO 2002).

Acrylamide produces chromosomal aberrations and micronuclei in somatic (Dobrzynska MM 2000) and germ cells (Shelby MD 1987) of male rodents, as well as AA inducing sperm head abnormalities (Dobrzynska MM 2000). AA is also a toxicant of the male reproductive system in rodents, but very little evidence is available about its toxic effects on the female reproductive system (Tyl and Friedman, 2003) The toxicities of AA in male animals include degeneration of the epithelial cells of the seminiferous tubules, decreased number of sperm, and abnormal sperm and result in decreased fertility rates and retarded development of pups (Tyl 2000). These toxic effects may be attributed to the interfering effect of AA on the kinesin motor proteins, which also exist in the flagella of sperm, resulting in the reduction in sperm motility and fertilization events (Tyl and Friedman, 2003; Tyl 2000). Acrylamide has been shown to be genotoxic to sperm. Positive results were reported with AA in the dominant lethal test and heritable translocation assay and AA produced unscheduled DNA synthesis in the germ cells of male mice (Adler 1994; Generoso 1996). Exposure to AA increased the occurrence of micronuclei in sperm cells of mice and rats (Lahdetie 1994). AA is therefore judged to be a classic clastogen, and its metabolite GA is required for this effect as there were no changes in fertility parameters with AA-treated CYP2E1-null male mice (Ghanayem 2005). Biotransformation of AA to GA is exclusively mediated by CYP2E1 (Sumner 1999). It has been postulated that the clastogenic effects of AA on sperm cells may not be by direct interaction with DNA; instead, these effects may be mediated through interference with the kinesin motor proteins that are involved in spindle fiber formation and chromosomal segregation during cell divisions or alkylation of protamines in sperm (Adler 2000). Despite the fact that AA by virtue of its metabolism to GA can induce base mutations in somatic cells and cause genetic damage to sperm cells through clastogenic effects. In order to estimate the genetic risk associated with AA and GA exposure, it is necessary to obtain additional experimental data for treatment of spermatogonia as well as female germ cells (Favor and Shelby, 2005).

Antioxidants are the main defense factors against oxidative stress induced by free radicals (Agarwal 2005). Vitamin E is believed to be the primary component of the antioxidant system of the spermatozoa and is one of the major membrane protectants against ROS and LPO attack (Yousef 2003). In the context of human reproduction, a balance normally exists between ROS production and antioxidant scavenging activities in the male reproductive tract. As a result of such balance, only minimal amounts of ROS remain, and they are needed for the regulation of normal sperm functions, such as sperm capacitation, the acrosome reaction, and sperm–oocyte fusion (Aitken RJ 1999). The production of excessive amounts of ROS in semen can overwhelm the antioxidant defense mechanisms of spermatozoa and seminal plasma and causes oxidative stress (Sikka SC 2001). There is a relationship between activity of these antioxidant and function of sperm (Zini A 1993). Vitamins E and C which are belong to non-enzymatic antioxidant are used as a supplemented drug to improve sperm quality in male infertility (Thiele JJ 1995).

Animals and treatments:

During the course of this experiment, we followed the recommendations set forth by our Institutional Animal Care and Use Committee for the handling, maintenance, treatment, and killing of the animals. Detailed information about animals and treatments has been reported previously (Manjanatha 2006). Briefly Totally 32 Adult male mice (10 weeks old,35g) that they divided to 4 group each containing 8 mice), . mice of group 1 served as control fed on basal diet ,group 2 received basal diet and acrylamide (10 mg/kg ,water solution) (Kermani-Alghoraishi M 2010) ,group 3 received basal diet and vitamin E (100 mg/kg , intraperitoneal)(Gavazza M 2001) , group 4 received basal diet, acrylamide and vitamin E for 35 days . They were held in cages and were housed in a controlled environment with a temperature range of 25±3℃ and mean relative humidity of 50±5%. The experimental proposal was agreed by our university ethics committee. Chemical analysis indicated that AA and GA were stable in water for at least 1 week, and dosing solutions were prepared and changed weekly. Weekly individual bw gains and water consumption in each cage were monitored throughout the course of the experiment to estimate the amount of the chemicals consumed per mouse per kilogram bw. On the 21st day after the last treatment, samples of blood were taken from the mice so that testosterone may be analyzed, afterward the mice were killed.

Epididymal sperm preparation:

After 35 days (one duration of spermatogenesis in mice is about 32 days), a small part of the cauda epididymis of each mouse was dissected and located in 1 mL of pre-warmed Hams F10 medium (37℃, 5% CO2). Gentle tearing of the tissue was done to make spermatozoa swim out into the culture medium. The dishes were placed in the incubator for 15 min.

Materials and methods:

Sperm count:

The dissected epididymis of each animal was transferred into 10 ml Ham's F10 medium and cut to small slices, in order to swim out the sperm into the medium. After 10 min of diffusion, 1 ml of the solution was diluted with 9 ml formaldehyde fixative. The diluted solution was transferred into each chamber of Neubauer hemocytometer and sperm heads was manually counted under a microscope. Sperm count was performed according to WHO guidelines (WHO 1999) and data were expressed as the number of sperm per ml.(Hamid Reza Momeni 2012)

Sperm motility:

Assessment of sperm motility was done according to WHO protocol (WHO 1999). In brief, 10 µl of the sperm suspension was placed on a microscopic slide and coversliped. A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 200 sperm for each animal. The percentage of sperm motility was analyzed for following motion parameters: Motility was expressed as percentage of progressive (fast and slow) and non-progressive spermatozoa (Hamid Reza Momeni 2012).

Sperm viability test:

Eosin-nigrosin staining was used to asses sperm viability according to WHO protocol (WHO 1999). Briefly, eosin (1%, Merck, Germany) and nigrosin (10%, Merck, Germany) was prepared in distilled water. One volume of sperm suspension was mixed with two volume of 1% eosin. After 30 second, an equal volume of nigrosin was added to this mixture. Thin smears were then prepared and observed under a light microscope at 100X magnification. Viable sperm remained colorless while nonviable sperm stained red. (Hamid Reza Momeni 2012).

Sperm morphology:

For studying the sperm morphology, a drop of sperm suspension was smeared onto a clean glass slide. The smear was then air dried and fixed in a mixture of equal parts ethanol and ether. The slides were then stained with Papanicolaou stain. Dried stained slides were scanned under oil immersion (100 objectives) for morphological abnormalities. A total of 100 sperms per sample were classified according to their morphology; such as normal, coiled mid piece, hair pin (a kink at the annulus, usually 180°), bent tail (a kink at the annulus, usually 90°), coiled tail, double head, amorphous head, triangular head, pin head and cytoplasmic droplet. Sperm abnormality was expressed as percent. (Zohre Zare 2010).

Statistical analysis:

Results are expressed as mean±SD for 8 animals per group. One-way analysis of variance (ANOVA) was used to assess the statistical significance of the data. $p<0.05$ was considered significant.

Results and Discussion:

Table 1 shows the means and statistical analysis of the various sperm parameters Between the 4 groups. Table 2 shows the means and statistical analysis of testosterone hormone Between the 4 groups.

Table 1: The results of semen analysis

Variables	Control group	Acrylamide group	Vitamin E group	Acrylamid+ vitamin E group	p-value
Rapid motility(%)(Grade a)	20.75±3.011	16.25±3.494	28.75±3.327	18.75±1.313	0.010
Slow motility(%)(Grade b)	23.25±4.862	14.375±3.662	27.625±4.627	23.25±5.775	0
Non progressive(%) (Grade c)	32±4.375	18.125±5.54	26±7.946	24.5±7.23	0.002
Immotile sperm(%)(Grade d)	24±4.276	50±7.69	17.625±5.998	33.5±8.451	0
Total motility(%)(Grade a,b,c)	76±4.276	50±7.69	82.375±5.998	64±10.889	0
Count($\times 10^6$)	110±17.493	90.12±10.006	132.5±19.116	98±11.071	0
Viability (%)	78.125±5.083	66.5±5.554	85.375±4.794	73.625±5.68	0
Normal morphology	75.875±6.728	68.75±5.035	83.75±7.382	74.5±5.732	0.001

Statistically significant, P value <0.005.

Table 2: The results of testosterone hormone analysis

Variables	Control group	Acrylamide group	Vitamin E group	Acrylamid+ vitamin E group	p-value
Testosterone	3.25±1.582	1.35±1.047	6.087±2.494	7.925±305	0

Statistically significant, P value <0.005.

Wang et al. evaluated the effect of acrylamide on development and reproductive performance of male rats and showed that acrylamide can induce histopathological lesions in the testis, has toxic effects on the seminiferous tubules and decreases the production of viable sperm cells in male rats (Yang HJ 2005 A). In another study acrylamide induced histopathological changes in the testis such as vacuolation and swelling of the round spermatids, necrosis of the late elongated spermatids, numerous apoptotic cells and formation of multinucleated giant cells in the seminiferous tubules which can affect the sperm parameters (Yang HJ 2005 A). Song et al. observed that subchronic exposure to acrylamide could affect the normal development of sperm, especially decreased sperm vitality, and increased the rates of abnormal morphology in sperm.Also, acrylamide directly damages Leydig cells and affects the endocrine function of the testis and spermatogenesis (Song HX 2008). In agreement with pervious study (Yang HJ 2005 A), our results also showed a significant decrease in the total sperm number in rats treated with Acrylamide. One possibility for this effect might be due to a decrease in hormones such as FSH, LH or testosterone which intern reduce sperm count (Yang HJ 2005 A). Yang et al. found reduced sperm concentration in the cauda epididymis, as well as increased morphological abnormalities of spermatozoa (Yang HJ 2005 A, Yang HJ 2005 B). In addition, it was found that testosterone concentration decreased in the serum of acrylamide-treated rats. Finally, they concluded that acrylamide can reduce the viability of Leydig cells, which in turn, diminishes spermatogenesis in the rat testis (Yang HJ 2005 A). Acrylamide also induce free radical such as ROS which exerts the peroxidation of polyunsaturated fatty acid in the sperm (Zhang JX 2010). It may consequently lead to the destruction of sperm mitochondria, resulting in sperm ATP depletion (Das J 2009) and reduced sperm motility and viability. It is therefore likely to assume that reduced sperm motility and viability induced by acrylamide has been due to the ability of this toxicant in the induction of oxidative stress.

An interesting finding in sperm viability and motility assay was that Vit.E alone increased these parameters compared to the control. This effective result of Vit.E might also be due to its antioxidant role. Therefore hypothesized that the toxic effect of AA on the reduction of sperm viability and motility could be as a result of acrylamide-induced stress oxidative. If our hypothesis was true, Vit.E, a well-known antioxidant (Yue D 2010), should have reversed hazardous effect of AA on sperm viability and motility. Interestingly, we showed that in AA+Vit.E group, Vit.E significantly ameliorated acrylamide -mediated decrease in sperm viability and motility. This vitamin plays an important protective role for preventing the production of lipid peroxides by scavenging free radicals which are toxic for biological membranes (Yue D 2010). Therefore, it could be speculated that this vitamin by improving the activity of sperm defense antioxidant system including superoxide dismutase, glutathione peroxidase and catalase exerted its role in increasing sperm viability and motility. Our results showed a significant increase in sperm morphological anomalies in rats treated with acrylamide. It is documented that ROS generation can induce abnormal sperm morphology (Venkatesh S 2009). Studies Cao showed that increased oxidative stress, enzymatic and non-enzymatic antioxidantis reduced levels in leydig cells and an important factor for impaired spermatogenesis and consequently a significant reduction in epididymal sperm count (Cao L 2004) Our results also raise the sperm count and normal sperm in the vitamin E group than the control group and This is due to the effect of vitamin E on antioxidant enzymes oxidant effects could improve. Vitamin E is quite an effective antioxidant which protects rabbit testis against lipid peroxidation, and, testosterone-induced lipid peroxidation could be improved by additional vitamin E treatment (N. Aydilek 2004).

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