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Which exposure stage (gestation or lactation) is more vulnerable to atrazine toxicity? Studies on mouse dams and their pups

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ABSTRACT

Either during gestation or lactation, the experimental mouse dams received one of the following treatments: (a) diet free of pesticide; (b) diet enriched with atrazine (ATZ); 31.0 μg kg^{-1}; (c) diet free of pesticide + oral vitamin E (α-tocopherol; 200 mg kg^{-1} per mouse); and (d) diet enriched with ATZ (31.0 μg kg^{-1}) + oral vitamin E (200 mg kg^{-1} per mouse). At the weaning, pups and dams were killed and selected organs and blood samples were collected for analyses. Compared with the control results, ATZ induced alteration in a number of biochemical and histopathological parameters either in the dams or their offspring. The ameliorative effect of vitamin E, based on estimating the "Ameliorative Index; AI" to malondialdehyde (MDA) and superoxide dismutase (SOD) ranged between 0.95 and 1.06 (≈1.0) for the dams and the pups either in gestational or lactational exposure routes. In general, the mouse pups were more vulnerable to ATZ toxicity than their mothers and exposure during gestation was suggested to be more effective than during lactation. The findings may support the need to further investigating the adverse effects of exposure to low doses of commonly used pesticides, especially during pregnancy and breast-feeding as well as effects on newborn child.

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1. Introduction

Atrazine, ATZ (6-chloro-*N*-ethyl-*N*-isopropyl-1,3,5-triazinediyl-2,4-diamine; IUPAC) is a chloro-S-triazine compound used as a selective pre-emergence and post-emergence herbicide for the control of weeds in a variety of agricultural crops, as well as in forestry and for non-selective weed control on non-crop areas [1]. In 1991, the US-EPA established a Maximum Contaminant Level (MCL) of 3.0 μg/l for ATZ in drinking water. The most significant regulatory decision in recent years occurred in 2003, after the EU, US, and other nations decided to re-examine the safety of ATZ in light of new evidence. At that time, research had expanded beyond toxicity studies and was beginning to investigate long-term, low-dose exposures in animals, particularly female and fetal animals [2].

Some European countries have included ATZ in the list of pesticide residues to be controlled because it is a potential contaminant due to its chemical characteristics, including lipophilicity, slow hydrolysis, and moderate to low water solubility, and high solubility in organic solvents with high absorption by organic matter, clay and fat tissues

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[3]. Due to inability to keep water contamination below 0.1 ppb, which is a uniform limit for the residue of any pesticide in drinking and groundwater in the European Union (EU), the EU regulators announced the ban of ATZ use. However, in the rest of the world the use of ATZ continues with few, if any, restrictions, and it is even the most frequently detected pesticide in ground- and surface water of the USA [4].

Possible exposure to ATZ can be assumed due to the application to agricultural land and contamination of surface and ground water [5]. ATZ is readily absorbed through gastrointestinal tract [6]. Occupational exposure may occur through both inhalation and dermal absorption during its manufacture, its formulation and its application by spraying. It is found widely, together with its dealkylated degradation products, in rivers, lakes, estuaries, groundwater and reservoirs. In drinking water, the levels rarely exceed 1 μg/l. Surveys of various foods and feeds have generally indicated no detectable ATZ residue. Although ATZ generally has low level of bioaccumulation in fish, it does accumulate in brain, gall bladder, liver and gut of some fishes [7]. Therefore, consumption of contaminated fish can also contribute to human exposure. The residues of ATZ were found in the farmers' blood and urine [8].

The major site for ATZ detoxification is liver, where it is undergoing biotransformation throughout phase I and phase II reactions. In rodent species, two cytochrome P450 (CYP) enzymes (CYP1A & CYP2B) are distinctively involved in ATZ biotransformation [9]. ATZ has a lot of adverse effect on health such as tumors, breast, ovarian, and uterine cancers as well as leukemia and lymphoma. It is an endocrine disrupting chemical interrupting regular hormone function and alters reproductive function not only in human [10], but also in many other species such as developing alligators [11], birds [12], goat [13], and as most vulnerable amphibians [14,15] and fish [16], causing birth defects, reproductive tumors, and weight loss in amphibians as well as humans. It also causes induction of the detoxifying hepatic microsomal oxidative enzymes, continual synthesis of esterases, physiological adaptation to decreased esterase levels, and adaptation of cholinergic receptors [17]. Thus, interfering with the biochemical pathways mechanisms including reproductive functions as a whole and also gene expression changes, ATZ inevitably affects biodiversity and causes environmental havoc [18].

With regard to fetal and childhood exposures, the US Department of Health and Human Services arrived at a conclusion that it is not known whether ATZ or its metabolites can be transferred from a pregnant mother to a developing fetus through the placenta or from a nursing mother to her offspring through breast milk [19]. In their article, Pathak and Dikshit [17] reported the same ATSDR's awareness. However, Stoker et al. [20] found that 3 h following administration of ^{14}C-ATZ to the Wistar rat, there was a distribution of ^{14}C-chlorotriazines (14C-ClTRI) [refer to all residues of the initial dose of ^{14}C atrazine], to the organs of the dam, with the highest amounts in the liver and kidney (1.1 and 0.3% of the administered dose, respectively). Recently, Fraites et al. [21] quantified the distribution of ATZ and its chlorinated metabolites in maternal, fetal, and neonatal fluid and tissue samples following gestational and/or lactational exposure of Sprague Dawley dams to ATZ at different doses. Dose-dependent levels of chlorotriazines, primarily diaminochlorotriazine (DACT), were present in most samples analyzed, including fetal tissue.

There is growing evidence that exposure to xenobiotics (either pharmaceuticals or environmental chemicals) during gestation may result in a variety of adverse outcomes when the developing organism reaches adulthood. Oral exposure to ATZ is known to alter endocrine and reproductive function in adult and peripubertal rodents of both sexes [22–25], but few studies have investigated the effects of ATZ exposure in utero or during the perinatal period. A number of studies have shown that ATZ can promote oxidative stress, by increasing the concentration of reactive oxygen species (ROS) and products of oxidative damage such as lipid peroxides, and therefore influencing the activity of antioxidant enzymes (AOE) [6,26–28]. Liver is the first target of ingested oxidants and also very important tissue in defense against oxidative stress. Testes also possess proper antioxidant defense, and changes in the activity of antioxidant enzymes have been recorded in xenobiotic-exposed animals [29].

Numerous foods provide vitamin E. Nuts, seeds, and vegetable oils are among the best sources of alpha-tocopherol, and significant amounts are available in green leafy vegetables and fortified cereals [30]. Vitamin E is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities [31]. Naturally occurring vitamin E exists in eight chemical forms (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol) that have varying levels of biological activity [31]. Alpha- (or α-) tocopherol is the only form that is recognized to meet human requirements. The research has not found any adverse effects from consuming vitamin E in food. However, high doses of α-tocopherol supplements can cause hemorrhage and interrupt blood coagulation in animals, and *in vitro* data suggest that high doses inhibit platelet aggregation [32]. Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species (ROS) formed when fat undergoes oxidation. Scientists are investigating whether, by limiting free-radical production and possibly through other mechanisms, vitamin E might help prevent or delay the chronic diseases associated with free radicals. In addition to its activities as an antioxidant, vitamin E is involved in immune function and, as shown primarily by *in vitro* studies of cells, cell signaling, regulation of gene expression, and other metabolic processes [31]. Vitamin E is an important biological free radical scavenger in the cell membranes [33]. Administration of vitamin E at 100 mg kg^{-1} bw to Wistar rats treated with ATZ (300 mg kg^{-1} bw) ameliorated the effects of ATZ suggesting it as potential antioxidant against ATZ -induced oxidative stress [6].

Most studies on ATZ toxicity to rodents during gestation/lactation were carried out on doses much higher than the dose characterized as the "acceptable daily intake, ADI" (0.005 mg kg^{-1} food per day). Examples for the doses used in this respect were 1, 5, 20 and 100 mg kg^{-1}/day [20,21,34,35]; 100 mg kg^{-1}/day [36]; 50–100 mg kg^{-1}/day [37]; and 50–200 mg kg^{-1}/day [18].

Women, during pregnancy and lactation, may expose to low doses of ATZ from different environmental sources. In the same protocol of the present study we recently investigated the effect of exposure of mouse dams to ATZ during the overall period of gestation and lactation, and the reflection of such exposure on the offspring. It was found of interest, in the present study, to elucidate and compare ATZ toxicity to each exposure stage, and to assess the ameliorative effect of vitamin E supplementation. In the course of the present study, we will base our investigation on the ADI of ATZ, which may be the lowest dose to be tested on pregnant and lactating mouse dams.

2. Materials and methods

2.1. Reagents

High purity PESTANAL® Analytical Standard of Atrazine (97.4%) was purchased from Fluka (Riedel-de Haën, France) and added as a component of rodent nuggets at a dose of 25 μg/kg of food. This dose allowed the mice to ingest the equivalent of 0.005 mg kg^{-1} food per day (ADI) defined for humans by Food & Agricultural Organization/World Health Organization [38] that was extrapolated for mice on the basis of mean body weight. Information on the toxicity of ATZ was obtained from ExToxNet (ExToxNet: http://extoxnet.orst.edu/).

D-L α-tocopherol acetate was purchased from Fluka (Riedel-de Haën, France). The vitamin was dissolved in corn oil (120 mg/3 mL). Each mouse (with a mean body weight of nearly equal to 20 g) received 100 μl by oral gavages twice a week on the first and fourth day. This dose equals 200 mg kg/mouse twice a week based on the dosage followed by Kalender et al. [39].

2.2. Preparation of pesticide enriched feed

ATZ (0.125 mg) was dissolved in 1 ml methanol. The resulting solution was mixed with acetone (1/9; v/v) and dispersed on 10 g of the vitamin mixture powder (vitaminic mixture 200, Scientific Animal Food Engineering, Safe, France). The vitamin powder containing 125 μg ATZ was then homogenized in a rotavapor (Laborota 4000 Fischer Bioblock, France) for 30 min at 45 °C to evaporate the solvent and then mixed for 50 min at room temperature with the remaining quantity of vitamin mixture (40 g) needed to make the rodent nuggets (5 kg). The vitamin powder enriched with ATZ was then sent to the National Institute of Agricultural Research (INRA) animal feed preparation unit (UPAEINRA, Paris, France) where vitamins (1%) and mineral components (7%) were mixed with the other components of the rodent nuggets. The presence of the ATZ was quantified by Eurofins (Nantes, France). The final quantity was around that we expected (i.e., 0.031 mg kg^{-1}; LC/MS/MS). The control feed was prepared as described above with a mixture of methanol/acetone (1/9, v/v) but lacking pesticide. It was analyzed for the presence of the endogenous pesticide (ATZ) and also for the presence of the most common pesticides found in the environment (including the organophosphorus, OC and pyrethroid pesticides, and polychlorobiphenyls). The results showed that nuggets in control food do not contain pesticides at the detectable level (0.01 mg kg^{-1}).

2.3. Animals

Ten-week-old female and male C57 BL/6J mice were purchased from Charles River Laboratories (Domaine des Oncins-BP 109, 69592 L'arbresle, Cedex, France). Mean body weights were 20 ± 2 g for females and males. The animals were acclimatized for 2 weeks before starting dosing. Thirty-two (32) virgin female mice were distributed into 16 cages. In each cage, one male was placed overnight and the presence of spermatozoa was checked in the vaginal smear in the following morning. This day was connoted as gestation day 0 (GD 0). At that time, pregnant females were divided into 2 major groups, each of 16 animals, individually housed in clean plastic cages in the laboratory animal room (23 ± 2 °C; 40% RH), fed on the prepared diet and tap water was allowed *ad libitum*. One of the two major groups was specified as a "Gestation Group (G)", while the other a "Lactation Group (L). The G group received the tested treatments during gestation only (20 days), while the L group was treated during lactation only (20 days). The day of parturition was considered day 0 of lactation, postnatal day 0 (PND 0). The offspring of each litter were counted, sexed and each litter was randomly reduced to 6 pups of equal number of sexes to maximize the lactation performance [40]. The experimental work on animals was performed in Toxalim Unit, INRA, Toulouse (France), and in accordance to its institutional ethical committees in an accredited animal house.

2.4. Experimental design

During gestation period, the experimental mouse dams (D) received one of the following treatments: (a) diet free of pesticide; control (CD); (b) diet enriched with ATZ; 31.0 μg kg^{-1} (AD); (c) diet free of pesticide + oral vitamin E (α-tocopherol); 200 mg/kg^{-1} per mouse (VD); and (d) diet enriched with ATZ; 31.0 μg kg^{-1} + oral vitamin E; 200 mg kg^{-1} per mouse (AVD). The dose of vitamin E was divided into 2 equal portions and given twice a week. Pups of the experimental dams were designated as CP, AP, VP and AVP, to sign control, ATZ, vit E and ATZ + vit E treated groups, respectively.

Similarly, the group of lactation (L) constituted of four subgroups of mouse dams with their pups and received the same treatments, mentioned above, during lactation period only.

At the end of weaning, blood samples were taken from the facial artery of each animal (dams and pups) and added to heparinized centrifuge tubes (Multivette®, SARSTED, Germany) for separating plasma. Centrifugation was performed on Sigma 1K15 Bioblock Scientific (Subra, France) for 10 min at 4 °C and 4000 rpm (1400 × *g*). The plasma were kept in eppendorf tubes and stored at (−80 °C). Then the animals were sacrificed by cervical dislocation, and the heart, spleen, liver, kidneys, testes or ovaries were removed and weighted. Small pieces of organs were kept in 10% formalin for histopathological studies. Other pieces of liver were packed in aluminium sheets and kept in nitrogen

(−80 °C) for certain biochemical estimations. The fractionated blood samples and specimens of internal organs were shipped frozen, in addition to formaldehyde – reserved specimens, to the National Research Centre (NRC), Cairo, Egypt for biochemical analyses and histopathological studies.

2.5. Biochemical analyses

These included butyryl cholinesterase (BuChE), alkaline phosphatase (ALP), urea, Malondialdehyde (MDA) and super oxide dismutase (SOD). Measurements were performed spectrophotometrically using a Shimadzu UV-VIS Recording 2401 PC (Japan), and in accordance with the manufacturer's instructions given in the pamphlets. The activity of plasma cholinesterase (BuChE; EC 3.1.1.8) was measured at 405 nm and expressed in units per liter using the method of Knedel and Böttger [41]. ALP (EC 3.1.1) was measured in plasma at 510 nm in terms of international units per liter according to Belfield and Goldberg [42]. Concentration of urea (milligram per deciliter) was determined in plasma at 550 nm using the method of Fawcett and Scott [43]. MDA and SOD activities were determined in liver tissues, based on methods published by Satoh [44] and Nishikimi et al. [45], respectively. Spectrophotometric measurements were carried out at 534 nm for lipid peroxide, in terms of MDA, and expressed in nanomole per gram tissue. SOD measurements at 560 nm were calculated in terms of units per gram liver tissue.

2.6. Histology studies

Liver, kidneys, ovaries and testes from the experimental mice were dissected and fixed in 10% neutral formalin, dehydrated in ascending grades of alcohol and imbedded in paraffin wax. Paraffin sections (5 mm thick) were stained for routine histological study using haematoxylin and eosin (H&E). Two slides were prepared for each mouse; each slide contained two sections for each organ. Ten field areas for each section were selected and examined for histopathological changes under light microscope (400× magnifications). The histopathology was carried out in the Pathology Department, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt. Tissue injury in the examined organs was scored in different ratings according to Önder et al. [46].

2.7. Statistical analysis

The data were analyzed by using GraphPad Prism 5 Demo (www.graphpad.com/downloads/docs/Prism5Regression.pdf), and expressed as means ± S.E. Paired samples (t) test was used to compare the data of the control with those of treatments, where $P < 0.05$ and $P < 0.01$ were considered for significant and high significant differences, respectively. When $P > 0.05$, values were not significantly different from one another.

3. Results

3.1. Signs of toxicity and observations

All animals were carefully observed during gestation and lactation periods. Generally, the experimental animals showed normal behavior except signs of restless on the dams during gestation period. The number of pups per each female ranged between 6 and 8 of nearly 1:1 sex ratio.

3.2. Body and relative organs weights

The initial starting body weight of mouse dams was approximately 20 g/animal. In the G experimental group, the mean body weight of control mothers (CD), at the end of experimental period, recorded 29.70 g which did not differ significantly than body weights of the other tested treatments. The body weight of male pups in ATZ treatment (AP; 11.84 g) and female pups in vitamin treatment (VP; 11.48 g) was significantly ($P < 0.05$) lower than that of the corresponding control values (Table 1). Generally, the internal organs weights relative to body weights showed no significant differences between the tested treatments, except for liver and heart of ATZ-treated dams which showed values significantly differed than those of the corresponding control values. Also, significant difference was recorded for the relative weight of kidneys from ATZ-female pups (1.47% compared to 1.296% for the control treatment; Table 1).

In the lactation experiment, L (Table 2), the mean body weight of control mothers (CD) or male and female pups (CP) did not differ significantly among the other tested treatments. The relative weight of liver from ATZ (AP; 6.342%) or ATZ + vitamin (AVP; 6.982%) – female pups showed significant ($P < 0.05$) and high significant ($P < 0.01$) elevations compared to control value (5.256%). The relative weight of heart from male and female pups exposed to ATZ + vitamin treatment showed significant decreases, while ovaries from female pups exposed to ATZ treatment (AP; 0.748%) showed high significant elevation compared to the corresponding control value (CP; 0.080%).

The relative weights of liver, heart, spleen and ovary/testes in the G experimental group, as well as kidneys, spleen and testes in the L experimental group did not show significant differences among the pups of these treatments (Tables 1 and 2).

3.3. Biochemical analyses

Due to the little amount of blood obtainable from the tested mouse, the estimated biomarkers were carried out on alkaline phosphatase (ALP), butyryl cholinesterase (BuChE) and urea in plasma, as well as malondialdehyde (MDA) and superoxide dismutase (SOD) in liver tissues. The results of these parameters are represented by column histograms designated by asterisks for the values of significant differences than the control values.

Control values of ALP recorded 75.3, 56.0 and 56.3 IU/l for the mouse dams (CD), male pups CP (M) and female pups CP (F), respectively, in gestational exposure experiment (Fig. 1a). The values recorded for ATZ-treated dams, as well as male and female pups were higher than the

Table 1
Body and relative organs weights of mouse dams and their pups exposed to atrazine, with and without vitamin E, during gestation.

Treatment groups	B. Wt. (g)	Relative organs weights (%)				
		Liver	Kidneys	Heart	Spleen	Ovary/testes
CD	29.70 ± 0.87	5.353 ± 0.33	1.387 ± 0.09	0.8733 ± 0.003	0.3900 ± 0.03	0.1200 ± 0.01
AD	30.43 ± 0.77	6.733 ± 0.33*	1.180 ± 0.07	0.7700 ± 0.03*	0.3300 ± 0.02	0.1133 ± 0.03
VD	28.00 ± 0.61	5.613 ± 0.59	1.330 ± 0.07	0.6833 ± 0.07	0.3433 ± 0.01	0.07667 ± 0.02
AVD	27.70 ± 0.47	5.953 ± 0.60	1.370 ± 0.08	0.7467 ± 0.13	0.4000 ± 0.03	0.1133 ± 0.01

Pups	M	F	M	F	M	F	M	F	M	F	M	F
CP	14.10 ± 0.62	12.78 ± 0.38	5.312 ± 0.51	5.298 ± 0.13	1.248 ± 0.05	1.296 ± 0.04	0.6240 ± 0.04	0.6960 ± 0.04	0.4820 ± 0.03	0.5060 ± 0.03	0.5600 ± 0.04	0.1300 ± 0.01
AP	11.84 ± 0.60*	12.68 ± 0.50	5.108 ± 0.71	5.150 ± 0.45	1.346 ± 0.03	1.470 ± 0.07*	0.6340 ± 0.03	0.6140 ± 0.04	0.5900 ± 0.05	0.5960 ± 0.07	0.5780 ± 0.02	0.1100 ± 0.01
VP	14.16 ± 0.71	11.48 ± 0.32*	4.992 ± 0.23	4.870 ± 0.19	1.192 ± 0.03	1.356 ± 0.03	0.5940 ± 0.02	0.7100 ± 0.09	0.4840 ± 0.04	0.5660 ± 0.04	0.6160 ± 0.05	0.1000 ± 0.02
AVP	12.44 ± 0.83	12.74 ± 0.81	4.956 ± 0.71	5.016 ± 0.39	1.262 ± 0.07	1.386 ± 0. 10	0.6340 ± 0.03	0.6840 ± 0.04	0.5400 ± 0.05	0.5160 ± 0.05	0.5260 ± 0.06	0.1240 ± 0.02

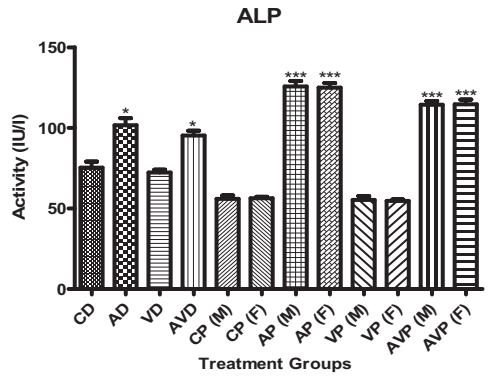
Group symbols: C = control; D = dams; P = pups (M: male and F: female); A = atrazine; V = vitamin E; AV = atrazine + vitamin E.
Statistical analysis:
Body wt. CP (M) versus AP (M): *significant difference at <0.05; CP (F) versus VP (F): *significant difference at <0.05.
Relative wt. of liver: CD versus AD: *significant difference at <0.05.
Relative wt. of kidneys: CP (F) versus AP (F): *significant difference at <0.05.
Relative wt. of heart: CD versus AD: *significant difference at <0.05.

Table 2
Body and relative organs weights of mouse dams and their pups exposed to atrazine, with and without vitamin E, during lactation.

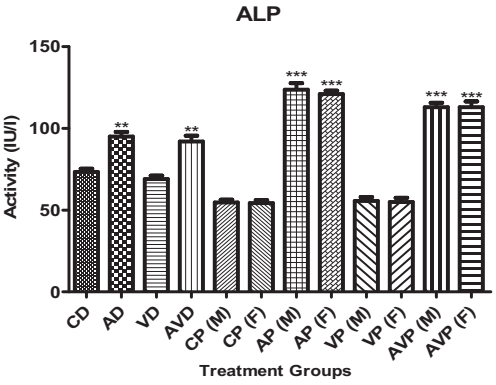
Treatment groups	B. wt. (g)	Relative organs weights (%)				
		Liver	Kidneys	Heart	Spleen	Ovary/testes
CD	27.63 ± 1.13	8.303 ± 0.84	1.470 ± 0.05	0.8067 ± 0.09	0.3633 ± 0.04	0.0800 ± 0.03
AD	29.43 ± 0.54	9.127 ± 0.22	1.560 ± 0.006	0.8000 ± 0.15	0.2767 ± 0.01	0.1067 ± 0.06
VD	27.20 ± 1.51	7.440 ± 0.90	1.377 ± 0.06	0.7200 ± 0.03	0.3833 ± 0.03	0.05333 ± 0.02
AVD	28.03 ± 0.18	9.653 ± 0.12	1.473 ± 0.024	0.8233 ± 0.14	0.2667 ± 0.05	0.08333 ± 0.05

Pups	M	F	M	F	M	F	M	F	M	F	M	F
CP	7.00 ± 0.58	7.88 ± 0.68	5.542 ± 0.36	5.256 ± 0.40	1.436 ± 0.17	1.486 ± 0.13	0.8360 ± 0.11	0.8740 ± 0.10	0.5700 ± 0.07	0.5840 ± 0.05	0.5760 ± 0.08	0.0800 ± 0.03
AP	8.16 ± 0.89	6.78 ± 0.28	6.180 ± 0.94	6.342 ± 0.19*	1.422 ± 0.15	1.450 ± 0.06	0.6840 ± 0.05	0.7200 ± 0.03	0.5300 ± 0.07	0.6060 ± 0.12	0.4780 ± 0.07	0.7480 ± 0.05***
VP	8.30 ± 0.41	8.16 ± 0.25	6.352 ± 0.34	6.198 ± 0.14	1.498 ± 0.08	1.524 ± 0.02	0.7740 ± 0.02	0.7700 ± 0.02	0.5960 ± 0.8	0.5420 ± 0.06	0.4680 ± 0.08	0.0980 ± 0.01
AVP	6.48 ± 0.58	6.76 ± 0.38	5.834 ± 1.02	6.982 ± 0.31**	1.462 ± 0.13	1.524 ± 0.08	0.6200 ± 0.05*	0.6660 ± 0.05*	0.3040 ± 0.04	0.3900 ± 0.05	0.4820 ± 0.08	0.1040 ± 0.01

Group symbols: C = control; D = dams; P = pups (M: male and F: female); A = atrazine; V = vitamin E; AV = atrazine + vitamin E.
Statistical analysis:
– No significant differences in body weights.
– Liver: high significant difference (<0.001): CP (F) versus AVP (F).
– Heart: significant difference (<0.05): CP (M) versus AVP (M) and CP (F) versus AVP (F).
– Ovary: high significant difference (<0.001): CP (F) versus AP (F).

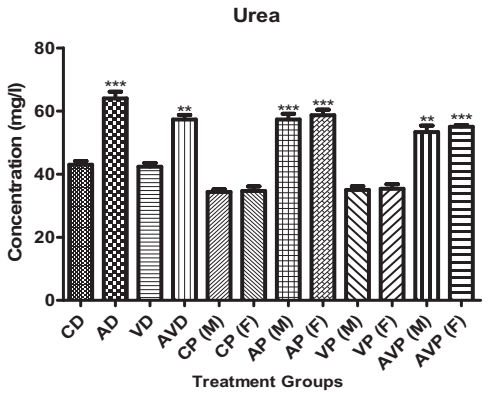


[a]: CD = 75.3; CP (M) = 56.0; CP (F) = 56.3 IU/l

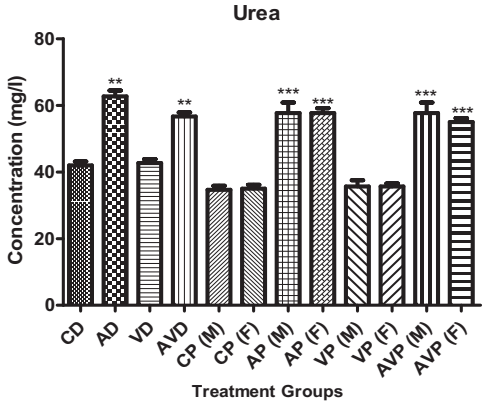


[b]: CD = 73.3; CP (M) = 54.7; CP (F) = 54.3 IU/l

Fig. 1. ALP activity in plasma of mouse dams and their pups exposed to atrazine, with and without vitamin E, during gestation (a) and lactation (b) periods. *Statistical analysis*: No significant difference; *significant difference at $P < 0.05$; **highly significant difference at $P < 0.01$. Group symbols: C = control; D = dams; P = pups (M: male and F: female); A = atrazine; V = vitamin E; AV = atrazine + vitamin E.



[a]: CD = 43.0; CP (M) = 34.3; CP (F) = 34.7 mg/dl



[b]: CD = 42.0; CP (M) = 34.7; CP (F) = 35.0 mg/dl

Fig. 2. Urea concentration in plasma of mouse dams and their pups exposed to atrazine, with and without vitamin E, during gestation (a) and lactation (b) periods. *Statistical analysis*: No significant difference; *significant difference at $P < 0.05$; **highly significant difference at $P < 0.01$. Group symbols: C = control; D = dams; P = pups (M: male and F: female); A = atrazine; V = vitamin E; AV = atrazine + vitamin E.

corresponding control values. The differences were statistically significant at $P < 0.05$ in case of dams, and were highly significant at $P < 0.01$ in case of pups. Similar pattern of ALP elevation was observed on mice of ATZ + vitamin E groups.

In lactational exposure experiment, control values of ALP recorded 73.3, 54.7 and 54.3 IU/l for the mouse dams (CD), male pups CP (M) and female pups CP (F), respectively (Fig. 1b). The values recorded for ATZ-treated dams and their pups were highly significant than the corresponding control values. Similar pattern of ALP elevation was observed on mice of ATZ + vitamin E groups.

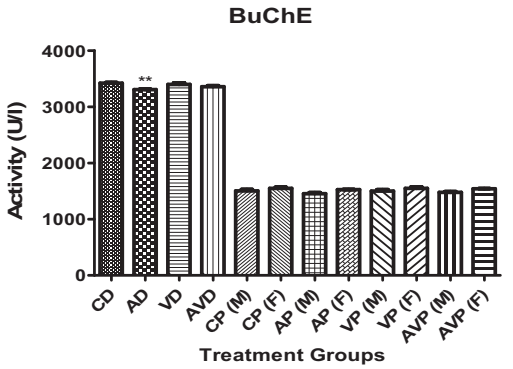
It seemed that the elevation of ALP activity in ATZ-treated group was higher in lactational than in gestational exposure route, and the mouse pups were much vulnerable to alteration in ALP activity than their mothers in both cases compared. On the other hand, the vitamin treatment did not cause detectable alteration in ALP activity, either in mouse dams or their offspring, while treatment of ATZ + vitamin E induced high elevation of ALP activity in the dams and their pups (Fig. 1).

In gestational exposure experiment (Fig. 2a), control values of urea recorded 43.0, 34.3 and 34.7 mg/dl for the mouse dams (CD), male pups CP (M) and female pups CP (F), respectively. ATZ treatment induced high significant increase ($P < 0.01$) accounting to 64.0, 57.3 and 58.7 mg/dl, respectively. Similar pattern of urea elevation was observed on mice of ATZ + vitamin E groups.

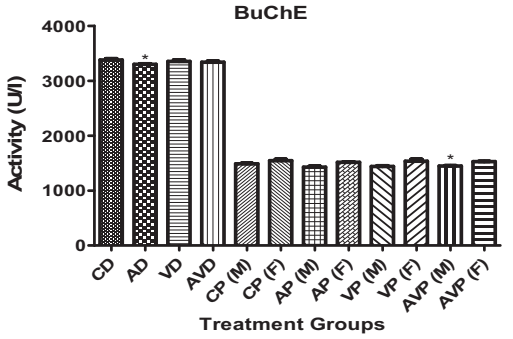
In lactational exposure experiment, control values of urea recorded 42.0, 34.7 and 35.0 mg/dl for the mouse dams (CD), male pups CP (M) and female pups CP (F), respectively (Fig. 2b). ATZ treatment induced high significant increase accounted to 62.7, 57.7 and 57.7 mg/dl, respectively.

The results obtained indicate that urea elevation after ATZ treatment of mouse dams was somewhat higher after gestational exposure than after lactational exposure. Mouse pups exhibited a similar pattern of urea alterations as their mothers, both after gestational and lactational exposure. While vitamin E treatment did not cause detectable alteration in urea concentration, either in mouse dams or their offspring, the treatment of ATZ + vitamin E induced high elevation of urea in the dams and their pups (Fig. 2).

In gestational exposure experiment, the butyryl cholinesterase (BuChE) recorded 3419, 1503 and 1548 U/l, respectively for control mouse dams, male offspring and female offspring. In ATZ-treated groups, the above mentioned values were generally declined and recorded



[a]: CD = 3419; CP (M) = 1503; CP (F) = 1548 U/l



[b]: CD = 3379; CP (M) = 1487; CP (F) = 1544 U/l

Fig. 3. Activity of BuChE in plasma of mouse dams and their pups exposed to atrazine, with and without vitamin E, during gestation (a) and lactation (b) periods. Statistical analysis: No significant difference; *significant difference at $P < 0.05$; **highly significant difference at $P < 0.01$. Group symbols: C = control; D = dams; P = pups (M: male and F: female); A = atrazine; V = vitamin E; AV = atrazine + vitamin E.

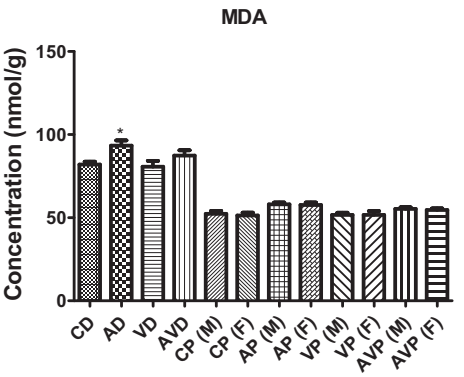
3305 U/l in dams with high significant difference at $P < 0.01$ (Fig. 3a).

In lactational exposure experiment, the butyryl cholinesterase (BuChE) recorded 3378, 1487 and 1544 U/l, respectively for control mouse dams, male offspring and female offspring. In ATZ-treated groups, the above mentioned values were generally declined and recorded 3298 U/l in dams, a value which was significantly lower ($P < 0.05$) than the corresponding control value (Fig. 3b).

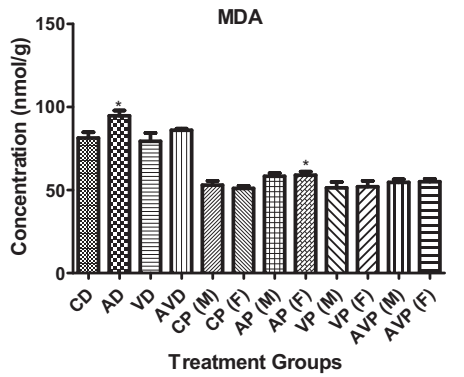
The offspring (male and female) either in gestational or lactational exposures, in comparison with the corresponding control values, did not show significant alteration in BuChE activity (Fig. 3).

Control values of malondialdehyde (MDA) concentration in the gestational exposure experiment were accounted to 82.0, 52.3 and 51.3 nmol/g tissue for mouse dams, male pups and female pups, respectively. In comparison, ATZ-treated groups recorded 93.3, 58.0 and 57.7 nmol/g tissue, respectively. Only, the value recorded for mouse dams was significantly ($P < 0.05$) higher than that recorded for control (Fig. 4a).

Concentration of MDA for control animals in the lactational exposure experiment equaled to 81.3, 53.0 and 51.0 nmol/g tissue for mouse dams, male pups and female pups, respectively. In comparison, ATZ-treated groups recorded 94.7, 58.3 and 59.0 nmol/g tissue, respectively.



[a]: CD = 82.0; CP (M) = 52.3; CP (F) = 51.3 nmol/g tissue



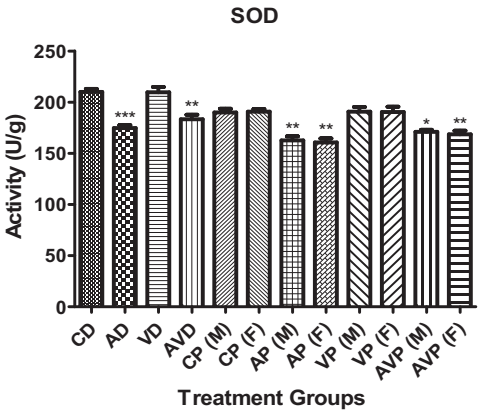
[b]: CD = 81.3; CP (M) = 53.0; CP (F) = 51.0 nmol/g tissue

Fig. 4. Concentration of MDA in liver tissue of mouse dams and their pups exposed to atrazine, with and without vitamin E, during gestation (a) and lactation (b) periods. *Statistical analysis*: No significant difference; *significant difference at $P < 0.05$; **highly significant difference at $P < 0.01$. Group symbols: C = control; D = dams; P = pups (M: male and F: female); A = atrazine; V = vitamin E; AV = atrazine + vitamin E.

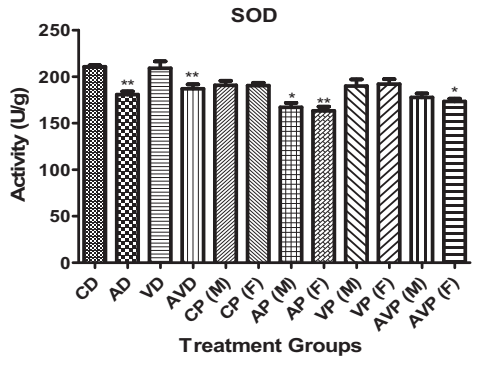
Only the values recorded for mouse dams and female pups were significantly ($P < 0.05$) higher than those recorded for control values (Fig. 4b).

Alterations of superoxide dismutase (SOD) activity in mouse dams and their offspring following administration of atrazine, with and without vitamin E, to the mothers during gestational and lactational periods separately are presented in Fig. 5. The general pattern of alteration was a decline of activity due to administration of ATZ. In gestational exposure, SOD activity in mouse dams recorded 174.7 U/g tissue compared to control value of 210.0 U/g tissue, achieving high significant difference at $P < 0.01$. The pups, either males or females, behaved similarly with respect to high significant decline of SOD activity. Also, female and male pups from mother groups treated with ATZ + vitamin E showed lower enzyme activity compared to control (Fig. 5a).

In lactational exposure experiment, control SOD activity recorded 210.7, 190.7 and 190.3 U/g tissue for mouse dams, male offspring and female offspring, respectively. In ATZ-treated groups, these values were 180.7, 167.0 and 163.3 U/g tissue, achieving high significant decline ($P < 0.01$) for mouse dams, significant decline ($P < 0.05$) for



[a]: CD = 210.0; CP (M) = 190.0; CP (F) = 190.7 μg/g tissue



[b]: CD = 210.7; CP (M) = 190.7; CP (F) = 190.3 μg/g tissue

Fig. 5. Activity of SOD in liver tissue of mouse dams and their pups exposed to atrazine, with and without vitamin E, during gestation (a) and lactation (b) periods. *Statistical analysis*: No significant difference; *significant difference at $P < 0.05$; **highly significant difference at $P < 0.01$. Group symbols: C = control; D = dams; P = pups (M: male and F: female); A = atrazine; V = vitamin E; AV = atrazine + vitamin E.

male pups, and high significant decline ($P < 0.01$) for female pups (Fig. 5b).

3.4. Histopathology examination

Microscopic examination of sections from liver, kidneys, ovaries and testes prepared from the animals subjected to different treatments in the present study was performed. Fig. 6 illustrates the histopathologic structure of the studied organs prepared from the normal (control) groups. The latter was considered as a base for comparing the histopathologic alterations in ATZ and ATZ + vitamin E groups. Such alterations were scored in terms of degree of change in cells showing damage or alterations, as follows: (0) = no change; (1) = mild change (e.g., <25% of cells showing damage); (2) = moderate change (e.g., 25–50% cell damage); and (3) = severe change (e.g., >50% cell damage). The results are presented in Tables 4 and 5.

Sections of control organs (Fig. 6) were characterized by normal histopathologic structure, observed on hepatic lobules, central veins and appearance of hepatocytes. Renal paranchyma and tubules showed normal histopathological structure of kidneys. Graffian follicle and corpus luteum were characterized in normal ovary. Sections of testes were characterized by normal seminiferous tubules, and ovaries showed normal corpus luteum (Fig. 6a–i).

In ATZ-treated groups during gestation (Table 4), the following histopathologic effects were observed:

- *Liver*: mild hepatic and moderate vacuolar degeneration of hepatocytes (dams, male and female pups); moderate perivascular mononuclear cells infiltration accompanied with mild Kupffer cells activation (dams) and moderate fibroblast proliferation around bile duct (female pups).
- *Kidneys:* moderate vacuolation of epithelial lining renal tubules and glomerular tuft (dams, male and female pups); moderate focal renal hemorrhage (dams); moderate thickening of parietal layer of Bowman's capsule (dams) and mild congestion of intertubular blood vessels (female pups).
- *Ovary*: moderate degeneration of corpus luteum and moderate congestion of blood vessels (dams).
- *Testis*: moderate degeneration of spermatogoneal cells; moderate thickening of basement membrane of seminiferous tubules, as well as mild small diameter seminiferous tubules.

In ATZ + vitamin E-treated groups during gestation (Table 4), most (if not all) of the above histopathologic changes disappeared, except for some mild changes represented by hepatic degeneration of hepatocytes (male pups); mild vacuolar degeneration of hepatocytes, as well as kupffer cells activation (dams).

In ATZ-treated groups during lactation (Table 5), the following histopathologic effects were observed:

- *Liver*: mild hepatic degeneration of hepatocytes (female pups); severe (dams) to moderate (pups) vacuolar degeneration of hepatocytes; mild perivascular mononuclear cells infiltration (male pups); mild fibroblast proliferation around bile duct (male pups) and severe focal hepatic hemorrhage (dams and male pups).
- *Kidneys*: severe vacuolation of epithelial lining renal tubules (dams and offspring); moderate vacuolation of epithelial lining glomerular tuft (dams and male pups), but mild for female pups; severe focal renal hemorrhage (dams) and moderate congestion of intertubular blood vessels (dams).
- *Ovary*: moderate (dams) and mild (female pups) degeneration of corpus luteum and severe congestion of blood vessels (dams).
- *Testis*: moderate degeneration of spermatogoneal cells, mild thickening of basement membrane of seminiferous tubules, moderate small diameter seminiferous tubules and severe intratubular odema.

In ATZ + vitamin E-treated groups during lactation (Table 5), most (if not all) of the above histopathologic changes disappeared or declined, except for some effects represented by mild kupffer cells activation (male pups); mild vacuolation of epithelial and endothelial lining renal tubules (dams) and mild degeneration of spermatogoneal

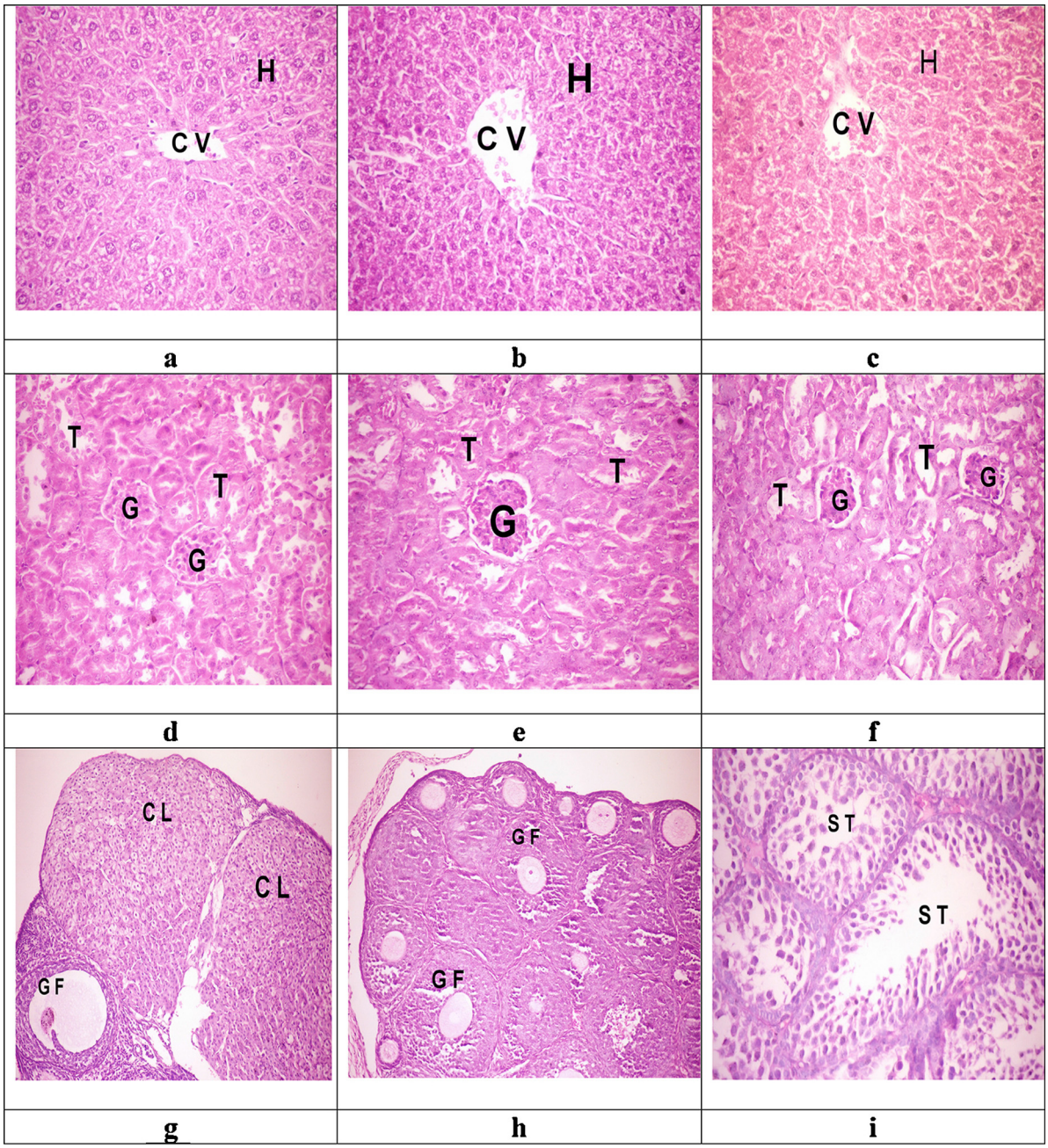


Fig. 6. Histopathological sections for organs (liver, kidney, ovary and testis) prepared from normal mice for comparison with atrazine-treated mice, with and without vitamin E, during gestation and lactation periods (H&E stain 400×). (a) Liver of control mouse dam showing the normal histological structure of hepatic lobule from central vein (CV) and normal hepatocytes (H) (H&E stain 400×). (b) Liver of control male offspring showing the normal histological structure of hepatic lobule from central vein (CV) and normal hepatocytes (H) (H&E stain 400×). (c) Liver of control female offspring showing the normal histological structure of hepatic lobule from central vein (CV) and normal hepatocytes (H) (H&E stain 400×). (d) Kidney of control mouse dam showing the normal histological structure of renal parenchyma. Note normal glomerulus (G) and normal renal tubules (T) (H&E 400×). (e) Kidney of control male offspring showing the normal histological structure of renal parenchyma. Note normal glomerulus (G) and normal renal tubules (T) (H&E 400×). (f) Kidney of control female offspring showing the normal histological structure of renal parenchyma. Note normal glomerulus (G) and normal renal tubules (T) (H&E 400×). (g) Ovary of control mouse dam showing normal graffian follicle (GF) and normal corpus luteum (CL). (H&E stain 400×). (h) Ovary of female offspring showing normal graffian follicle (GF) (H&E stain 400×). (i) Testis of control male offspring showing normal seminiferous tubules (ST) (H&E stain 400×).

cells (male pups). Numerous follicles were seen in a section of ovary from the pups.

4. Discussion

In toxicological studies, organ and relative organs weights are important criteria for evaluation of organ toxicity [47], and increase or decrease of organs weights than normal may be considered as a sign of toxicity [48]. Several investigators (e.g., [36,37,49,50]) have reported the effect of ATZ on body and internal organs weights, as well as on the function of liver and kidney. Therefore, our findings may add further awareness to possible effects of the tested dose in the present study which was extremely lower than the doses used by the previous investigators. In lactational exposure experiment, the relative weights of liver and heart from the ATZ + vitamin E group of pups (AVP; Table 2) have exercised significant differences compared to the corresponding control values. Such result may refer to interaction between the pesticide and vitamin E which appeared as "potentiating effect" for liver and "antagonistic effect" for heart, a result which may need further investigation in future studies.

Hepatic effects of ATZ in terms of enzymatic alteration had been studied in different animal species. In the present study, elevation ($P < 0.05$) of ALP activity was observed in ATZ-treated dams during gestation (group AD; Fig. 1a), and high elevation ($P < 0.01$) for those treated during lactation (group AD; Fig. 1b), results which coincide with that of Santa Maria et al. [49] on Wister rats treated with ATZ at 100 mg kg^{-1}/day for 14 days. Either in gestational or lactational exposure, the mouse pups showed elevation of ALP activity very higher than that in the dams, a result reflecting high vulnerability of the young mice to indirect exposure to ATZ via their mothers.

Alkaline phosphatases are a group of enzymes found primarily in the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). There are also small amounts produced by cells lining the intestines (isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules). What is measured in the blood is the total amount of ALP released from these tissues into the blood. The primary importance of measuring ALP is to check the possibility of bone disease or liver disease. When the liver, bile ducts or gallbladder system are not functioning properly or are blocked, this enzyme is not excreted through the bile and ALP is released into the blood stream [51]. On the other side, the observed high elevation ($P < 0.01$) of urea concentration may be considered as an indication of kidney dysfunction due to exposure to ATZ at such very low dose used in the present study. Our results coincide with those previously reported regarding the profound effect of ATZ on liver and kidney functions in a variety of experimental animals [36,37,49,50].

Cholinesterase (ChE, 3.1.1.8), also known as pseudocholinesterase, has been recognized as an enzyme that hydrolyzes choline esters. It is synthesized mainly in hepatocytes and secreted into the blood stream [52]. ChE activity is reduced in liver dysfunction due to reduced synthesis, in contrast to other serum enzymes associated with the clinical assessment of liver function whose activities increase as a result of increased release from their cellular sources following cell membrane damage [53]. Cholinesterases had been long recognized as biomarkers of effect induced mainly by organophosphorus and carbamate pesticides [54]; so, investigations on other anticholinesterases were being limited. To the best of our knowledge, the literature offers very little information about the effect of atrazine on mammals ChEs activity, while much studies on aquatic vertebrate organisms are available in the literature with special concern to joint action of ATZ and other pesticides [55–58]. This led Boonthai et al. [55] to suggest that AChE as a biomarker for organophosphorus and carbamate pesticides may be valid to use for other chemical groups.

In a single paper, it was reported that administration of ATZ to rats at 300 mg kg^{-1} affected erythrocyte membranes causing significant inhibition of AChE activity and induction of oxidative stress in terms of increased malondialdehyde (MDA) levels. However, administration of vitamin E (100 mg kg^{-1} bw daily) ameliorated the oxidative stress and changes in the erythrocyte membranes induced by ATZ [59]. Such results corroborated our findings regarding high significant ($P < 0.01$) decline of BuChE activity in ATZ-treated mouse dams during gestation (Fig. 3a), and significant ($P < 0.05$) decline in ATZ-treated mouse dams during lactation (Fig. 3b). In both cases of exposure to ATZ, co-administration of vitamin E normalized the enzyme activity to that of the control values. It seemed that there were no significant alteration in BuChE activities in the pups whose mothers were treated with ATZ either during gestation or lactation stages (Fig. 3). This may be referred to the administered low dose of ATZ which is not strong inhibitor to cholinesterase enzymes [58].

In fact, the toxicity of many xenobiotics is associated with the production of oxygen-free radicals, more generally known as "reactive oxygen species" (ROS), which are not only toxic themselves but are also implicated in the pathophysiology of many diseases [60]. The harmful effects of ROS are balanced by the antioxidant action of nonenzymatic antioxidants in addition to antioxidant enzymes [61]. Antioxidants protect cells from the damaging effects of free radicals, which are molecules that contain an unshared electron [62]. It has been reported that many pesticides may induce oxidative stress following acute exposure in humans [63] and animals [64–67]. Increased lipid peroxidation (LPO) in various tissues may be one of the molecular mechanisms involved in the ATZ-induced toxicity. SOD provides the first line of defense against oxygen derived free radicals and decreases oxidative stress by dismutation of O^{2-} [68]. Previous studies in our laboratory on rat (*Rattus norvegicus*), using different insecticides, revealed increase of LPO and decrease of SOD levels following the insecticidal treatments [64–67,69–71], and corroborate our findings on ATZ herbicide. Also, oral administration of ATZ to rats at 120 mg kg^{-1} was found to impair the antioxidant defense and increased LPO (in terms of MDA concentration) in the liver and significantly decreased SOD activity [72].

Vitamin E is an important biological free radical scavenger in the cell membranes [33]. In the present investigation we studied whether vitamin E has the potential to attenuate ATZ-induced oxidative stress, either in

mouse dams exposed during gestation or during lactation as well as on their offspring. In mouse dams, the significant ($P < 0.05$) elevation of MDA induced by ATZ and the high significant decline ($P < 0.01$) of SOD were normalized with co-administration of vitamin E (Figs. 4 and 5). Moreover, the female pups from lactation experiment (Fig. 4b) showed significant elevation of MDA activity which was returned to its normal value by co-administration of vitamin E. Such results coincide with Singh et al. [6] who found a significant increase in hepatic LPO following ATZ administration to male Wistar rats at 300 mg kg^{-1} bw, but administration of vitamin E (100 mg kg^{-1} bw) attenuated ATZ-induced LPO in liver. According to Pascoe et al. [73], vitamin E allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids (PUFA), thus breaking the chain of free radical reactions. The resulting antioxidant radical is relatively a non- reactive species.

To assess the effect of ATZ on antioxidative stress enzymes (e.g., MDA and SOD) in a "quantitative manner", we calculated the percentage of change in ATZ-treated groups relative to control untreated groups, either for the dams or their offspring, both in cases of gestational and lactational exposures. According to the data presented in Table 3, the average of change in MDA and SOD in the pups of gestational exposure was accounted to 11.6% and 15.1%, respectively, compared to 12.8% and 13.3%, respectively for the pups of lactational exposure (based on calculating the average of the values of male and female pups given in the table). For the dams, the change accounted to 13.8% and 16.8% in gestation and 16.4% and 14.3% in lactation. This means that the effect on MDA was more pronounced in ATZ exposure during lactation, while the opposite for SOD which was more pronounced in ATZ exposure during gestation. Interestingly, such trend was obtained either for the dams or their offspring (Table 3). Such findings clearly demonstrate that pups have exercised degree of effects nearly equaled to that their mothers were affected. Indeed, the differences between the two cases compared were small, may be due to the low dose of ATZ used in the present study (31.0 μg kg^{-1}). On the other hand, we compared the results of MDA and SOD in ATZ/vit. E groups with the results of control groups, to assess the ameliorative effect of vitamin E, based on calculating the "Amelioration Index; AI". As AI approaching "1", as the amelioration reaching high degree of normalization to the control value. The obtained results revealed that the AI of MDA and SOD ranged between 0.95 and 1.06 (≈1.0) for the dams and pups either in gestational or lactational exposure routes. Similar assessment could be carried out on BuChE changes due to exposure to ATZ, and the ameliorative efficiency of co-administration of vitamin E. Generally, changes due to ATZ were little and accounted to 1.5–3.4% in gestation and 1.9–4.0% in lactation. The AI was accounted to approximately 1.0 for the two studied cases (Table 3).

Pregnant women are a special risk group, given the findings showing increased risk of childhood acute lymphocytic leukemia when women use pesticides in the home and garden during pregnancy [74]. On the other hand, lactation may pose stress to the mothers and such mothers will be more vulnerable, than non-lactating mothers, to other chemical stressors [75]. It is documented that the fetus is more vulnerable to the toxic effects of a number of environmental exposures than are children or adults [76]. Highly lipophilic compounds are expected to cross the placenta and reach the fetus during pregnancy [77,78]. Also, there are many factors involved in the transfer of chemicals into the milk and subsequently to the suckling neonate, including lipophilicity, ionization and maternal plasma protein binding, which can all influence transport of the compound during lactation [79]. ATZ is a lipophilic compound and has a low molecular weight compatible with passive diffusion and transfer from the dam to the pup [80,81]. In their studies on gestational and/or lactational exposure of Sprague Dawley dams to ATZ (0, 1, 5, 20, or 100 mg kg^{-1}/day), Fraites et al. [21] concluded that placenta does not appear to limit the transfer of ATZ and its metabolites from the maternal circulation to the developing fetus. Also, levels of ATZ and the metabolites were found in neonatal milk, plasma and tissues. It worthy to mention that chlorinated metabolites of ATZ [e.g., diethylatrazine (DEA), di-isopropyl-atrazine (DIA), and diaminochlorotriazine (DACT)] are considered equivalent in toxicity to ATZ, and exposure to metabolites are also of concern [19,82]. Atrazine and its metabolites were found to contaminate human blood in Japan [83], women mammary fat tissue in Argentina [84] and milk in British mothers (around 0.5.2.3 μg/g) [85].

In the present study, the pups were exposed to ATZ intoxication via placenta and breast milk in the experiment of gestational exposure, and via breast milk only in the second experiment (lactational exposure). In an attempt to elucidate which stage of exposure (gestation or lactation) to ATZ was more affected, we estimated alteration of MDA and SOD (as examples) following ATZ treatments, based on the data presented in Table 3. The average of change in MDA and SOD in the pups of gestational exposure was accounted to 11.6% and 15.1%, respectively, compared to 12.8% and 13.3%, respectively for the pups of lactational exposure (based on calculating average of the values of male and female pups given in the table). For the dams, the change accounted to 13.8% and 16.8% in gestation and 16.4% and 14.3% in lactation. This means that the effect of ATZ on MDA was more pronounced during lactation, while the opposite for SOD which was more pronounced during gestation. Interestingly, such trend was obtained either for the dams or their offspring (Table 3). Performing similar calculations with ALP may give additional picture. In gestational exposure experiment, ALP activity equaled 75.3 and 56.1 IU/l for control dams and their pups (without sex differentiation), respectively. ATZ-treated groups showed 101.7 and 125.3 IU/l, respectively (Fig. 1a). In lactational exposure experiment, these values equaled 73.3 and 54.5 IU/l, respectively for control animals, and 95 and 122.3 IU/l for ATZ-treated groups (Fig. 1b). This means that, in gestation experiment, the change in ALP activity was accounted to 35.1% and 123.4% for dams and their pups, respectively, while changes in ALP activity in lactation experiment reached 29.6% and 124.4%, respectively for dams and their pups. This means that the effect of ATZ on ALP in mouse dams was higher during gestation than during lactation. The effect on pups-ALP was nearly equal in both gestational and lactational exposure.

Table 3
Assessment of oxidative stress of atrazine and the ameliorative effect of vitamin E, based on measured biochemical parameters from gestation and lactation experimental results.

Mice/parameter	Control (a)	ATZ (b)	ATZ + E (c)	% of change[a]	Ameliorative Index (AI)[b]
Gestation[c]					
MDA (nmol/g tissue)					
Dams	82.00	93.33	87.33	13.8	1.06
Pups (M)	52.33	58.00	55.33	10.8	0.95
Pups (F)	51.33	57.70	54.70	12.4	0.95
SOD (U/g tissue)					
Dams	210.00	174.60	183.33	−16.8	1.05
Pups (M)	190.00	162.70	171.00	−14.4	1.05
Pups (F)	190.70	160.60	168.60	−15.8	1.05
BuChE (U/l)					
Dams	3419.0	3305	3358	−3.3	1.02
Pups (M)	1503.3	1452	1477	−3.4	1.02
Pups (F)	1548.0	1525	1540	−1.5	1.00
Lactation[c]					
MDA (nmol/g tissue)					
Dams	81.33	94.7	86.0	16.4	0.91
Pups (M)	53.00	58.3	54.7	10.0	0.94
Pups (F)	51.00	59.0	55.0	15.7	0.93
SOD (U/g tissue)					
Dams	210.7	180.6	187.0	−14.3	1.03
Pups (M)	190.6	167.0	177.6	−12.4	1.06
Pups (F)	190.3	163.3	173.3	−14.2	1.06
BuChE (U/l)					
Dams	3379	3298	3341	−2.4	1.01
Pups (M)	1487	1427	1448	−4.0	1.01
Pups (F)	1544	1514	1527	−1.9	1.00

[a] Percent of change = $(b - a)/a \times 100$.
[b] Ameliorative Index (AI) = c/a.
[c] Data under gestation and lactation are mean values for the respective biochemical parameters.

Table 4
Histopathologic changes based on scoring severity of injury in different organs from mouse dams and their pups following exposure to atrazine with and without vitamin E during gestation.

Histopathological changes	Treatments					
	ATZ			ATZ + vit. E		
	Dams	Pups (M)	Pups (F)	Dams	Pups (M)	Pups (F)
Liver						
Hepatic degeneration of hepatocytes	1	1	1	0	1	0
Vacuolar degeneration of hepatocytes	2	2	2	1	0	0
Perivascular mononuclear cells infiltration	2	0	0	0	0	0
Fibroblast proliferation around bile duct	0	0	2	0	0	0
Focal hepatic haemorrhage	0	0	0	0	0	0
Kupffer cells activation	1	0	0	1	0	0
Kidneys						
Vacuolation of epithelial lining renal tubules	2	3	3	0	0	0
Vacuolation of endothelial lining glomerular tuft	2	2	2	0	0	0
Focal renal haemorrhage	2	0	0	0	0	0
Thickening of parietal layer of Bowman's capsule	2	0	0	0	0	0
Congestion of intertubular blood vessels	0	0	1	0	0	0
Ovary						
Degeneration of corpus luteum	2	0	0	0		
Congestion of blood vessels	2	0	0	0		
Numerous follicles	0	0	0	2		
Testis						
Degeneration of spermatogoneal cells	2	0				
Thickening of basement membrane of seminiferous tubules	2	0				
Small diameter seminiferous tubules	1	0				
Intratubular odema	0	0				

Degree of changes: (0) = no change; (1) = mild change (e.g., <25% of cells showing damage); (2) = moderate change (e.g., 25–50% cell damage); and (3) = severe change (e.g., >50% cell damage).

Table 5
Histopathologic changes based on scoring severity of injury in different organs from mouse dams and their pups following exposure to atrazine with and without vitamin E during lactation.

Histopathological changes	Treatments					
	ATZ			ATZ + vit. E		
	Dams	Pups (M)	Pups (F)	Dams	Pups (M)	Pups (F)
Liver						
Hepatic degeneration of hepatocytes	0	0	1	0	0	0
Vacuolar degeneration of hepatocytes	3	2	2	0	0	0
Perivascular mononuclear cells infiltration	0	1	0	0	0	0
Fibroblast proliferation around bile duct	0	1	0	0	0	0
Focal hepatic haemorrhage	3	3	0	0	0	0
Kupffer cells activation	0	0	0	0	1	0
Kidneys						
Vacuolation of epithelial lining renal tubules	3	3	3	1	0	0
Vacuolation of endothelial lining glomerular tuft	2	2	1	1	0	0
Focal renal haemorrhage	3	0	0	0	0	0
Thickening of parietal layer of Bowman's capsule	0	0	0	0	0	0
Congestion of intertubular blood vessels	2	0	0	0	0	0
Ovary						
Degeneration of corpus luteum	2	1	0		0	
Congestion of blood vessels	3	0	0		0	
Numerous follicles	0	0	0		3	
Testis						
Degeneration of spermatogoneal cells	2	1				
Thickening of basement membrane of seminiferous tubules	1	0				
Small diameter seminiferous tubules	2	0				
Intratubular odema	3	0				

Degree of changes: (0) = no change; (1) = mild change (e.g., <25% of cells showing damage); (2) = moderate change (e.g., 25–50% cell damage); and (3) = severe change (e.g., >50% cell damage).

Indeed, the response of the antioxidant enzymes (e.g., MDA and SOD) to ATZ was lower than the response of ALP, and that may refer to the low dose of ATZ used in the present study (31.0 $\mu g\,kg^{-1}$), and the different response of such biomarkers to a toxicant such as ATZ.

Exposure of mice dams to ATZ at the equivalent dose to its ADI (ca. 0.005 $mg\,kg^{-1}$ bw/d) has resulted in noticeable histopathological effects on liver, kidney, ovary and testis as illustrated in Fig. 6 and Tables 4 and 5. The offspring from the treated dams generally exercised nearly similar effects. Histologically, it has been reported that ATZ caused liver degeneration represented by chronic interstitial inflammation, lymphocyte and eosinophil infiltration, and narrowing and irregular forms of bile canaliculi [49,86]. Also, it induced multiple ovarian follicular cysts and persistence of corpus luteum [87], as well as kidney dysfunction represented by an increase in the protein content of the urine in rats treated for 14 days with 10 $mg\,kg^{-1}$ of ATZ per day [88]. In the present study, biochemical alterations observed in ATZ-treated mice and normalization levels of these biochemical parameters after administration of vitamin E corroborated the results of histopathological findings, where vitamin E supplementation repaired the impairment of ATZ to the studied organs.

5. Conclusion

In light of the results of the present study, it can be concluded that exposure of adult female mice to contaminated food with ATZ at its acceptable daily intake (ADI) level can induce hepatic and renal oxidative stress and histopathological effects. Since mouse pups from dams exposed to ATZ during lactation were dependent only on breast feeding, the findings of the present investigation reveal that toxic effects of ATZ, as well as ameliorative effect of vitamin E, occurred via lactation process. In pups from dams exposed to ATZ during gestation, the toxic effects of ATZ, as well as ameliorative effect of vitamin E, occurred mainly via gestation and probably through the mother's milk also. In light of the results of the present study, it may be difficult to conclude, in a general statement, which exposure stage was much affected following exposure to ATZ. But we can suggest which stage was more vulnerable to alteration of a specific parameter (e.g., MDA, SOD, ALP, etc.). For instance, the effect of ATZ on ALP in mouse dams was higher during gestation than during lactation. The effect on MDA was more pronounced in ATZ exposure during lactation, while the opposite for SOD which was more pronounced in ATZ exposure during gestation. The response of MDA and SOD was similar either for the dams or their offspring. Histologically, ATZ induced impairment in tissues of the experimental dams and their offspring, both in those exposed during gestation and lactation. Taking into consideration that exposure of dams to ATZ during gestation was followed with a withdrawal period (ca. 21 days), this may lead us to consider that gestation stage was more affected than lactation stage. The findings may support the need to further investigating the adverse effects of exposure to low doses of commonly used pesticides, especially during pregnancy and breast-feeding as well as effects on newborn child.

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Conflict of interest

The authors declare that no conflicts of interest.

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