TIME-DEPENDENT EFFECTS OF ACUTE ORAL ADMINISTRATION OF AMITRAZ ON SERUM ENZYME ACTIVITY IN WISTAR RATS

Njomza SHAQIRI[1], Zehra HAJRULAI-MUSLIU[2], Icko GJORGOSKI[3], Jane VLAHOV[2], Velimir STOJKOVSKI[2]

[1] *PhD candidate at Faculty of Veterinary Medicine, Ss. Cyril and Methodius University, Skopje*
[2] *Faculty of Veterinary Medicine, Ss. Cyril and Methodius University, Skopje*
[3] *Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, Skopje*
[*] *Corresponding author email*: *nj.shaqiri@gmail.com*

Abstract

This study is based on the investigation of the time-dependent effects of a single oral administration of amitraz (50 mg/kg) on serum enzyme activity in Wistar rats. Amitraz is a widely used acaricide with known neurotoxic and metabolic effects; however, its short-term biochemical impact requires further investigation. In this study, enzymatic activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase (AMY) and lactate dehydrogenase (LDH) were assessed with an automated biochemical analyzer, at the following time points: 2 h, 24 h, 48 h and 7 days after one-time oral administration of amitraz 50mg/kg b.w. Using one-way analysis of variance (one-way ANOVA) to assess differences between experimental groups compared to the control group, the results showed no statistically significant changes in the hepatic and metabolic enzymatic activities assessed at 2 h and 7 days ($p > 0.05$) and significant at 24 h and 48 h time points (from $p < 0.01$ to $p < 0.001$). The results showed a consistent pattern, with the maximum peak observed at 48 h, while after 7 days, although in some cases there is still a slight increase, it is not significant and indicates a normalization of their activity. The results showed, that despite a single oral administration of amitraz, it can still cause hepatotoxicity and pancreatic alterations which appear to be reversible. It can be concluded that oral administration of amitraz causes moderate, time-dependent and reversible biochemical changes, mainly affecting hepatic, pancreatic and metabolic functions. These findings contribute to a better understanding of the acute toxicological profile of amitraz and highlight the importance of assessing the time course in toxicological studies.

Keywords: Amitraz, Wistar rats, Hepatotoxicity, Enzymes, Toxicology

Introduction

Amitraz is a formaldehyde-type pesticide widely used as an acaricide and insecticide in veterinary and agricultural practices [21] with known neurotoxic and metabolic effects. This study is based on the analysis of its acute biochemical impact over time, trying to clarify its toxic effects on animal organs. Previous studies have shown that exposure to amitraz can affect multiple physiological systems, including hepatic and pancreatic functions [1,5]. Pathological mechanisms are mainly observed through α2-adrenergic receptor agonism, leading to central nervous system depression, altered metabolic activity and possible oxidative stress [2,8,13,14]. The liver plays a central role in xenobiotic metabolism and is particularly sensitive to toxic damage, therefore enzymes considered as biomarkers of hepatic function have been tested, such as: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), as well as amylase reflecting pancreatic activity and lactate dehydrogenase (LDH) serving as a general marker of cellular damage [7,12].

Although there are a large number of studies on the acute toxicity effects of amitraz, data on time-dependent biochemical responses are limited. Most previous investigations have focused on subacute or chronic exposure, leaving a gap in understanding the early biochemical changes induced by amitraz. Therefore, the aim of this study was to evaluate the temporal changes in key biochemical parameters in Wistar rats following a single oral administration of amitraz at a dose of 50 mg/kg.

Aim

The study focuses on the acute toxicity of amitraz in ***Wistar*** rats, specifically on the modulation of enzymatic activities and disruption of enzymatic parameters, with the aim of developing further strategies for safe application and control of exposure in animals and potentially in humans, as well as prevention of its toxicity.

Materials and Methods

In this experiment, 50 male Wistar rats with a body weight of 200–250 g were used [22,23]. During the seven-day experiment, the rats were kept under standard laboratory animal conditions and were fed standard laboratory animal diet *ad libitum* [18,19]. Fifty male Wistar rats were divided into five groups, 10 animals each. The first group served as a control and was treated with 2 mL of 0.85% NaCl, while the remaining groups were treated with oral administration of 50 mg/kg body weight of amitraz. Measurements were taken at 2 h, 24 h, 48 h, and after 7 days. For euthanasia, an overdose of ketamine (500 mg/kg, i.p.) and xylazine (50 mg/kg, i.p.) was administered [18,19]. Blood samples were collected from the abdominal artery according to established methods [20]. Enzyme analyses were performed using an automated biochemical analyzer (Chem 200, Gesan, Italy) according to standard clinical chemistry procedures [7].
Statistical analysis was conducted using one-way analysis of variance (one-way ANOVA) to assess differences among control and experimental groups, where the significance of the obtained values is expressed by the *p* value, where values with $p > 0.05$ are insignificant, and those with $p < 0.05$ are significant.

Results

The results of the study on the effects of a single dose application of amitraz on serum enzymes activity in Wistar rats are presented graphically. The statistical significance is compared for each group separately, by comparing the values for the control group with the values for the respective enzyme 2 h, 24 h, 48 h and 7 days after application.

Alkaline phosphatase (ALP): The results of the alkaline phosphatase examination are shown in Figure 1.
The results obtained show that 2 h after treatment in the animals, no significant changes were observed in the activity of alkaline phosphatase compared to the control group (196±21 U/L in the control and 201±21 U/L in the group 2 h after treatment; $p > 0.05$).
As for the group 24 h after treatment, significant changes were observed, with AP activity compared to the control group being increased by 70.4% ($p < 0.001$), from 196±21 U/L in the control to 334±64 U/L in the treated group.
In the third examined group (48 h after treatment), highly significant changes were observed, namely an increase from 196±21 U/L in the control to 476±77 U/L in the examined group, i.e. an increase of 142.9% ($p < 0.001$).
ALP activity tests after 7 days of treatment show slight changes in enzyme activity, but these changes are not statistically significant ($p > 0.05$). Thus, in this group the values are 214±29 U/L compared to the control where they are 196±21 U/L.

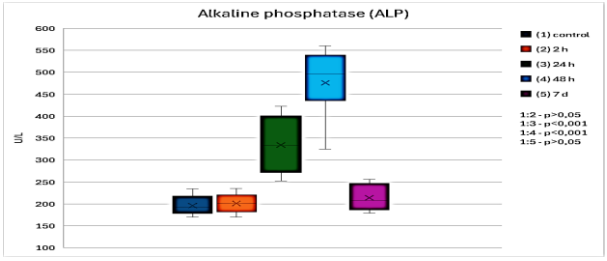

Fig. 1. The effect of a single oral administration of amitraz in Wistar rats on alkaline phosphatase serum activity.

Aspartate aminotransferase (AST): The results of the amitraz examination (Fig. 2) on aspartate aminotransferase activity 2 h after treatment do not show significant changes. The values in the examined group are 138±19 U/L compared to the control where they are 130±20 U/L ($p > 0.05$).

Unlike the previous group, in the group 24 h after treatment, an increase of 80% ($p < 0.001$) was registered, from 130±20 U/L in the control, to 234±55 U/L in the examined group.

A significant increase in aspartate aminotransferase activity was also observed in the third examined group at 48 h after treatment. In this group, the values were 294±81 U/L, i.e. they were increased by 126.2% compared to the control values of 130±20 U/L ($p < 0.001$).

The results obtained from the study 7 days after treatment did not show significant changes compared to the control group (141±22 U/L, compared to 130±20 U/L in the control; $p>0.05$).

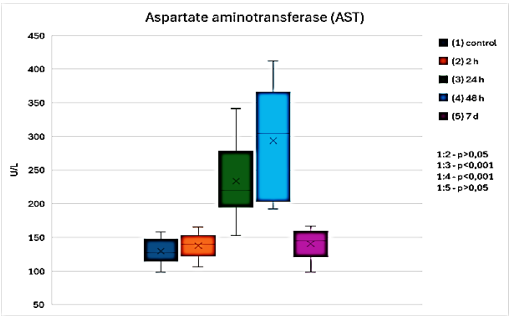

Fig. 2. The effect of a single oral administration of amitraz in Wistar rats on aspartate aminotransferase serum activity.

Alanine aminotransferase (ALT): The results of alanine aminotransferase obtained show that 2 h after treatment, no significant changes were observed in the activity of enzyme compared to the control group (43±7 U/L in the control and 49±9 U/L in the group 2 h after treatment; $p > 0.05$; Fig. 3).

In the group 24 h after treatment, an increase of 39,5 ($p < 0.01$) was registered, from 43±7 U/L in the control, to 60±9 U/L in the examined group.

In the examined group, 48 h after treatment, a significant increase in enzyme activity by 79.1% ($p < 0.001$) was also observed. The values obtained in the examined group were 77±15 U/L, compared to 43±7 U/L in the control.

Unlike the previous two groups, no changes were observed in the group 7 days after treatment, with the obtained values being 47±7 U/L, compared to 43±7 U/L in the control ($p > 0.05$).

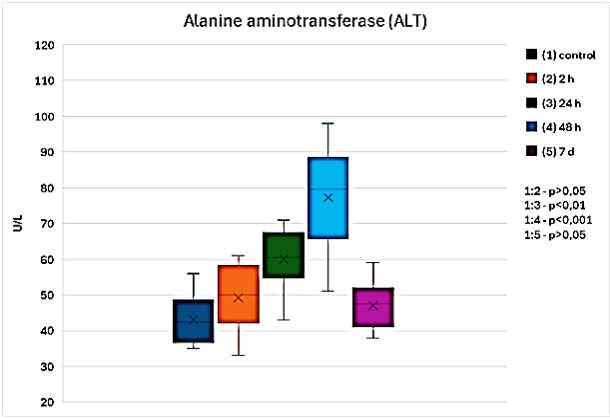

Fig. 3. The effect of a single oral administration of amitraz in Wistar rats on alanine aminotransferase serum activity.

Amylase (AMY): The results of the amylase examination are shown in Figure 4.

The results of the examination on amylase activity 2 h after treatment do not show significant changes. The values in the examined group are 1139±118 U/L compared to the control where they are 1055±117 U/L ($p > 0.05$).

In the group 24 h after treatment, an increase of 46.6 ($p < 0.001$) was registered, from 1055±117 U/L in the control, to 1547±152 U/L in the examined group.

Significant changes were also registered in the group 48 h after treatment. In this group, the enzyme activity is elevated by 119.8% ($p < 0.001$). The values obtained in this group were 2319±352 U/L, compared to 1055±117 U/L in the control.

Unlike the previous two groups, even though there is a slight increase, no changes were observed in the group 7 days after treatment, with the obtained values being 1210±108 U/L, compared to 1055±117 U/L in the control ($p > 0.05$).

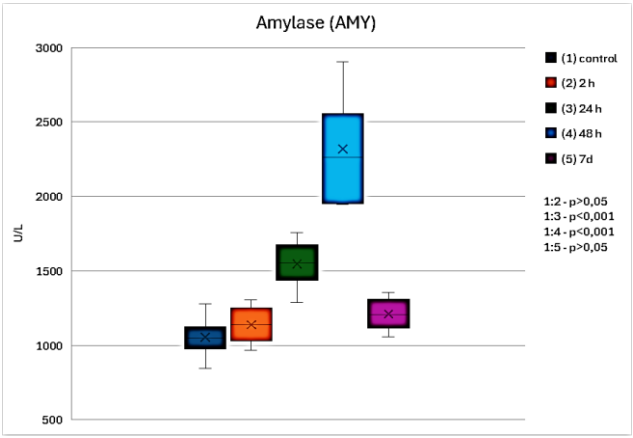

Fig. 4. The effect of a single oral administration of amitraz in Wistar rats on amylase serum activity.

Lactate dehydrogenase (LDH): The results of the amitraz examination (Fig. 5) on lactate dehydrogenase activity 2 h after treatment do not show significant changes. The values in the examined group are 919±136 U/L compared to the control where they are 869±95 U/L ($p > 0.05$).

Unlike the previous group, in the group 24 h after amitraz administration, an increase of 43.3% ($p < 0.001$) was registered, from 869±95 U/L in the control, to 1228±141 U/L in the examined group.

A significant increase in lactate dehydrogenase activity was also observed in the third examined group at 48 h after treatment. In this group, the values were 1820±201 U/L, i.e. they were increased by 109.4% compared to the control values of 869±95 U/L ($p < 0.001$).
The results obtained from the study 7 days after treatment did not show significant changes compared to the control group (928±95 U/L, compared to 869±95 U/L in the control; $p > 0.05$).

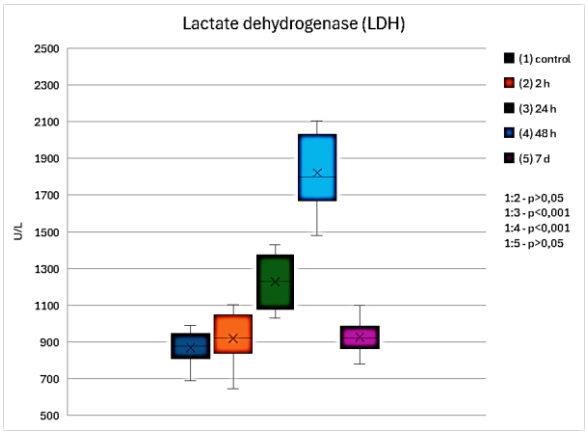

Fig. 5. The effect of a single oral administration of amitraz in Wistar rats on lactate dehydrogenase serum activity.

Discussion

Amitraz is a pharmacologically active compound which has α2-agonist actions. The stimulatory effects of α2-receptors are in partly responsible for neurotoxic and preconvulsant effects [15]. Adverse reactions and side effects have been reported in animals exposed to the product but only a limited number of human intoxication cases have been published in the literature [4]. US Environmental Protection Agency classifies amitraz as slightly toxic by the oral and inhalation routes (Toxicity Category III) and moderately toxic by the dermal route (Toxicity Category II) [10]. The clinical manifestations of amitraz (impaired consciousness, drowsiness, vomiting, disorientation, miosis, mydriasis, hypotension, bradycardia, respiratory depression, hypothermia, generalized seizures, hyperglycemia and glycosuria) can be explained by the agonist action of amitraz on α1 and α2 receptors [11,16]. Management of amitraz poisoning is still considered to be supportive and symptomatic with monitoring of nervous system, cardiovascular and respiratory systems [11].
The reversion of amitraz poisoning in animals is based on supportive therapy, gastrointestinal decontamination, and the use of α2-adrenoceptor antagonists such as yohimbine [3,23] and atipamezole [3]. Yohimbine is reported for reversing the alterations caused by amitraz administration in high concentrations, such as hypotension, bradycardia, emesis, salivation, mydriasis, moderate sedation, hyperglycemia, and in some cases, sinus arrhythmia [23]. Atipamezole is a potent selective α2-adrenoceptor antagonist, not interacting with other receptors. It is a great antagonist of xylazine and medetomidine drugs known for their agonistic activity on α2- adrenergic receptors, as is amitraz [9].
In this study, the primary objective was to analyze the time-dependent effects of amitraz toxicity on the activity of hepatic enzymes AST, ALT and ALP, as well as AMY and LDH.
The available literature contains data on the effect of amitraz on the activity of serum enzymes. However, direct comparison with the results of most studies is not feasible, because the work

was carried out on different animal species and with different concentrations of amitraz (from 50 to 500 mg/kg bw) [6,17].

The results of our research showed that the dose of 50 mg/kg body weight of amitraz caused significant time-dependent changes in biochemical parameters that reflected in a clinical picture of acute and subacute toxicity with liver involvement and systemic involvement, whereby the severity of the changes is different at different time intervals after the administration of amitraz.

Compared to the values of the control group, after 2 h, a not very pronounced increase in enzyme activity is observed, but which indicates cellular stress and mild functional disorders. This is confirmed by the increase in AST and LDH values that determine the early stages of oxidative stress, metabolic disorders and instability in the permeability of cell membranes.

Unlike previous measurements, measurements of enzyme activity after 24 h of treatment, and especially those at 48 h after treatment, show highly significant changes in their activity. The increase in the activity of the examined enzymes indicates that a certain retention of amitraz or its metabolites in the body is possible, for a sufficient time to cause evident changes, i.e. this pattern of changes in enzyme activity is dependent on the kinetics of amitraz degradation in the tissues. However, we will know more precisely what type of mechanism is involved when we examine the concentrations of amitraz and its metabolites in our further research. Also, histopathological analyses of tissues will be of valuable assistance in the comprehensive interpretation of the results, as a direct indicator of their damage. In any case, it can be concluded that a single dose of oral administration of amitraz in Wistar rats causes an increase in enzyme activity, as a basic indicator of changes in tissues and organs, especially in the liver and pancreas.

Conclusion

This study provides evidence that oral administration of amitraz at a single dose of 50 mg/kg body weight causes a time-dependent toxic response that includes marked hepatocellular and systemic damage. The progression of changes in enzymatic activity confirms the transition from early subclinical cellular stress to obvious biochemical damage, peaking after 48 h, followed by partial recovery after 7 days. The simultaneous increase in hepatic (AST, ALT, ALP) and non-hepatic (AMY and LDH) biomarkers indicates that amitraz toxicity involves organs other than the liver, primarily the pancreas, and that the underlying mechanisms are not limited to local hepatic damage, but to widespread oxidative stress and other metabolic deregulation.

The partial biochemical recovery after 7 days highlights the possibility of prolonged and unresolved subclinical damage, which leads to studies on the long-term consequences of acute exposure to amitraz, which may escape short-term studies.

This study continues with histopathological and molecular analyses (of amitraz metabolites) of organs of Wistar rats exposed to a single dose of amitraz, such as brain, liver, kidney, adipose tissue and muscle, which will provide a deeper understanding of the mechanisms of toxicity and specific tissue damage.

From the results obtained in this study, we can conclude that acute amitraz poisoning in Wistar rats causes moderate toxicity, with hepatic and systemic implications, characterized by reversibility in enzyme activity after 7 days of application.

Ethical Approval: *The experiment was performed and the animals were treated in accordance with Approval UP I No. 10-641 dated 22.05.2025 from the Food and Veterinary Agency of the Republic of North Macedonia*

Nomenclature

ALP – Alkaline phosphatase
AST – Aspartate aminotransferase
ALT – Alanine aminotransferase
AMY – Amylase
LDH – Lactate dehydrogenase
U/L – Unite per Liter
SD – Standard deviation
X̄ - Mean
n- number of experimental animals
h - hours
i.p – international administration
NaCL – Sodiom chloride
ANOVA – Analysis of Variance
p - probability

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