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Research Article

Pathological Findings in Acute Amitraz Intoxication in Mice

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Abstract: This study examined the pathological changes in tissues and organs with acute intoxications with amitraz and its veterinary medicinal formulation. In total 312 healthy white English mice (*Mus musculus*), 156 female and 156 male, were used for this purpose. The solutions were given to the mice by gavage at the following dosages: 500, 1000, 1500, 1800, 2200 or 2500 mg of technical amitraz/kg and 50, 100, 250, 500, 750 or 1000 mg of amitraz/kg in a veterinary formulation. There was an interval of 1 week after the administration of the study medication. Necropsies were performed immediately on the mice that died during this period and after 7 days on the surviving mice and those in the control group after ether euthanasia. The body weights and liver and kidney weights of all mice were determined.

It was observed that amitraz was metabolised in a high degree in the liver, and xylene that was added to the formulations was eliminated from the kidneys and increased the toxicity of amitraz. It was concluded that amitraz caused weight loss in mice.

Key Words: Amitraz, mice, toxicity, pathology

Farelerde Akut Amitraz Zehirlenmelerinde Patolojik Bulgular

Özet: Bu çalışmada amitraz ve ticari veteriner müstahzarı ile farelerde akut zehirlenme durumlarında doku ve organlardaki hispatolojik bulgular ortaya konulmuştur. Yarısı erkek ve yarısı dişi 312 adet sağlıklı beyaz İngiliz ırkı fareye amitraz müstahzarı 50, 100, 250, 500, 750 and 1000 mg/kg ve teknik amitraz 500, 1000, 1500, 1800, 2200 and 2500 mg/kg dozlarda ağızdan tek sefer verilmiştir. Bir hafta içinde ölen farelerde hemen, ölmeyenler ve kontrol grubundakilerde ötanazi uygulanarak ölüm ağırlıkları, karaciğer ve böbrek ağırlıkları belirlenmiştir. Ayrıca, karaciğer ve böbreklerde de histopatolojik inceleme yapılmıştır.

Çalışma sonucunda tek dozda bile amitrazın farelerde kilo kaybına yol açtığı ve ksilenin amitrazın zehirliliğini artırdığı, karaciğer ve böbrek ağırlıklarını değiştirmediği ortaya konulmuştur.

Anahtar Sözcükler: Amitraz, fare, zehirlenme patoloji

Introduction

Amitraz is the common name for N-N-di-(2,4-xylyl-iminomethyl) methylamine, a formamidine pesticide currently marketed worldwide as an acaricide, larvicide and insecticide for use against a variety of plant and animal pests (1). This pesticide is especially effective against ticks in cattle and against lice and mange in sheep and dogs. Furthermore, it is also effective against *Varroa jacobsoni* Oudemans, which is the agent of varroa diseases in bees. Although this compound has been used in veterinary practice for a long time, few studies have been conducted on its toxicity (2).

Amitraz is well absorbed when administered orally or applied as a bath on the skin; therefore, there is a high risk of toxicity (3-5). After absorption, symptoms such as depression and sedation in the central nervous system, bradycardia, decreases in body temperature and blood pressure, bloating, decreases in gastrointestinal motility and slowing of the passage of chyme, polyuria, vomiting, anorexia, mydriasis and hyperglycaemia may occur (6-9).

It is known that many pesticides are formulised with organic solvents. Xylene is used in formulising amitraz. Therefore, xylene should also be taken into consideration in cases of intoxications with commercial products of amitraz. Depression in the central nervous system, ataxia, weakness in the motor functions, muscle twitching in the eyes, blurring of consciousness, coma and nervous irritation of unknown origin are seen in acute intoxication with xylene, which is an aromatic hydrocarbon (10,11). In fact, in the intoxication cases reported by Bonsall and Turnbull (12) xylene was demonstrated to be a more toxic compound in the formulation than amitraz.

No study has been conducted so far to demonstrate the pathological changes that may occur in organs and tissues in cases of acute intoxication with amitraz and its commercial products. The aim of the present study was to demonstrate the pathological changes in tissues and organs in cases of acute intoxication with amitraz and its veterinary medicinal product.

Materials and Methods

Animals

This study was conducted on 312 healthy white English mice (*Mus musculus*) (156 male, 156 female). After 12 male and 12 female mice were identified as the control group (Group 1), the remaining animals were divided into 2 main groups each containing 72 female and 72 male mice (Groups 2 and 3), which were then divided into 12 subgroups each containing 12 female and 12 male mice (Table 1).

Medication

Technical amitraz standard* (97%) and veterinary medicinal product containing 120 mg of amitraz in 1 ml [Kenecid EC** (12.5% amitraz, 57.5% xylene and 30% soya oil plus emulgator) formulation was approved by the Ankara University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology according to the gas chromatography results] were used in this study. These were diluted with dimethylsulphoxide to administer 0.3 ml to each mouse.

Gas Chromatography working conditions

Detector: Nitrogen Phosphorus Detector (NPD), Temperature 250 °C

Colon: DB WAX colon, 30 m x 0.32 mm, film thickness 0.5 µm

Oven and injection port temperature: 250 °C

Carrier gas: Nitrogen, 1 ml/min

The solutions of the medicinal product were prepared based on a 120 mg/ml amitraz concentration. Solutions of the medicinal product were given to the mice via a gastric tube in single 1-time doses (Table 1).

Table 1. Animal groups and doses of amitraz solutions.

Animal Groups		Doses, mg/kg
Group 1 (Control)	-	-
Group 2 (Technical Amitraz)	2A	500
	2B	1000
	2C	1500
	2D	1800
	2E	2200
	2F	2500
Group 3 (Veterinary Medicinal formulation)	3A	50
	3B	100
	3C	250
	3D	500
	3E	750
	3F	1000

n = 24

Histopathological examination

There was an interval of 1 week after the administration of the medication. Necropsies were performed immediately on the mice that died during this period and after 7 days on the surviving mice and those in the control group after euthanasia using ether. Body weight after death and the weights of liver and kidneys of all mice were calculated. The kidneys and a sample slice from the liver were fixed in buffered 10% formaldehyde solution. Paraffin blocks were prepared after passing through ethyl alcohol and xylol stages. Slices of 5-6 micron thickness were cut by a microtome and stained with haematoxylin-eosin and examined under a light microscope.

* Atabay Drug Ltd. Company, Turkey ** Tay Drug Ltd. Company, Turkey

Statistical analysis

The statistical significance was assessed using one-way ANOVA followed by the Newman-Keuls multiple comparison test (13).

Results

Following amitraz administration, deaths started to occur on day 3 in Group 2B, in the 6th h in Group 2C, in the 2nd h in Group 2D, in the 1st h in Group 2E and in the 45th min in Group 2F; all of the mice in Group 2F died within 6 h. After the administration of the veterinary medicinal formulation deaths started to occur in the 18th h in Group 3B, in the 4th h in Group 3C, in the 2nd h in Group 3D, in the 1st h in Group 3E and in the 30th min in Group 3F and ended on the 4th day. No deaths were observed in Groups 2A or 3A.

During necropsies of the mice that died a subcutaneous fluid collection and loss of hair related to this in Group 2 and a green coloration in both the subcutis and other tissues were observed in Group 3. Mice from both groups had distension due to gaseous collection in the stomach and intestines. In Groups 2C, 2D, 2E and 2F, an off-white coloration in some lobes of the liver was observed and no gross pathological findings were found in the kidneys. While Groups 3A and 3B did not have any gross change in their livers and kidneys, there was an off-white coloration in some lobes of the livers of mice from other groups and especially a white, localised region in the left renal pelvis (a lighter coloration in the right kidney). At the end of the 7th day, surviving mice from Groups 2A, 2B, 2C, 2D, 2E-female, 3A, 3B, 3C, 3D and 3E-male and the control group were sacrificed by ether euthanasia and similar findings were found in mice from Groups 2C, 2D, 2E-female, 3C, 3D and 3E-male. Body weights (Table 2) and liver and kidney weights (Table 3) of all the groups were determined.

The body weights of Group 1 mice sacrificed by euthanasia at the end of the 7th day (WMAD) were slightly higher than their body weights measured on the 1st day (WMA), while there was a significant weight loss in Groups 2A, 2B, 2C, 2D, 2E (only females), 3A, 3B and 3C ($P < 0.05$). There was no significant difference between the body weights in groups 2E-males, 3D, 3E and 3F between the 1st and 7th days ($P > 0.05$).

Liver and kidney weights (g) of mice measured after death due to the agent or euthanasia are presented in Table 3.

Table 2. Body weights (g) of mice measured alive, before the agent was administered and measured after death due to the drug or euthanasia.

Group	Male		Female	
	WMA[1]	WMAD[2]	WMA[1]	WMAD[2]
1	24.01 ± 3.1	25.11 ± 3.8	22.10 ± 3.2	23.04 ± 2.9
2A	25.80 ± 2.3	21.20 ± 2.9*	26.30 ± 1.8	21.10 ± 3.2*
2B	24.30 ± 2.5	20.10 ± 3.3*	25.40 ± 2.7	19.80 ± 2.4*
2C	26.60 ± 3.1	21.10 ± 2.9*	24.40 ± 1.8	18.10 ± 3.4*
2D	25.40 ± 2.9	17.80 ± 3.6*	28.10 ± 3.9	19.10 ± 3.1*
2E	27.50 ± 3.2	25.20 ± 3.8	26.90 ± 2.2	20.10 ± 3.1*
2F	28.10 ± 3.4	27.20 ± 3.1	27.30 ± 2.8	26.10 ± 3.1
3A	26.80 ± 3.2	22.3 ± 4.0*	27.60 ± 2.8	22.20 ± 3.9*
3B	25.30 ± 2.4	21.30 ± 4.6*	28.10 ± 3.3	22.50 ± 4.9*
3C	24.60 ± 1.7	21.20 ± 2.4*	24.90 ± 2.1	21.30 ± 3.9*
3D	25.60 ± 2.4	24.50 ± 2.8	25.90 ± 2.5	24.70 ± 3.0
3E	28.40 ± 3.6	27.70 ± 3.4	26.60 ± 2.9	25.20 ± 3.2
3F	27.8 0± 3.1	27.10 ± 3.4	27.70 ± 3.2	27.50 ± 3.4

1.Weight measured alive (g), 2. Weight measured after death (g), *.$P < 0.05$

n = 24

Table 3. Liver and kidney weights (g) of mice measured after death due to the agent or euthanasia.

	Liver		Kidney	
Group	Male	Female	Male	Female
1	2.01 ± 0.26	1.92 ± 0.21	0.38 ± 0.07	0.35 ± 0.06
2A	2.11 ± 0.31	1.99 ± 0.20	0.36 ± 0.02	0.38 ± 0.09
2B	2.19 ± 0.28	2.10 ± 0.19	0.36 ± 0.06	0.34 ± 0.04
2C	2.04 ± 0.19	2.02 ± 0.22	0.37 ± 0.09	0.39 ± 0.08
2D	2.14 ± 0.18	2.01 ± 0.19	0.37 ± 0.06	0.38 ± 0.07
2E	2.07 ± 0.24	1.98 ± 0.24	0.40 ± 0.10	0.35 ± 0.04
2F	2.09 ± 0.18	2.03 ± 0.28	0.36 ± 0.08	0.37 ± 0.08
3A	1.98 ± 0.23	2.05 ± 0.17	0.40 ± 0.09	0.39 ± 0.12
3B	2.08 ± 0.31	2.06 ± 0.20	0.39 ± 0.10	0.39 ± 0.13
3C	2.10 ± 0.33	1.99 ± 0.19	0.36 ± 0.06	0.36 ± 0.09
3D	2.07 ± 0.28	2.03 ± 0.31	0.38 ± 0.11	0.34 ± 0.09
3E	2.08 ± 0.20	2.00 ± 0.24	0.37 ± 0.08	0.35 ± 0.10
3F	2.02 ± 0.27	2.04 ± 0.30	0.36 ± 0.10	0.37 ± 0.11

n = 24

There was a slight increase in the liver weights of the mice exposed to either study agent, but the difference did not attain statistical significance ($P > 0.05$). There were no differences in the weights of the kidneys between the groups.

No pathological changes were detected in the kidneys of Group 2 and the livers of the Groups 2A and 2B, while degenerative changes were detected in the groups exposed to doses of 1500 mg/kg and over (2C, 2D, 2E and 2F) in the histopathological examination of livers. Hydropic degeneration in the hepatocytes of the liver in Group 2C, and fatty changes as well as hydropic degeneration were observed in the livers of Group 2D. Liver degeneration was severe in Groups 2E and 2F (Figure 1). Similar findings were observed in the animals

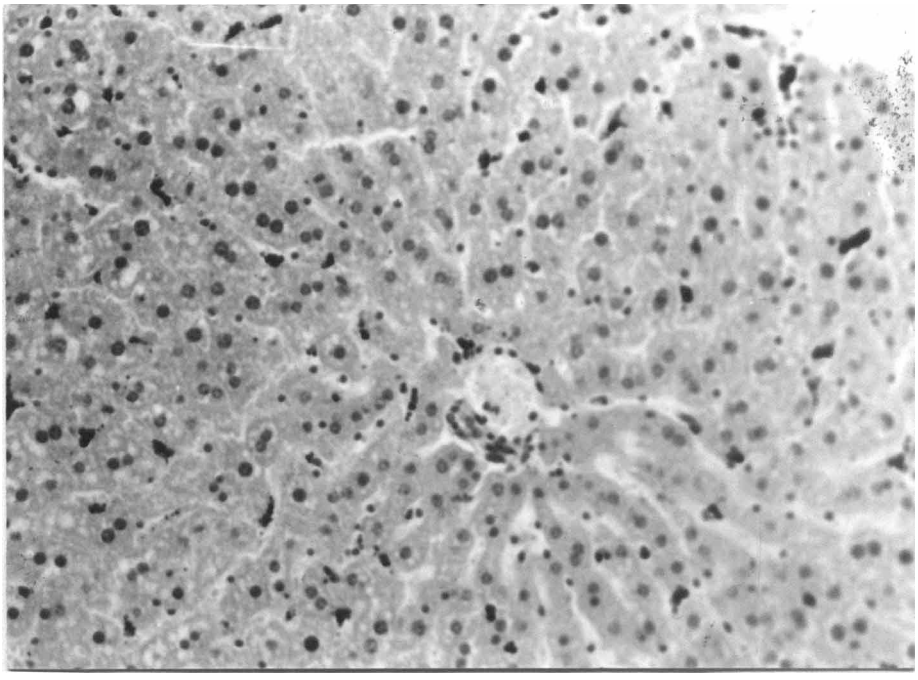

Figure 1. Vacuolar degeneration and fatty changes in liver epithelial cells. H&E. Bar, 50 µm.

exposed to doses of 250 mg/kg and over in Group 3. Hydropic degeneration in the liver in Group 3C, and hydropic degeneration and fatty liver were observed in Groups 3D, 3E and 3F. Tubulonephrosis in the kidneys of Group 3D and necrosis in the tubulus epithelial cells of the kidneys of Groups 3E and 3F were observed. The tubular basal membranes of the kidneys were intact (Figure 2).

Discussion

In acute intoxication induced with technical amitraz standard and its veterinary product, the difference between the body weights of mice measured on the 1st day when alive and those measured after death was significant ($P < 0.05$) in Groups 2A, 2B, 2C, 2D, 2E (only females), 3A, 3B and 3C and was not significant ($P > 0.05$) in the others. Amitraz is therefore thought to stimulate α2-adrenergic receptors (14), which induce bloating and constipation (15), causing a decrease in appetite and resulting in weight loss. The absence of weight loss in some groups can be explained by death occurring on the day following the administration of the study agent, thereby limiting the time for a loss in weight.

When the liver and kidney weights were compared, there was no significant difference between the mice that were exposed to the veterinary medicinal product and technical amitraz and the control animals ($P > 0.05$). However, there was an increase in liver weights, albeit small.

No pathological changes were observed in the kidneys of mice exposed to technical amitraz standard, but as the dose proportionally increased severe hydropic degeneration and fatty changes in the livers were observed. It was reported that amitraz converted quickly into a more toxic substance called mono-N-methyl derivative in the stomach contents after being given orally. This substance is metabolised in the liver mainly to 4-amino-3-benzoic acid, which is not toxic, and then eliminated by urine and bile (5). When the liver is exposed to this substance in an amount above which it cannot be detoxified, a disturbance results. Furthermore , it was reported that the conversion of amitraz after being administered orally to mono-N-benzoic derivative quickly shows that the stasis in the digestive tract is actually a result of this metabolite and it plays an important role in amitraz intoxication (16).

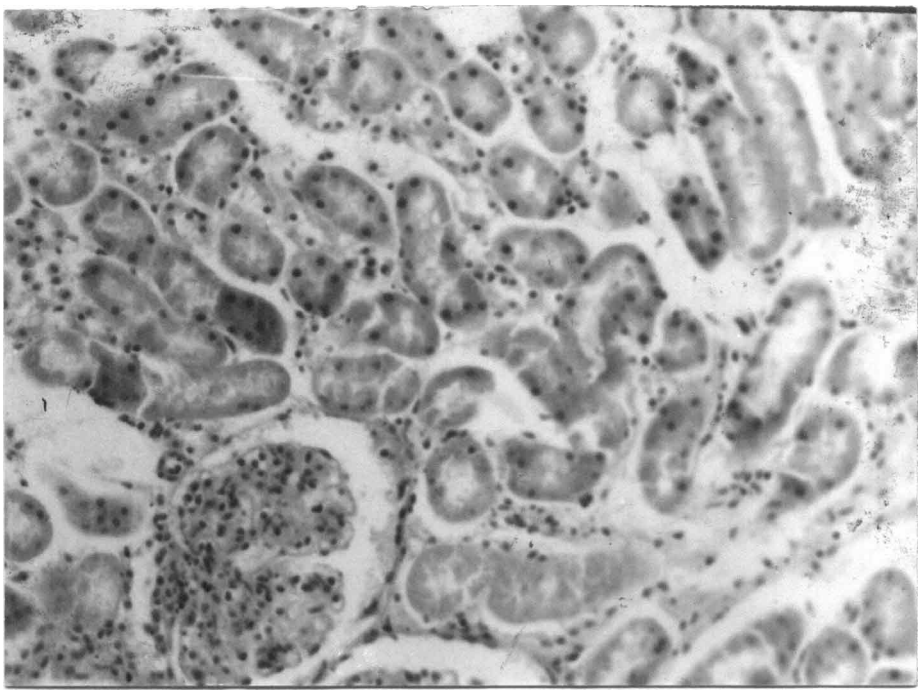

Figure 2. Degeneration and necrosis in tubulus epithelial cells of the kidney. H&E. Bar, 50 µm.

Lesions that are directly proportional to the dose given result in lesions in the livers and kidneys of mice exposed to veterinary medicinal formulation. This shows that xylene and other substances that are put in the formulation with amitraz are more toxic than amitraz and also have adverse affects on the kidneys. It is known that the carrier substances (soy oil and emulgator) are not toxic. Ninety-five percent of the absorbed amount of xylene is eliminated in the urine and less than 5% is eliminated in unchanged form by way of the lungs (11). Thus, lesions in the kidneys result from xylene, and lesions in the livers result mainly from amitraz. Hydropic degeneration and fatty changes in the hepatocytes of the liver are common changes in toxic material intake (17). For this reason, no specific change due to amitraz could be detected.

Another interesting point is that liver lesions were seen in animals given technical amitraz standard at doses of 1500 mg/kg and higher, while liver lesions were seen in animals given veterinary medicinal formulation at doses of 250 mg/kg and higher. As pointed in another study (15), xylene probably causes the amplification of the intoxication by increasing the absorption of amitraz.

In conclusion, it is observed that amitraz is metabolised in a high degree in the liver and xylene added to the formulations is eliminated from the kidneys and increases the toxicity of amitraz. It is also concluded that amitraz causes weight loss in mice.

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