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Toxicopathological, Cytogenetic Effects of Acetothioamide on Female Albino Mice Reproductive System

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Abstract

AIM: To determine toxicopathological and cytogenetic effects of Acetothioamide (ATA) on the female reproductive system.

METHODS: Twenty albino female mice were divided equally into two groups: the first group (control) fed with diet pellet. The second group (treated) were inoculated intraperitoneally with a single dose of ATA (100 mg/kg Bw) for 15 days. All mice were sacrificed at the end of the experiment and blood was collected for evaluation of (FSH and LH), serum peroxy nitrate radical concentration. Cytogenetic analysis (chromosomal aberration, micronuclei, mitotic index and blast index) and the histopathological examination on ovary and uterus were done.

RESULTS: ATA causes significant reduction ($p < 0.0001$) for FSH, LH and serum peroxy nitrate radical concentration among treated females. Oophoritis, pyometria, thrombosis and endometrial hyperplasia with granulomatous reaction were reported among treated females mainly in uterus tissue and ovary.

CONCLUSION: ATA causes significant reduction for FSH, LH and serum peroxy nitrate concentration among treated females. Oophoritis, pyometria, thrombosis and endometrial hyperplasia with granulomatous reaction were the main pathological changes in uterus tissue and ovary among treated females.

Introduction

Acetothioamide (ATA) is the most dangerous source of pollution [1], which used as a fungicide, chemical reagent; organic solvents dye [2]. The national occupational Hazard Survey (1972-1974) estimated that 1,130 workers potentially were exposed to ATA [3]. According to the U.S. Environmental protection agency, Toxics release inventory, 500 Ib of ATA was released in the environment [3], in 2009. ATA was produced by seven manufacture in East Asia [4] and was available from 45 suppliers, including U.S. suppliers [5]. ATA is known to induce acute or chronic liver disease (fibrosis and cirrhosis) in the experimental animal model. Its administration in the rat induces hepatic encephalopathy, metabolic acidosis, increased levels of transaminases, abnormal coagulopathy, and centrilobular necrosis, which are the main features of the clinical chronic liver disease so ATA can precisely replicate the initiation and progression of human liver disease in an experimental animal model [6]. The material is not thought to produce either adverse health effects or irritation of the respiratory tract following inhalation (as classified by EC Directives using animal models). Nevertheless, adverse systemic effects have been produced following exposure of animals by at least one other route, and good hygiene practice requires that exposure be kept to a minimum and that suitable control measures be used in an occupational setting. ! Persons with impaired respiratory function, airway diseases and conditions such as emphysema or chronic bronchitis, may incur further disability if excessive concentrations of particulate are inhaled. If prior damage to the circulatory or nervous systems has occurred or if kidney damage has been sustained, proper

screenings should be conducted on individuals who may be exposed to further risk if handling and use of the material result in excessive exposures [7]. Accidental ingestion of the ATA may be harmful; animal experiments indicate that ingestion of less than 150 gram may be fatal or may produce serious damage to the health of the individual.

Long term exposure to high dust concentrations may cause changes in lung function, i.e. pneumoconiosis; caused by particles less than 0.5 microns penetrating and remaining in the lung. The prime symptom is breathlessness; lung shadows show on X-ray. When administered to the diet thioacetamide-induced hepatocellular carcinomas in mice of both sexes, hepatocellular neoplasms in male rats and bile duct or cholangiocellular neoplasms in rats of both sexes. ATA is a strong hepatotoxic and hepatocarcinogenic chemical. ATA cause various structural and functional change in spleen, lung, brain and stomach, with an increase of oxidative stress by overproduction of reactive oxygen species (ROS) and nitrogen together [8].

The current study was designed to determine toxicopathological and cytogenetic effects of Acetothioamide (ATA) on the female reproductive system.

Material and Methods

Experimental Animal and Management

Twenty female albino mice with age of three months and body weight 30-35 gm were housed in plastic cages 60 x 60 x 10 cm³ in the animal house at Al-Razi centre, ministry of industry, Baghdad, Iraq. All animals were provided with identical management protocol.

Preparation of ATA

ATA obtained from biomeriexns-France, was administrated at a dose of 100 mg/kg B.w I/P according to [9]. ATA was freshly dissolved in distilled water and injected intraperitoneally (subchronic dose). The biochemical and hormonal analysis of (follicular stimulation hormone, FSH and luteal hormone, LH), chromosomal aberrations and histopathological examination for reproductive system done at Department of Pathology, College of Veterinary Medicine, University of Baghdad, Iraq.

The intoxication of Albino Female Mice

The albino female mice were divided equally into two groups: a control group with 10 mice received a normal diet (pellets). While the treated group contain 10 mice that injected intraperitoneally for 15 successive days (subchronic dose) with 100 mg/kg B.w. All mice were sacrificed for postmortem examination, biochemical, hormonal analysis, pathological and cytogenetic examination at the end of 40 days of the experiment.

Blood collection

Blood collected for biochemical and cytogenetic analysis at the end of 40 days of the experiment via cardiac puncture technique in the test tube and left 15 minutes to stand and coagulate in the refrigerator. Serum was separated by centrifugation at 500rpm for 15 minutes and kept at -20C° until use.

Determination of hormonal assay (FSH and LH) (ng/ml)

Vaginal smear was taken to detect the diestrus phase of the estrus cycle for female mice [10]. Radioimmunoassay technique used for evaluation of FSH and LH levels in serum samples.

Determination of serum peroxy nitrate radical concentration (m/l) in serum samples

Peroxynitrate ONOO-from nitrophenol was absorbed in 412 nm. The amount of nitrophenol that was formed in the serum cells reflects the level of peroxynitrite in the serum [11].

Preparation of bone marrow

The cytogenetic analysis was performed according to [12] with few modification, as following: colchicine solution was made by dissolving 1 mg of colchicine tablets in 10 ml of PBS (phosphate buffer saline), then hypotonic solution was made by dissolving 6 gm of KCL powder in 1.073 L, add D.W. (5.191HM/L) (0.075), the fixative was freshly prepared as a mixture of absolute methanol and glacial acetic acid in the ratio 3:1(v/v). Giemsa stain was prepared by dissolving 2 gm of Giemsa stain powder in 100 ml of methanol then stirred constantly using a magnetic stirrer at room temperature for two hr. Then lifted at least for one week before filtration by Whatman number 1 filter paper. The solution was stored in a dark bottle. To stain one ml of stock solution was added to 4 ml of Sorenson buffer (PH: 6.8).

Cytogenetic Analysis (Micronuclei) Mitotic Index, Blast Index and Chromosomal Aberrations

A. **Blast index (B I):** the slides were examined under (40x) of light microscope and (1000)

cells were counted to calculate the percentage of blast cells according to the following equation:

$$B1 = number\ of\ blast \div total\ number\ of\ cells \times 100$$

B. Mitotic Index (MI): The slides were examined under (40 x) of light microscope and (1000) of divided and non divided cells were counted, and the percentage was calculated only for the dividing cells according to the following:

$$\mathbf{MI = number\ of\ dividing\ cells \div total\ number\ of\ cells\ (1000)\ x100}$$

C. Chromosomal Aberrations: Each mouse, 100 metaphase spread were examined microscopically (1000x) for chromosomal aberrations. Only cells with spread chromosomes were selected for scoring. All metaphase spreads, were examined for structural aberrations.

D. Micronuclei Examination: the number of micronuclei was calculated in 1000 cell/animal (40x).

Pathological technique

Uterus, the ovary was taken for fixed in 10 % formalin at day forty of the experiment from the mice and fixed for 72 hours, then processed according to [13]. Histopathological lesions were examined under a light microscope.

Statistic analysis

Standard deviation and Standard error were used to compare the significant difference between means. Data were analysed using the statistical analysis system program [14].

Results

Table 1 showed significant decrease (p < 0.0001) in serum level of glutathione peroxidase concentration (ml/l) at day forty in albino female mice (2.689 ± 0.018) compared with control group (6.764 ± 0.007).

Table 1: Effect of ATA on Antioxidant Enzyme Glutathione Peroxidase Radical Concentration (Ml/ l) of Albino Female Mice At Day 40

Group	Glutathione peroxidase radical Concentration (ml/l)	P
Control	6.764 ± 0.007	P < 0.0001
Treated	2.689±0.018	P < 0.0001

N= 10, significant between groups (p < 0.0001).

The levels of FSH, (0.95 ± 0.023 (ng/ml)) and LH (0.98 ± 0.015 (ng/ml)) illustrated in Table 2 indicated significant (p < 0.0001) decrease among ATA treated group at day 40 compared with control group (1.68 ± 0.0003 (ng/ml)), (1.75 ± 0.023 (ng/ml)) respectively.

Table 2: The effects of ATA on serum FSH, LH concentration (ng/ml) in albino female mice (at day 40)

Group	Hormone		P-value
	FSH	LH	
Control	1.68 ± 0.003	1.75 ± 0.023	P < 0.0001
Treated	0.95 ± 0.023	0.98 ± 0.015	P < 0.0001

N= 10, significant between groups (p < 0.0001).

Cytogenetic analysis revealed that all form of chromosomal aberrations were reported significantly in the increased manner (16.10 ± 0.23) among the treated group (p < 0.0001) as shown in Table 3, especially central fusion, ring form, gaps, breaks and deletion compared with the control group (1.80 ± 0.29).

Table 3: Effect of ATA on Chromosomal Aberration in Albino Female Mice (At Day 40)

Group	Chromosomal abrasions							
	Fragment	C.F	Ring	Gap	Break	Deletion	Total	P
Control	0.50 ± 0.16	0.00 ± 0.00	0.00 ± 0.00	0.600 ± 0.163	0.70 ± 0.153	0.00 ± 0.00	1.80 ± 0.29	P<0.0001
Treated	2.30 ± 0.26	1.50± 0.16	1.70 ± 0.153	3.80 ± 0.13	2.80 ± 0.13	3.90 ± 0.10	16.10± 0.23	P<0.0001

N=10, significant between groups (P<0.0001).

Table 4 revealed that ATA treated female mice have significant increase (p < 0.0001) in micronuclei (3.200 ± 0.249); mitotic index (4.700 ± 0.152) and blast index (34.400 ± 0.266) compared with control group.

Table 4: Effect of ATA on micronuclei, mitotic index, blast index on albino female mice (at 40 days)

Group	Micronuclei	Mitotic index	Blast index	P
Control	1.100 ±0.179	2.700 ± 0.152	13.500 ±0.166	P<0.0001
Treated	3.200 ± 0.249	4.700 ± 0.152	34.400 ± 0.266	P<0.0001

N= 10, significant between groups (P<0.0001)

Histopathological changes

No important lesion was determined in uterus and ovary of the control group.

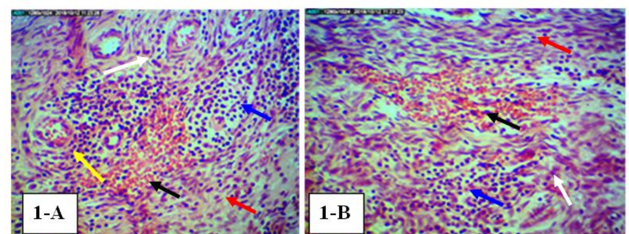

Figure 1: Histopathological section of the ovary of albino female mice treated with 100 mg/ kg.bw. ATA showed (A) oophoritis characterised by fibrinous exudate (red arrow) mononuclear cells mostly macrophage and lymphocyte and neutrophils (blue arrow), congested blood vessels (yellow arrow), haemorrhage (black arrow), necrosis and apoptosis (white arrow) (H and E stain X40). (B) oophoritis characterised by fibrinous exudate (red arrow) mononuclear cells mostly macrophage and lymphocyte and neutrophils (blue arrow), haemorrhage (black arrow), necrosis and apoptosis (white arrow) (H and E stain X40).

Among ATA treated female mice, the ovary characterised by oophoritis with varying amount of fibrinous inflammation surrounded by varying degree of neutrophils, lymphocytes and mostly macrophages infiltration with sever congestion and haemorrhage in cortex and medulla. Numerous areas of necrosis and apoptosis were reported as shown in Figure 1- A, B. Diffuse infiltration of mixed inflammatory cells mostly (macrophage) in the endometrium and myometrium was recorded. Sever sloughing of endometrium cells with necrotic areas filled with dead neutrophil (pyometra) were reported as illustrated in Figure 2-A, B, C.

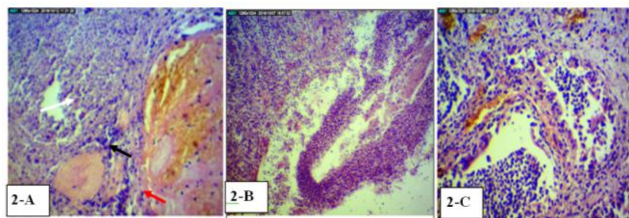

Figure 2: Histopathological section of female uterus mice treated with 100 mg/kg.bw. ATA showed mixed inflammatory mostly macrophage (red arrow), dead neutrophils in the endometrium (black arrow), necrotic area (white arrow) (H and E stain X40).

Sever haemorrhage showed in endometrium cells and myometrium with blood vessels thrombosis. Multiple irregular areas of hemosiderosis mostly engulfed by macrophage with brown to yellow pigment, as shown in Figure 3-A, B, C.

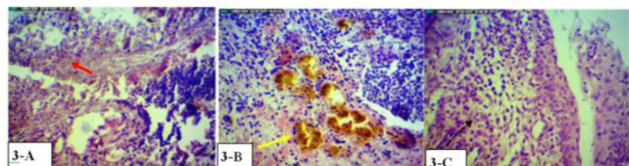

Figure 3: Histopathological section of female uterus mice treated with 100 mg/kg.bw. ATA showed (A) necrosis (red arrow), (B), free and engulfed hemosiderin pigment by macrophage (yellow arrow) (c) necrosis with polymorphic nuclear cells (black arrow) (H and E stain X40).

All areas of uterus were characterised by severe necrosis, vacuolation with endometrium hyperplasia (4-A, B).

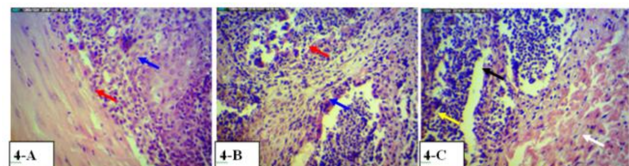

Figure 4: Histopathological section of female uterus mice treated with 100 mg/kg.bw. ATA showed (A, B) sever necrotic area of uterus layer (red arrow) endometrium hyperplasia (blue arrow) (C) necrotic area (white arrow), lymphocytic granuloma composed of severing lymphocyte aggregation (yellow arrow) fibrous connective tissue (black arrow) (H and E stain X40)

Diffuse and focal lymphocytic granuloma present in endometrium and myometrium with serosa, and sometimes extend to the peritoneum, these granuloma surrounded by fibrous tissue as shown in Figure 4-C.

Discussion

The results indicated that ATA causing a significant decrease in peroxynitrite radical concentration. One of the most important toxic roles for ATA is oxidative stress which promotes the generation of reactive oxygen species (ROS) which are the important agents responsible for cellular necrosis and are interfering with body immunity [15]. The decrease in the serum concentration of FSH and LH hormone among ATA treated group probably due to is due to the suppressive effect of ATA on the centre of hypothalamus axis and hypothalamic gonadotropin-releasing hormone (GNRH) secretion which leads to a reduction in the FSH and LH serum levels. Our suggestion showed that ATA toxicity interferes director due to its metabolites on the neurotransmitters, which have an important role in the regulation of FSH and LH hormone secretion. The effects of ATA on induction of chromosomal aberrations may be due to cytotoxic effect on the bone marrow cells by increasing the chromosomal breaks and gaps as well as increase in micronuclei which may be related to severe destruction of the tissues leading to formation of higher number of pro-inflammatory cytokines that are facilitate the extravasations of inflammatory cells from blood flow to vessels and finally stimulates the starting of chromosomal aberrations due to high levels of preformed ROS which leads to destruction for the basis of chromosomal strands.ATA which have the ability to combine with macromolecules of tissue, especially liver and cause the formation of ROS responsible for cell injury [16].

Regarding the pathological changes, ATA has highly toxic effects which cause a reduction in antioxidant level for many exposed tissues with peroxidation derivatives. So the vacuolisation, necrosis and apoptosis with congestion and haemorrhage in exposed tissues indicated that ATA causing the formation of the high level of free radicals injuries [17] causing oxidative stress which decreases the body defence mechanism and minimising the lipid peroxidation process and starting of tissue necrosis and apoptosis [18]. When the oxidative insult was continued for a long time, the enzymes depleted and the body unable to act against free radicals [19]. ATA cause cells injury and formation of reactive metabolic activation by cytochrome p450 enzyme in the liver, which combines with an elevation of oxidative free radical. ATA metabolism produces ATA-S-oxide

producing oxidative stress in cells, causing apoptosis and necrosis [20-23]. Our results indicated that genotoxicity of ATA causing a complex granulomatous reaction which may lead to uterine and ovarian cancer in case of prolonged exposure.

In conclusion, ATA causes significant reduction for FSH, LH and serum peroxy nitrate concentration among treated females. Oophoritis, pyometria, thrombosis and endometrial hyperplasia with granulomatous reaction were the main pathological changes in uterus tissue and ovary among treated females.

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Author's Contributions

All the authors played a key role in carrying out the study to a fruitful outcome. Ethical approval, implementation of the research, study design, and data collection were done by the first author. Data analysis was done by the third author, interpretation with proof reading were done by the second author. All the authors contributed in conceptualization of the research, revisions of the article and final approval of the version to be published.

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