

Research Paper:

Histopathological Assessment of Subacute Doses of Tebuconazole on Hepatotoxicity in Rats



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ABSTRACT

Introduction: Tebuconazole is a systemic fungicide whose toxicity has been reported in animals and humans due to direct and indirect exposure. Our study aimed to assess the hepatotoxic effects of tebuconazole at subacute doses in rats.

Methods: Tebuconazole was administered by oral gavage at doses of 6, 12, 25, 50, and 100 mg/kg daily. Serum levels of ALT, AST, and ALP were determined. Histopathological analysis was performed using liver sections stained with hematoxylin and eosin. Parameters such as cell inflammation, accumulation of lipid vacuoles, and hepatocyte necrosis were assessed.

Results: The results showed that the serum levels of AST, ALT, and ALP enzymes elevated in all Tebuconazole doses compared to the control group. This elevation was statistically significant in the 50 and 100 mg/kg groups compared to the control group. Histopathological results revealed the increased necrosis and destruction of hepatocytes and accumulation of lipid vacuoles in the study groups compared to the control group.

Conclusion: The study's results showed significant hepatotoxic effects at subacute doses of tebuconazole. These results are alarming in the widespread use of tebuconazole as a fungicide. It is recommended to take the necessary precautions, including wearing gloves or a mask.

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1. Introduction

Tebuconazole (TEB) is a systemic triazole fungicide that inhibits ergosterol biosynthesis in the cell membrane of fungi and stops the development of these pathogens [1]. TEB is today widely used in crops and ornamental plants.

The use of pesticides in agricultural environments is a requirement for crop protection. On the other hand, the same safety for crops also endangers human and wildlife health [2]. TEB toxicity occurs when humans or animals have either direct exposure to the toxin or indirect exposure to infected plants. Symptoms of TEB toxicity have previously been described in rats, including restlessness, imbalance, spasm in some organs, and severe asthenia [3]. Metabolism of conazole-triazole fungicides occurs in hepatic microsomes [4].

Triazole fungicides increase the production of free radicals and oxidative stress in the body by significantly reducing glutathione-S transferase (GST) activity. Free radicals produced by oxidative stress lead to lipids, proteins, and DNA oxidation. These alterations eventually lead to cell death through apoptosis or necrosis, causing significant disorders in the body [5].

Moser et al. investigated the effect of TEB on rats' immune, reproductive, and nervous systems in a study, according to which the impact on the nervous system was observed in the behavioral and neuropathological alterations [6]. Sancho et al. showed that short-term exposure to TEB at subacute doses causes physiological disorders in male fish, and the fungicide is a potential neurotoxin [7]. Recently, several case reports have been provided for the extensive hepatotoxic effects of TEB in animals and humans [8, 9]. Accordingly, it seems required to investigate the hepatotoxic effects of unintentional exposure to TEB. The results of this study point out the dangers of this drug and the need for more precautions when using it in agriculture.

2. Materials and Methods

Animals

Sixty male Wistar rats weighing 200-220 g were provided from the animal house of Baqiyatallah University of Medical Sciences, Tehran, Iran. The animals were kept under controlled conditions, including $22\pm 2^{\circ}\text{C}$ and a 12-12h light-dark cycle photoperiod. All institutional and national guidelines for the care and use of laboratory animals were followed. All experiments

were performed following the principles of working with laboratory animals approved by the Ethics Committee of Baqiyatallah University of Medical Sciences.

Study design

The experimental rats were randomly divided into 6 groups (n=10 per group) using a random number table. Rats in each group were placed in special and similar cages of $40\times 20\times 20\text{cm}$. One group was a control group, and the other 5 were considered experimental groups. TEB was administered for each experimental group, dosing by oral gavage at 6, 12, 25, 50, and 100 mg/kg for 30 days. To assess the short-term effects of the drug, five rats were randomly selected at the end of the first week from all groups and deeply anesthetized by injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). A liver biopsy was then performed after blood sampling from the heart. Liver tissue samples were taken from other animals by the above method after the last dose of TEB at the end of the fourth week.

Biochemical analysis

The animals were anesthetized with ketamine-xylazine administration, and a direct blood sample was taken from the heart using a 5ml syringe, with 3-5 ml of blood from each animal. The blood samples were incubated at room temperature for a few minutes and then centrifuged at 5000 rpm for 10 min. Serum was separated from the clot, and samples were stored at -20°C to measure enzymes and biochemical parameters. Serum concentrations of AST, ALT, and ALP were measured by spectrophotometer, and the values of desired enzymes were measured by Pars Azmun ELISA kit.

Histopathological analysis

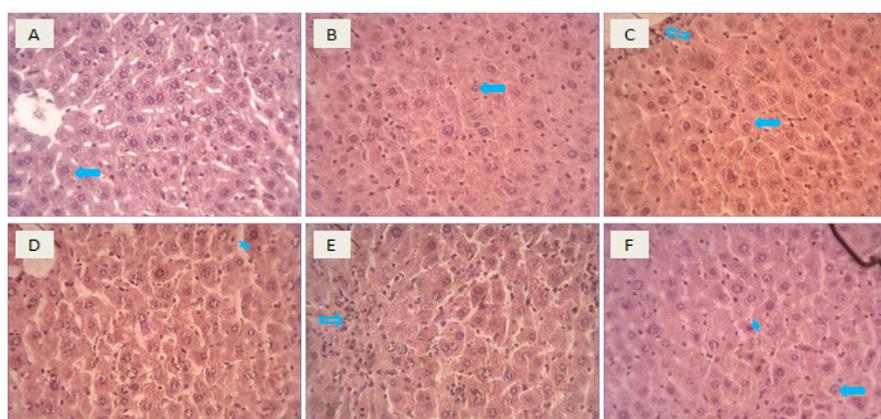
Liver samples were taken from all groups, and they were immediately placed in 10% formalin for fixation and preparation of tissue sections after rinsing with physiological serum. Tissue processing of fixed samples was performed, and tissue sections with a thickness of $5\ \mu\text{m}$ were prepared as a serial section using a rotary microtome. Liver tissues in all study groups were stained with hematoxylin and eosin to analyze their histopathological alterations. Alterations in parameters such as cell inflammation, lipid vacuole accumulation, cell necrosis, disintegration in the portal space, and a considerable distance between cells were analyzed.

Table 1. Mean comparisons of rat serum levels of liver enzymes AST, ALP, and ALT in the groups

Groups	Mean±SEM		
	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control	72±7	158±23	243±31
6 mg/kg	74±9	170±16	250±14
12 mg/kg	79±5	189±23	285±17
25 mg/kg	81±6	266±13*	341±20*
50 mg/kg	93±8*	346±17*	393±32*
100 mg/kg	98±10*	403±21*	460±29*

* P<0.05 compared to the control group.

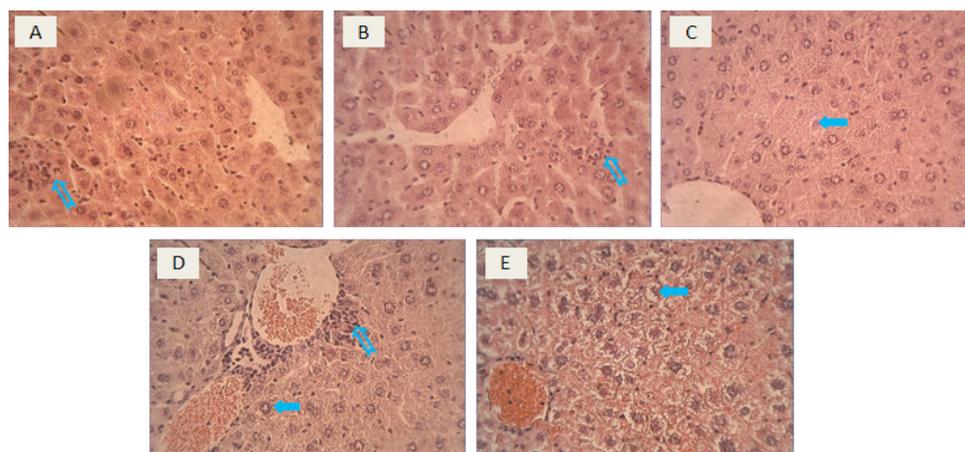
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Figure 1. Photomicrographs of liver sections of rats at the end of the first week (H&E staining)

A: Control group with normal hepatocytes (arrow) arranged in cords and normal sinusoids. B to F: Treatment groups with 6, 12, 25, 50 and 100 mg/kg Tebuconazole respectively. Accumulation of inflammatory cells (hollow arrow) and damaged hepatocytes with eosinophilic cytoplasm and pyknotic nucleus (arrowhead) was shown. (Magnification×400)



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Figure 2. Photomicrographs of liver sections of rats at the end of 4th week (H&E staining)

A to E: Treatment groups with 6, 12, 25, 50, and 100 mg/kg tebuconazole, respectively. Accumulation of inflammatory cells (hollow arrow) and damaged hepatocytes with ballooning degeneration and diffuse fatty changes (arrow) was shown. Microvesicular steatosis is evident in liver tissue. (magnification×400)

Statistic analysis

Data were analyzed by SPSS software using analytical statistics and one-way analysis of variance (One-way ANOVA) using a blind analysis method. The significant level was considered at $P < 0.05$.

3. Results

Statistical analysis of the data showed that serum levels of AST, ALT, and ALP enzymes elevated in all doses of TEB compared to the control group; however, this elevation was not significant at all doses (Table 1). The increase in ALT in the 50 and 100 mg/kg groups was statistically significant compared to the control group. The results also showed that the increase in AST and ALP was significant in the groups receiving 25, 50, and 100 mg/kg TEB compared to the control group.

The histopathological analysis of the liver in different study groups indicated that the liver tissue structure was undamaged and normal in the control group. In the groups receiving oral TEB, various tissue alterations were observed, including accumulation of inflammatory cells around the central vein, sinusoidal dilatation, irregular hepatocyte plates (Remark string), and pyknotic hepatocyte in liver tissue, which were dose-dependent.

Assessment of the short-term (1 week) effects of TEB on liver tissue shows the hepatotoxic effects of this substance even at low doses (Figure 1). Histopathological alterations of the liver in the long-term exposure (4 weeks) to TEB are shown in Figure 2. The results suggested an increase in hepatocyte necrosis, destruction, and accumulation of lipid vacuoles and microvesicular steatosis in the study groups compared to short-term alterations.

4. Discussion

The current study showed that very low doses of TEB could cause significant hepatotoxic alterations in rats. According to our results, TEB elevates serum levels of AST, ALP, and ALT in rats. The elevation in these enzymes, indicators of liver damage, is directly related to the dose of TEB. The results of our study also showed various histopathological alterations in the liver tissue of animals exposed to TEB. The hepatotoxic effects of TEB in the early days are primarily seen in the accumulation of inflammatory cells and alterations in the order of hepatocytes and sinusoids. In the long-term exposure, these alterations were observed in the form

of lipid accumulation, vascular and sinusoidal dilatation, and finally, extensive necrosis of hepatocytes.

As a result, the most destructive effects of these toxins are observed in the liver due to the metabolism location of conazole-triazole fungicides in liver microsomes. However, previous studies have also shown the destructive effects of TEB on other organs in the body. Ronis et al. in a study, identified multiple lesions of renal tissue exposed to TEB. This report indicates the occurrence of diffuse necrosis and severe hyperemia, which may be due to the effect of TEB on cells and their destruction through the mechanisms of inhibition and induction of cytochrome P450 [10]. Yargholi et al. investigated the histopathological effects of TEB on dog liver [8]. The researchers reported that the hepatocytes underwent adipose tissue changes, necrosis, and extensive destruction, which is consistent with our results.

The destructive effects of conazole-triazole fungicides have been reported to a limited extent in humans. Alaa et al. reported that oxidative stress and increased lipid peroxidation were observed in farm workers spraying conazole fungicide. This study showed that exposure to fungicides significantly reduces the activity of the glutathione-S transferase enzyme and induces oxidative stress in the human body by producing free radicals [11].

The hepatotoxic effects of TEB at subacute doses in humans were reported in another case report. In this case report, unintentional exposure of a farmer to a subacute dose of TEB caused a severe elevation in liver enzymes AST, ALP, and ALT in this person. The researchers concluded that unintentional exposure to low doses of TEB could increase an individual's risk of developing hepatitis [9]. Our study results also showed a sharp elevation in liver enzymes during exposure to TEB, which was consistent with this study.

5. Conclusion

The results of our study showed significant hepatotoxic effects at subacute doses of tebuconazole. It is recommended to take the required precautions, including personal protection tools, due to the hazardous effects of this fungicide. It is also recommended to use other fungicides with less toxicity to mammals.

Ethical Considerations

Compliance with ethical guidelines

The Ethics Committee of Baqiyatallah University of Medical Sciences approved this study (Ethical ID: IRIR.BMSU.REC.1398.384).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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References

- [1] Shen Z, Zhu W, Liu D, Xu X, Zhang P, Zhou Z. Stereoselective degradation of tebuconazole in rat liver microsomes. *Chirality*. 2012; 24(1):67-71. [DOI:10.1002/chir.21027] [PMID]
- [2] Aktar MW, Sengupta D, Chowdhury A. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdisciplinary Toxicology*. 2009; 2:1-12. [DOI:10.2478/v10102-009-0001-7] [PMID] [PMCID]
- [3] Fustinoni S, Mercadante R, Polledri E, Rubino F, Mandic-Rajcevic S, Vianello G, et al. Biological monitoring of exposure to tebuconazole in winegrowers. *Journal of Exposure Science & Environmental Epidemiology*. 2014; 24(6):643-9. [DOI:10.1038/jes.2014.14] [PMID]
- [4] Ward W, Delker D, Hester S, Thai s-f, Wolf D, Allen J, et al. Transcriptional Profiles in liver from mice treated with hepatotumorigenic and nonhepatotumorigenic triazole conazole fungicides: Propiconazole, triadimefon, and myclobutanil. *Toxicologic Pathology*. 2006; 34:863-78. [DOI:10.1080/01926230601047832] [PMID]
- [5] Torres MCL, Soares NdFF, Maia JF. Kinetics parameters of Glutathione S-Transferase and its activation by vegetable extracts. *Food Science and Technology*. 2004; 24(2):243-8. [DOI:10.1590/S0101-20612004000200014]
- [6] Barone S, Moser V. The effects of perinatal tebuconazole exposure on adult neurological, immunological, and reproductive function in rats. *Toxicological Sciences*. 2004; 77(1):183. [DOI:10.1093/toxsci/kfh036] [PMID]
- [7] Sancho E, Villarroel M, Fernández C, Andreu-Moliner E, Fernando M. Short-term exposure to sublethal tebuconazole induces physiological impairment in male zebrafish (*Danio rerio*). *Ecotoxicology and Environmental Safety*. 2009; 73(3):370-6. [DOI:10.1016/j.ecoenv.2009.09.020] [PMID]
- [8] Yargholi M, Ali Esfehiani T, Musavi Ss, Erfani AM, Salar Amoli J. [Poisoning of dog by tebuconazole fungicide-a case report (Persian)]. *Iranian Veterinary Journal*. 2016; 11(4):114-9. [DOI:10.22055/IVJ.2016.13022]
- [9] Habibzadeh S, Yazdanbod A, Mohammadshahi J, Maleki N, Ataei S. Hepatotoxicity after exposure to tebuconazole: A case report and brief review. *Hepatitis Monthly*. 2019; 19(7):e94548. [DOI:10.5812/hepatmon.94548]
- [10] Ronis MJJ, Celander M, Badger TM. Cytochrome P450 enzymes in the kidney of the bobwhite quail (*Colinus virginianus*): Induction and inhibition by ergosterol biosynthesis inhibiting. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology, and Endocrinology*. 1998; 121(1):221-9. [DOI:10.1016/S0742-8413(98)10043-9]
- [11] Meccdad AA, Ahmed MH, ElHalwagy MEA, Afify MMM. A study on oxidative stress biomarkers and immunomodulatory effects of pesticides in pesticide-sprayers. *Egyptian Journal of Forensic Sciences*. 2011; 1(2):93-8. [DOI:10.1016/j.ejfs.2011.04.012]

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