HİSTOPATHOLOGİCAL CHANGES OF THE TESTES İN COMMONLY USED HERBİCİDES (GLUFOSİNATE AND IMAZAMOX) EXPOSURE

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ABSTRACT

Glufosinate and imazamox are the most widely used synthetic insect pesticides. The widespread use of these pesticides has led to more research into the possible effects of reproductive toxic activities. Therefore, this study was conducted to evaluate and compare the acute effects of glufosinate and imazamox on the testis, the main organ of male reproduction. We studied the acute toxicity of an imazamox and glufosinate based herbicide at 7, 14 and 21 mg/kg bw/day imazamox and glufosinate equivalent dose on the testes tissue in Sprague Dawley rats. Rats were injected with 3 different doses of glufosinate and imazamox and the animals were sacrificed after 48 hours. Rats were decapitated rapidly under deep anesthesia and the testes were fixed in 10% formaldehyde and hematoxylin and eosin (HE) used stain in histopathology. According to our results, there was a difference between the control group and imazamox and glufosinate groups. The testis of control rats had a normal histological appearance whereas necrotic and degenerative lesions and decrease in spermatocytes, spermatids were detected in the 7 and 21 mg/kg bw/day groups. It was observed that treated with imazamox 21 mg/kg bw/day was seen higher pathological changes than the glufosinate group. Our data shows that commercial formulations of imazamox and glufosinate containing various co-formulants can have testicular toxic effects.

Keywords: glufosinate, imazamox, histopathology, testes

1.Introduction

Testicular toxicity due to pesticides often used in agriculture to kill harmful insects such as organophosphates is a serious concern [1]. Studies reported that pesticides such as dimethoate [2], phorate [3], phosphamidon [4], diazinon [5], malathion [6] and methyl parathion [7, 8] cause degenerative effects on the testicular functions in experimental animals.

Imazamox is a member of the imidazolinone class of chemicals including imazapic, imazapyr, imazethapyr, imazamethabenz and imazaquinine [9, 10]. Glufosinate is a salt of 2-amino-4-(hydroxymethylphosphinyl)-butanoic acid [11]. Because of the increasing number of resistant weeds, alternate herbicide-resistant crops and herbicides with different modes of action are required to protect crop yield [12]. They are the most important pesticides and has a wide diversity of uses in agriculture.

According to the world health organization (WHO) and United States Environmental Protection Agency this compound is categorized as a “Class III, slightly hazardous” for Glufosinate, “Class III, IV, slightly-to-moderately hazardous’’ for Imazamox for acute term toxication[13, 14] KEMI stated that glufosinate is toxic to reproduction and possible risk of impaired fertility[15].

In the present study to investigate the effects of an glufosinate and imazamox-based herbicide on testes tissues on rats. Testes tissues were assessed by organ histology. We found clear evidence of testis structure damage at high dose tested, In 7, 14 and 21 mg/kg bw/day imazamox-based herbicide groups, reduced germ cells, spermatocytes, spermatids and desquamations were observed.

2. Material-Method

Animal preparation and experimental design: This study was conducted at the Medical Experimental Research Center in Ataturk University (Erzurum, Turkey). The ethical committee of Ataturk University approved the study protocol (42190979-01—02/2411). 35 adult male Sprague-Dawley rats (8-12 weeks old, 250-300 g, DAYTAM, Erzurum, Turkey) were used in the present study, and rats divided 7 groups that each group has five rats; group 1 (control), group 2 (Glufosinate 40%), group 3 (Glufosinate 80%), group 4 (Glufosinate 120%,), group 5 (Imazamox 40%), group 6 (Imazamox 80%), group 7 (Imazamox 120%). The rats were decapitated in terms of 48 hours after undergoing sevoflurane anesthesia then testes were isolated and put formaldehite.

Histopathological analyses: For this purpose, testes tissue were fixed in 10% formalin. After 48 h of fixation, the tissue samples were dehydrated, cleared, and embedded in paraffin. Paraffin blocks were cut into 5 µm thick using a Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany) and stained in Hematoxylin and Eosin. The stained specimens were examined under a light microscope (Nikon eclipse i50, Tokyo, Japan) and photo images were taken for histopathological evaluation. Johnsen testis biopsy score was used for histopathological evaluation. Presence of spermatozoa scores 10, 9 or 8; spermatids (and no further) 7 or 6; spermatocytes (and no further) 5 or 4; only spermatogonia 3, only Sertoli cells 2 and no cells 1.

Toxic agent: An Imazamox-based herbicide (Intervix® Pro) was purchased from BASF company (Turkey) and contained 40 g/L of imazamox (5-(methoxymethyl)-2-(4-methyl-5-oxo-4-propan-2-yl-1H-imidazol-2-yl)pyridine-3-carboxylic acid). Glufosinate-based herbicide was purchased from Agrobest LTD, Turkey and contained pure glufosinate (CAS Number: 51276-47-2, Product code: FP16278) was used in the experiments.

Statistical analysis: For histopathological analysis, differences in measured parameters between the groups were analyzed with a nonparametric test (Kruskal–Wallis). Dual comparisons between groups exhibiting significant values were evaluated with the Mann–Whitney U-test (P <0.05).

3. Results and Discussion

In the present study, we investigated the glufosinate and imazamox-based herbicide toxicity on the testes. The results of the histopathological examination of the testes tissues are presented in Figure 1 and 2, Table 1 and 2. Our data show a statistically significant difference between the control group and the groups that received the all dose of glufosinate and imazamox-based herbicide 36 mg/kg for 48 and 72 hours (Fig. 1 and Fig. 2, Table 1 and Table 2; P <0.05). There was a significant difference between 7 and 21 mg/kg bw/day glufosinate-based herbicide group while no significant difference were between 7, 14 and 21 mg/kg bw/day groups (Table 1, P<0.05). While there was a significant difference between 7 and 21 mg/kg bw/day imazamox-based herbicide group, there was no difference between 14 mg/kg bw/day group (Table 2, P<0.05).

Histopathologically, both control groups had normal histological appearance (Fig 1a, Fig 2a). The decrease in germ cells was observed in the glufosinate-based herbicide treated with 7, 14 and 21 mg/kg bw/day groups. Spermatocytes, spermatids decreased in number and degenerative changes were found (Fig1 a, b, c, d). In 7, 14 and 21 mg/kg bw/day imazamox-based herbicide groups, reduced germ cells, spermatocytes, spermatids and desquamations were observed (Fig. 2 b,c,d). It was observed that the pathological changes were higher in the 21 mg/kg bw/day imazamox-based herbicide group than glufosinate-based herbicide group (Fig 1d, Fig 2. d).

4. Discussion

Testicular degeneration is a common cause of subfertility and infertility. Testicular degeneration (TD) can be defined as a process that occurs as a result of a deterioration in the structure of the testis or testicular function[16]. In some cases, TD is occured as secondary after testicular trauma,

exposure to heat, cold, radiation, toxins or ischemia, some nutritional deficiencies, exogenous androgens, infection, autoimmune disease, sperm outflow obstructions and neoplasia[17-19]. One of the causes of testicular degeneration is pesticides. Joshi et al reported that chlorpyrifos lead toxic/ suppressive effects on testicular function and causes to infertility in rats[1], while Latchoumycandane et al shown that the adverse effect of methoxychlor on the male reproduction system [20]. In men with no history of infertility of environmental chemicals, the mean sperm count has decreased significantly from 113×10^6/mL to 66×10^6/mL [21, 22]. These are called endocrine disrupters and are known to have an effect on the reproductive potential [23]. Due to the persistence of these substances, soil and water are at risk of for human and wildlife [24]. In our experiment, the exposure of glufosinate and imazamox-based herbiside (7-21mg/kg bw/day, for 48h) reduced germ cells, spermatocytes, spermatids and desquamations were observed. These results agree with the well-known toxic effects of the herbicides tested. Recently, we have demonstrated that glufosinate-based herbicide has the most toxic effect on the testis degeneration than the imazamox-based herbicide. Further studies are necessary whether these acute herbicides toxicity effect on infertility or not.

5. Conclusion

This present study insights into the toxicity of imazamox and glufosinate based herbicide induced degeneration in testis and its toxicity in spermatocytes and spermatids. The present evidence shows a decrease in spermatocytes and spermatid numbers. Thus, our study has demonstrated toxic effects of an imazamox and glufosinate herbicide formulation, which is widely used in agriculture and suggest that this pesticide should be used with caution.

Figure legends:

Table 1. Dose-dependent changes in testis of rats exposed to glufosinate-based herbicide

Table 2. Dose-dependent changes in testis of rats exposed to imazamox-based herbicide

Figure 1. The effect of Imazamox on acut toxicity induced alterations in rat testis tissues. a) Normal spermatogenesis in control group b) 21 mg/kg bw/day glufosinate-based herbicide group, reduced number and degeneration in primary spermatocytes (thin arrow) and spermatids (thick arrow). c) 14 mg/kg bw/day glufosinate-based herbicide group, reduced number of primary spermatocytes and spermatids (arrow). d) 21 mg/kg bw/day glufosinate-based herbicide group, moderate degeneration (arrow) and desquamation (arrowhead). HE.

Figure 2. a) Normal spermatogenesis in the control group b) Slight decreased in number of primary spermatocytes (arrow) and spermatids (arrowhead) in the 7 mg/kg bw/day imazamox-based herbicide group. c) Decreased in number and degeneration of primary spermatocytes (arrow) and spermatids (arrowhead) in the 14 mg/kg bw/day imazamox-based herbicide group. d) 21 mg/kg bw/day imazamox-based herbicide group, severe degeneration (arrow) and desquamation (arrowhead). HE.

Table 1

Groups	**(Mean±std)**
Control	9.80±0.44[a]
Glufosinate (7 mg/kg)	8.20±1.48[b]
Glufosinate (14 mg/kg)	6.20±2.28[bc]
Glufosinate (21 mg/kg)	5.00±1.58[c]

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Table 2

Groups	**(Mean±std)**
Control	9.80±0.44[a]
Imazamox (7 mg/kg)	6.40±1.67[d]
Imazamox (14 mg/kg)	6.00±1.58[de]
Imazamox (21 mg/kg)	4.60±0.54[e]

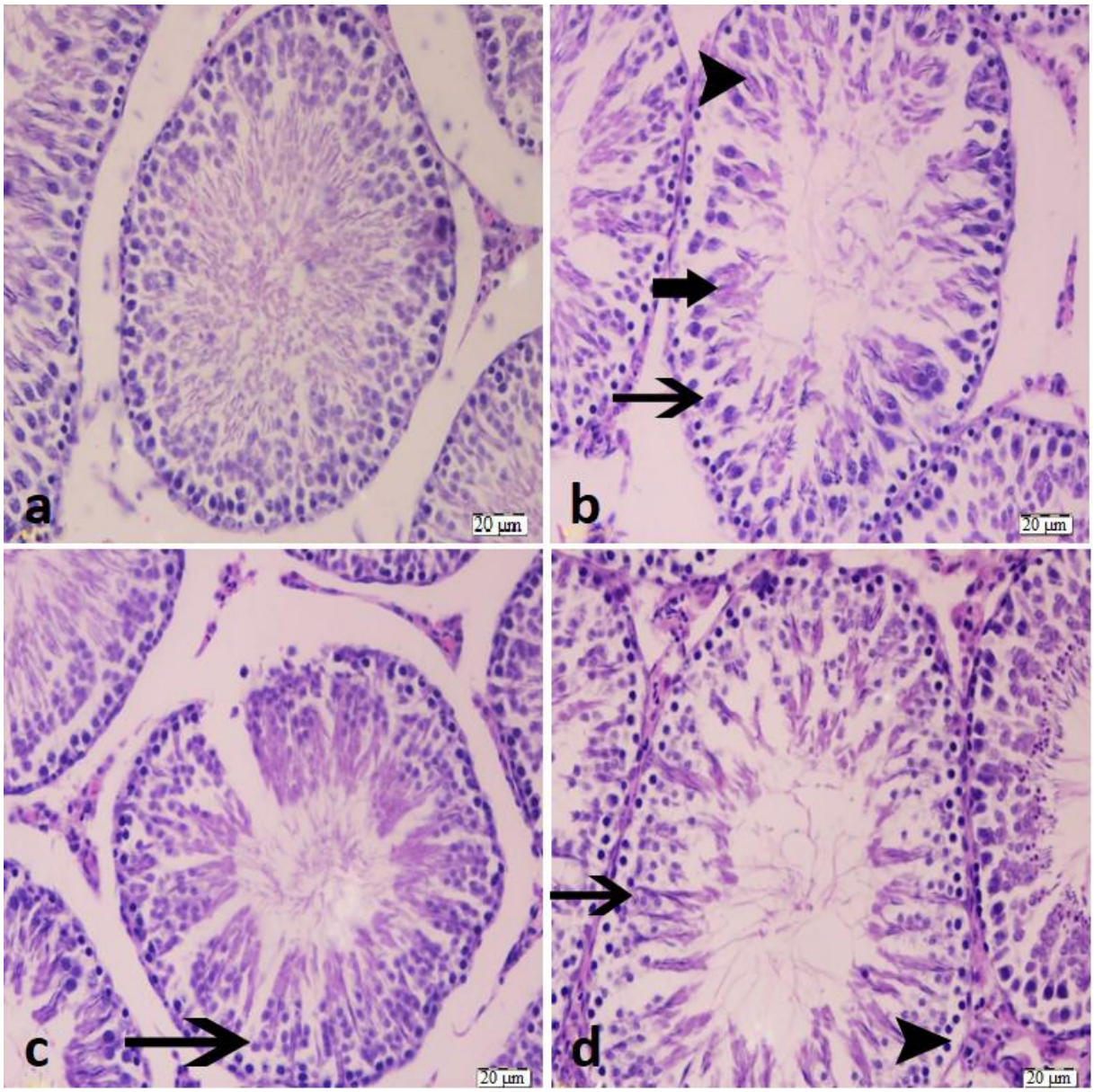

Fig.1.

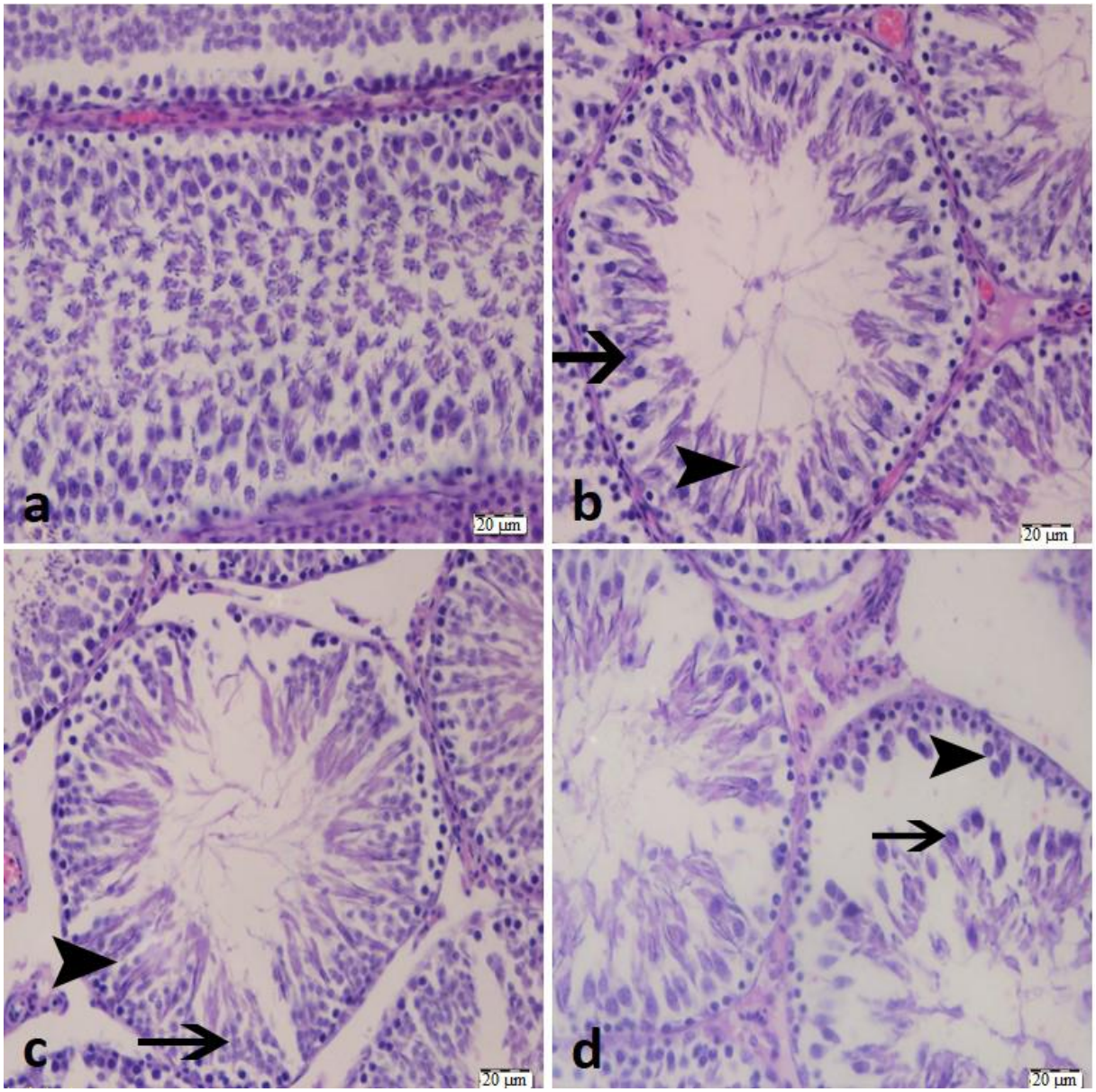

Fig.2.

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