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Histophysiological alterations in some tissues of male wistar albino rats exposed to atrazine

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Abstract
Atrazine is one of the most commonly used herbicides in the US and the world. More than 76 million pounds are used on US fields every year, particularly in the Central Plains. Because of its selective nature in India the use of Atrazine is tremendously increased, but unawareness and accidents may result into toxic effects. Therefore an attempt was made to study the sub chronic effect on histophysiology of some tissues in male Wistar Albino rats exposed to Atrazine. Experimental rats were given oral dose of 25% (124 mg/kg/body weight) of LD50 (3090 mg/kg/body weight) of the Atrazine dissolved in water for 120 days. The study shows histological alterations in testis, kidney and liver of rats. The testis exhibited dilation of the seminiferous tubules and atrophy of testis and various degenerative changes. Examination of kidney sections showed atrophy of a glomerulus and degenerated renal tubules. Hepatic cells of liver show vacuolar degeneration of hepatocytes diffuse necrosis and degeneration of the hepatic portal vein.

Keywords: Herbicides, Atrazine, Histology, liver, kidney, Testis.

1. Introduction
Atrazine is an organophosphate herbicide which is extensively used against long and broad leaf herbaceous plants in corn fields and gardens. This herbicide is most efficient against weeds when applied prior to growth. In some areas, Atrazine is used for the selective control of weeds in re-establishment of pine forest, cultivation of Christmas trees, long leaf seed fields. In addition, it is used in dry areas as a nonselective herbicide. Atrazine is a common name for 2-chloro-4-(ethylamino)-4-(isopropylamino)-s-triazine. Its chemical formula is C8H14ClN5 (Cai *et. al*. (2003) [1]. This herbicide was first introduced in 1958. Its commercial name is 'Atrax' and in Iran is marketed under the name of 'Gesaprim'. This compound is a white crystalline solid with the solubility of 33 ppm in water at 27˚C. Its molecular weight is 215.68gr/M; Atrazine does not degrade significantly in ground water and in surface water. It has a half-life of more than 200 days to 2 years (Agency for Toxic Substances and Disease Registry, 2003) [2]. Atrazine is absorbed through roots and transmitted via apoplast; it is also absorbed through leaves. This herbicide inhibits plant growth through interference with photosynthesis and leaf death. Other effects on the leaves include membrane and chloroplast destruction. The translocation of Atrazine from soil and water to plants and aquatic animals, results in its entry into food chain and bioaccumulation. Chronic exposure of this herbicide leads to non-Hodgkin type lymphoma, multiple myeloma and sarcoma in agricultural workers (Chiu *et. al.* 2004) [3]. Since Atrazine is now most commonly used in the world to control weeds therefore the relative risk and benefits of this herbicide must be compared to the existing herbicides. Studies show that Atrazine causes adverse effects on the liver, kidney and cardiovascular system in animals exposed to it (Chan *et al*. 2006) [4]. However, the patterns of accumulation of xenobiotics varies depending on the organism, characteristics of the chemical compound, a quantity of this substance present in the environment, and the balance between assimilation and metabolic rates (Nwani *et al*., 2011) [5]. The demand of herbicide is increasing day by day in India. Anything that is in excess is always harmful, so with the tremendous and repeated use of herbicides now adding one more head to environmental pollution. Accumulation bioaccumulation of herbicides may have toxic effects on animals, plants or human beings, those who directly or indirectly get exposed to it. So considering the effect of

herbicide, work was carried out to study the effect of sub-chronic exposure of Atrazine on albino male Wistar rats with reference to variation in histophysiology of testis, kidney and liver.

2. Material and methods

Healthy Wistar rats (male) were acclimatized to laboratory condition. During acclimatization rats were provided with food and water ad libitum. The animals were kept in clean polypropylene cages (measuring 12"x10"x8") with chrome plates grills. The rats were grouped in to two groups, six rats in each group; one group was kept as control whiles other as experimental. Experimental rats were given the 25% (124 mg/kg/body weight) of LD50 (3090 mg/kg/bw) dose of the Atrazine (dissolved in tap water) for 120 days. The control rats were sacrificed on 120th day. Whereas experimental rats were sacrificed on the 121st day after giving 120 days oral dose (according to rules of ethical committee registration no. is 1060/ac/07/CPCSEA). The tissues like liver, testis & kidney of male rats were removed, fixed in Bouin's fixative for at least 24 hrs, processed by paraffin wax impregnation method, cut using a rotary microtome at 5 µm thickness and stained with Hematoxylin and Eosin (H X E) for light microscopic examination.

3. Results

Male Wister rats were exposed to Atrazine by giving a dose 25% (124 mg/ kg/bw/day) of LD50 (3090 mg/kg/bw), for 120 days and then the animals were sacrificed for a tissue. When rats were dissected it has been observed that the testis were shrunk, (comparative reduction in weight), development of edema on kidney and liver, stomach filled with fluid having pungent smell was observed in all most all experimental rats.

3.1 Histological changes

3.1.1 Alteration in Testis architecture

T.S. of testis shows normal cellular architecture, with normal seminiferous tubules, germinal epithelial cells, primary and secondary spermatocytes in control rats (Fig. 1a). T.S. of testis of Atrazine treated rats exhibited atrophy of testis, dilation of seminiferous tubules and various degenerative changes (Fig. 1b).

3.1.2. Alteration in kidney architecture

Transverse section of kidney of control rats revealed normal renal tubule and glomerulus (Fig. 2a). In Atrazine treated rats kidney shows atrophy of a glomerulus and degenerated renal tubules. (Fig. 2b).

3.1.3. Alteration in Liver architecture:

Hepatocytes of liver in control rats were polygonal in shape, mononucleate or binucleate (Fig.3a). In Atrazine herbicide treated rats liver shows vacuolar degeneration of hepatocytes, diffuse necrosis and degeneration of the hepatic portal vein. (Fig.3b).

4 Discussion

Male Wistar rats were exposed to Atrazine by giving a dose 25% (124 mg/ kg/bw/day) of LD50 (3090 mg/kg/bw) for 120 days and then the animals were sacrificed for tissues. During the period of exposure of Atrazine rats were observed for their behavioral changes. No Change in food intake and no significant change in weight were observed. According to Cantemir many rat studies involving acute, intermediate or chronic exposure to Atrazine in the diet or by gavage showed mild to severe weight loss Cantemir C *et al.* [6]. No mortality was observed during exposure period. After giving the oral dose, rats showed symptoms of lethargy, red nasal and ocular discharge, dehydration and pasty diarrhea. Hyperactivity was seen just after the administration of dose for 5-10 minutes and rats became more aggressive. After long term exposure (nearly after 90 days of exposure) hair fall has been observed. Williams observed that Atrazine is a skin sensitizer and cyanazine which is toxic by the oral route Williams SC *et al.* [7]. Overexposure to triazine herbicides (atrazine, simazine, propazine) may induce fatigue, dizziness, nausea, irritation of the skin, eyes and respiratory tract, allergic eczema or asthma. Schlicher revealed that a 40-year-old white male farmer developed blisters on his hands and forearms, one afternoon after having applied Atrazine to crops in the morning using a spray ring and cleaning the plugged nozzles several times with his hands Schlicher JE *et al.* [8].

In case of Atrazine treated rats it has been also observed that excreta were semisolid with pungent smell. Bakke investigated specific data on elimination and excretion of Atrazine by any route was limited. However, the primary route of excretion appears to be in urine, as indicated by the detection of urinary Atrazine and its metabolites in a number of species exposed via oral and dermal routes Bakke JE *et al.* [9].

When rats were dissected it has been observed that the testis were shrunk, (comparative reduction in weight), development of edema on kidney and liver, stomach filled with fluid having a pungent smell was observed in all most all experimental rats. Wilhelms findings indicate that the absorption of Atrazine in humans following oral exposure was indicated in a single case report of a 38-year-old man who died of progressive organ failure three days after ingesting 500 ml of a weed killer that contained 100 g Atrazine, 25 g of aminotriazole, 25 g of ethylene glycol, and 0.15 g of formaldehyde Wilhelms KW *et al.* [10].

T.S. of testis shows normal cellular architecture, with normal seminiferous tubules, germinal epithelial cells, primary and secondary spermatocytes in control rats (Fig. 1a). T.S. of testis of Atrazine treated rats exhibited atrophy of testis, dilation of seminiferous tubules and various degenerative changes (Fig. 1b). According to Kniewald Atrazine causes changes in sperm morphology and a reduction in sperm motility [11].

T.S. of kidney of control rats revealed normal renal tubule and glomerulus (Fig. 2a). Atrazine treated rats show atrophy of a glomerulus and degenerated renal tubules (Fig. 2b). Toxicological Profile for Atrazine were observed sub-acute glomerulitis and degeneration and desquamation of the proximal tubules in female pigs receiving 2 mg/kg/day atrazine in the diet for 19 days Toxicological Profile for Atrazine [12].

Hepatocytes of liver of control rats were observed polygonal in shape, mononucleate or binucleate. (Fig.3a). While Atrazine herbicide treated rats, shows vacuolar degeneration of hepatocytes, diffuse necrosis and degeneration of the hepatic portal vein. (Fig.3b). Toxicological Profile for Atrazine reported Intermediate-duration exposure of pigs to 2 mg/kg/day resulted in a 35% increase in serum γ-glutamyltransferase activity and mild histopathological changes, including chronic interstitial inflammation, lymphocyte and eosinophil infiltration, and narrowing and irregular forms of bile canaliculi in liver Toxicological Profile for Atrazine [12].

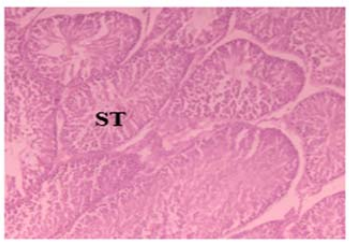
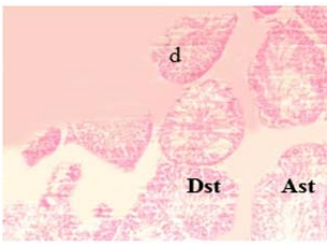


Fig. 1a:



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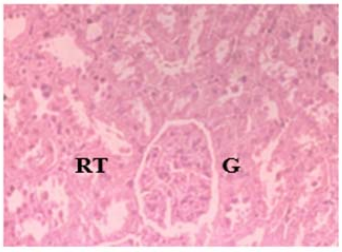

Fig. 2a:

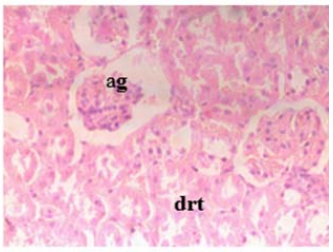

Fig. 2b:

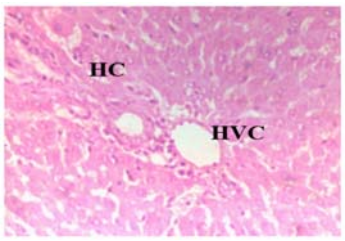

Fig. 3a:

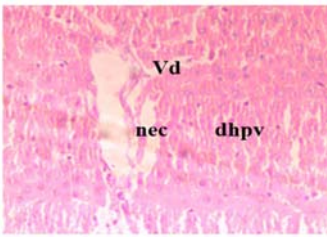

Fig. 3b:

(**Fig 1a):** T.S. Testis of control rat showing normal seminiferous tubule (**ST**), **(Fig. 1b):** T.S. Testis of experimental rat showing distraction of seminiferous tubule **(Dst),** atrophy of testis **(At)** various degenerative changes **(d) (Fig. 2a)**: T.S. Kidney of control rat showing Glomerulus (**G**), Renal tubule **(RT) (Fig. 2b):** T.S Kidney of experimental rat showing atrophy of a glomerulus **(ag)** and degenerated renal tubules **(drt) (Fig. 3a):** T.S. Liver of control rat showing regular hepatic cords (**HC**), Hepatic portal vein (**HPV**), **(Fig. 3b):** T.S. Liver of experimental rat showing vacuolar degeneration of hepatocytes **(Vd)**, diffuse necrosis **(nec)**, degeneration of the hepatic portal vein **(dhpv).** (Stain- Hematoxylin- eosin, magnification- X 100**)**

5 Conclusion

Atrazine Herbicide Causes considerable variation in histological structure of Liver, Kidney and Testis therefore there should be banned or restriction on the use is essential in an Agricultural field as well as public places.

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