

Effects of experimental amitraz intoxication in cats

[Efeitos da intoxicação experimental por amitraz em gatos]

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ABSTRACT

This work studied the effects of experimental amitraz intoxication in cats. Sixteen cats were randomly divided equally into two groups: amitraz group - animals received 1.5% amitraz at 1mg/kg IV; and the control group - animals without amitraz. Physiological parameters from blood, cardiorespiratory system, and sedation indicators were quantified over time up to 360 minutes. Blood profile, urea, creatinine, alanine aminotransferase and aspartate aminotransferase were not affected by amitraz. Sedation, loss of reflexes, hypothermia, bradycardia, bradyarrhythmia, hypotension, bradypnea, mydriasis, besides transitory hyperglycemia, hypoinsulinemia and decrease of cortisol levels were observed in cats experimentally exposed to amitraz. The α_2 -adrenergic effects induced by amitraz intoxication in cats are very similar to the same effects reported in others species, contributing with more information about this type of intoxication to veterinary toxicology.

Keywords: cat, amitraz, experimental intoxication

RESUMO

Este trabalho estudou os efeitos da intoxicação experimental por amitraz em 16 gatos, distribuídos, aleatoriamente, em dois grupos: grupo amitraz - animais receberam amitraz a 1,5% na dose de 1,0 mg/kg IV; e grupo controle - animais sem amitraz. Parâmetros fisiológicos sanguíneos, do sistema cardiorespiratório e de sedação foram aferidos até 360min. Perfil sanguíneo, uréia, creatinina, alanina aminotransferase e aspartato aminotransferase não foram afetados pelo amitraz. Sedação, perda de reflexos, hipotermia, bradicardia, bradiarritmias, hipotensão, bradipnéia, midríase, além de transitória hiperglicemia, hipoinsulinemia e diminuição dos níveis de cortisol, foram observados nos gatos experimentalmente expostos ao amitraz. Os efeitos α_2 -adrenérgicos induzidos pela intoxicação por amitraz em gatos são muito similares aos mesmos efeitos relatados em outras espécies, contribuindo com mais informações dessa intoxicação para a toxicologia veterinária.

Palavras-chave: gato, amitraz, intoxicação experimental

INTRODUCTION

Amitraz is an insecticide of the formamidine group, initially synthesized in England in 1969, that is used as an acaricide and tickicide in veterinary medicine (Andrade and Sakate, 2004).

The mechanism of action of amitraz in arthropods occurs by the activation of octopaminergic receptors. In mammals, the main mechanism of action consists of activation of α_2 -adrenoceptors similar to the mechanism of action of xylazine and clonidine, agonist α_2 -

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adrenergics, and in the inhibition of the enzyme monoaminoxidase (MAO) (Hsu, 1996).

Clinical signs occur due the α_2 -adrenergic effects induced by amitraz and include: sedation, loss of reflexes, lethargy and incoordination, bradycardia, bradyarrhythmia, hypotension, hypothermia, polyuria, transitory hyperglycemia, emesis, mydriasis and decrease of intestinal motility (Hsu, 1996; Andrade et al., 2004).

Amitraz is approved in Brazil by the Ministry of Agriculture for use in bovines, swine, sheep and dogs (Andrade and Sakate, 2004). Although its use is not recommended for cats by the manufacturer, it is an effective treatment of feline scabies and demodicosis (Cowan and Campbell, 1988; Scott et al., 1996) and is a very popular product, cheap and readily available, which could be an excellent alternative for acaricide treatment in this species (Andrade et al., 2004). However, few reports of amitraz intoxication are related to cats (Gunaratnam et al., 1983; Soli and Braseth, 1992; Andrade et al., 2004). Thus, the objectives of the present study were to investigate experimental amitraz intoxication and to analyze the α_2 -adrenergic effects induced by this acaricide in cats.

MATERIAL AND METHODS

The experiment was approved by the Ethical Committee (Protocol no. 47/2001). Sixteen mixed-breed adult cats, weighing 3.0 ± 0.5 kg, obtained from the cat pound were used. The cats were sorted by health condition and only those with physical (Lorens, 1996), hematological and biochemical (Willard et al., 1999) normal values were used. The day before running the experiment, the cats were socially isolated and held in individual stainless steel cages under 12:12 artificial light-dark cycle, room temperature at about 25°C , and fed *ad libitum*.

The cats were randomly divided into two groups of eight animals each (four males and four females): a) amitraz group = cats were administered 1mg amitraz/kg IV as a 1.5% concentration (by dilution of 0.6ml of amitraz¹,

75mg, in 4.4ml of bi-distilled water²); and b) control group = cats without any drug. This dose and dilution of amitraz were adapted from the amitraz intoxication model by iv route for dogs described in Andrade and Sakate (2003) and for cats in Andrade et al. (2006).

The following physical parameters were measured at 24 hours before and 0, 30, 60, 120, 180, 240 and 360min, and 24 hours after intoxication with amitraz: rectal temperature (T), respiratory rate (RR), heart rate (HR), systolic arterial pressure (SAP), electrocardiogram (ECG), pupil diameter (PD), degree of sedation (DS) and mean interval for sedation return (MISR).

The following scores were established for PD: normal (1), mydriasis (2), and myosis (3), and was evaluated by direct punctiform light toward the pupil. To measure DS the following scores were used: absent (0), mild (1), moderate (2), and high (3). Before intoxication all animals presented a score of 0. MISR was the time necessary for the animal to recover protective pupillary, palpebral and interdigital reflexes after amitraz administration. SAP was measured by an indirect and non-invasive method, with Doppler Ultrasonic equipment³. ECG was obtained for cats in the right lateral recumbence, recorded by an automated electrocardiograph⁴. For each heart activity parameter, the mean value of 5 consecutive heartbeats was recorded on lead II. To evaluate cardiac rhythm, the following scores were used: a) sinus; b) sinus arrhythmia; c) sinus bradycardia; d) 1st degree A-V block; and e) *sinus arrest* (Goodwin, 2002).

For laboratory determinations, 24 hours before and after intoxication with amitraz, 3.5ml of blood samples were collected by jugular puncture with vacutainer 23G scalp⁵ and a 5ml BD vacutainer tube⁵ without additive, 0.5ml were transferred to a 1.0ml Eppendorf tube⁵ containing 20 μ l with EDTA (ethylenediaminetetraacetic acid) as anticoagulant for red blood cell (RBC) and white (WBC) blood cell counts. The 3.0ml of remaining blood were centrifuged for serum to obtain biochemical parameters of urea, creatinine, alanine aminotransferase (ALT), and aspartate

¹Mitraz[®] - (1000ml/12.5g amitraz) Agribands Purina - Paulinia, Brazil.

²Aster - Sorocaba, Brazil.

³Parks Medical (841-A) - Aloha, USA.

⁴Cardiotest EK 51 - USA.

⁵BD - Becton Dickison - USA.

aminotransferase (AST). The total WBC and RBC counts were determined using a hemocytometer. The WBC count used liquid of Turk as the diluent and differential counting of WBC used Diff-Quick. Manual counting of RBCs was accomplished using, as diluent, Hayen liquid. For hemoglobin concentration the cyanometemoglobin method, and for hematocrit the microhematocrit method. The following biochemical proofs was determined by colorimetric methods: urea (urease)⁶, creatinine (fluid of the picric acid method)⁶, alanine aminotransferase (ALT) (Reitman-Frankel unit)⁶ and aspartate aminotransferase (AST) (Reitman-Frankel unit)⁶.

At time intervals of 0, 60, 180 and 360min after amitraz intoxication for plasmatic glucose analysis and serum insulin and cortisol analysis, 4ml of blood samples were collected by jugular puncture with 23G 3/4 vacutainer brand safety-lok blood collection sets⁵ and a 5ml BD vacutainer no additive tube⁵, being transferred 0.5ml for a 1.0ml Eppendorf tube⁵ containing 20 μ l with sodium fluoride for glucose dosage by colorimetric method (god-trinder oxidase) with spectrophotometric⁷ analysis. The remaining 3.5 ml of blood in the tube without anticoagulant were centrifuged and 1.0ml of serum sample was transferred to another tube and stored frozen at -40°C and were then submitted to the laboratory⁸ for insulin and cortisol analysis using a commercial radioimmunoassay⁹ (RIA) kit previously validated for cats by Nelson et al. (1990).

For each variable, a profile analysis (Morrison, 1990) was used to compare group and time effects. For non-parametric data, Friedmam (time effect evaluation) and Kruskal-Wallis (group effect evaluation) were used (Siegel, 1975). A significance level of P<0.05 was adopted.

RESULTS AND DISCUSSION

The effects of amitraz on rectal temperature, respiration and heart activities are shown in Fig. 1. Amitraz reduced temperature after 60min compared to the control group (P<0.05). This hypothermia is predictable, since temperature decreases with the use of α_2 -adrenergic agonist

affect the thermoregulation center in the hypothalamus (Hsu, 1996).

Mean respiratory and heart rates were slightly increased in the beginning of the experiment in both groups, reaching normal values from the 60thmin of the experiment. In the group control, changes in respiratory and heart rates may be increased by the influence of the sympathetic nervous system during stress (Noble, 2002). Amitraz decreased the respiratory rate at times 180, 240 and 360min relative to the respective controls (Fig. 1). Respiratory depression induced by amitraz is possibly due to the central α_2 -adrenergic action of this acaricide (Cockburn et al., 1993).

Amitraz administration induced a heart-rate decrease from 30 min to 360min (Fig. 2), with the greatest reduction at 60min, corresponding to a decrease of 37.7% in relation to time zero. SAP was decreased in control cats from times 180 to 240min. A more pronounced decrease was induced by amitraz, with SAP decreasing from 30min until the end of the experiment (Fig. 1). Among the groups, significant differences were detected (P<0.05) from 30 to 360min after amitraz administration. Bradycardia and hypotension after administration of amitraz may be due to activation of central pre-synaptic α_2 receptors, decreasing release of dopamine and noradrenaline and reducing sympathetic tonus (Hoffman and Lefkowitz, 1996).

The ECG average values were normal at all times in the two experimental groups. Although the values were within normality, four cats individually presented abnormal values after amitraz administration: one cat prolonged PR interval (0.10s) [cat No. 2 at 20 and 180min], and three cats presented QT larger than 0.18s [cat No. 3 at 30 and 60min; cat No. 5 at 120 and 180min; and cat No. 8 at 120 and 240min].

All control cats presented normal sinusal rhythm. Amitraz, however, induced several arrhythmias, such as sinusal bradycardia, sinusal arrhythmia and first A-V block (1st AVB), shown in Fig. 2. Cat No. 2 presented sinusal bradycardia at times 30, 60 (Fig. 2) and 240min, first A-V block at times 120 and 180min (Fig. 2), and sinusal arrhythmia at 360min. Cat No. 5 had sinusal bradycardia at 180min and 240min. Cat No. 7 revealed bradycardia at 30min and sinusal arrhythmia at times 120min and 180min. Cat No. 8 showed sinusal arrhythmia at time 240min.

⁶LABTEST - Lagoa Santa, Brazil.

⁷CELM 225D - São Paulo, Brazil.

⁸Laboratório de Radioimmunoensaio - CRIESP - São Paulo, Brazil.

⁹Diagnostic Products Inc. - Los Angeles, USA.

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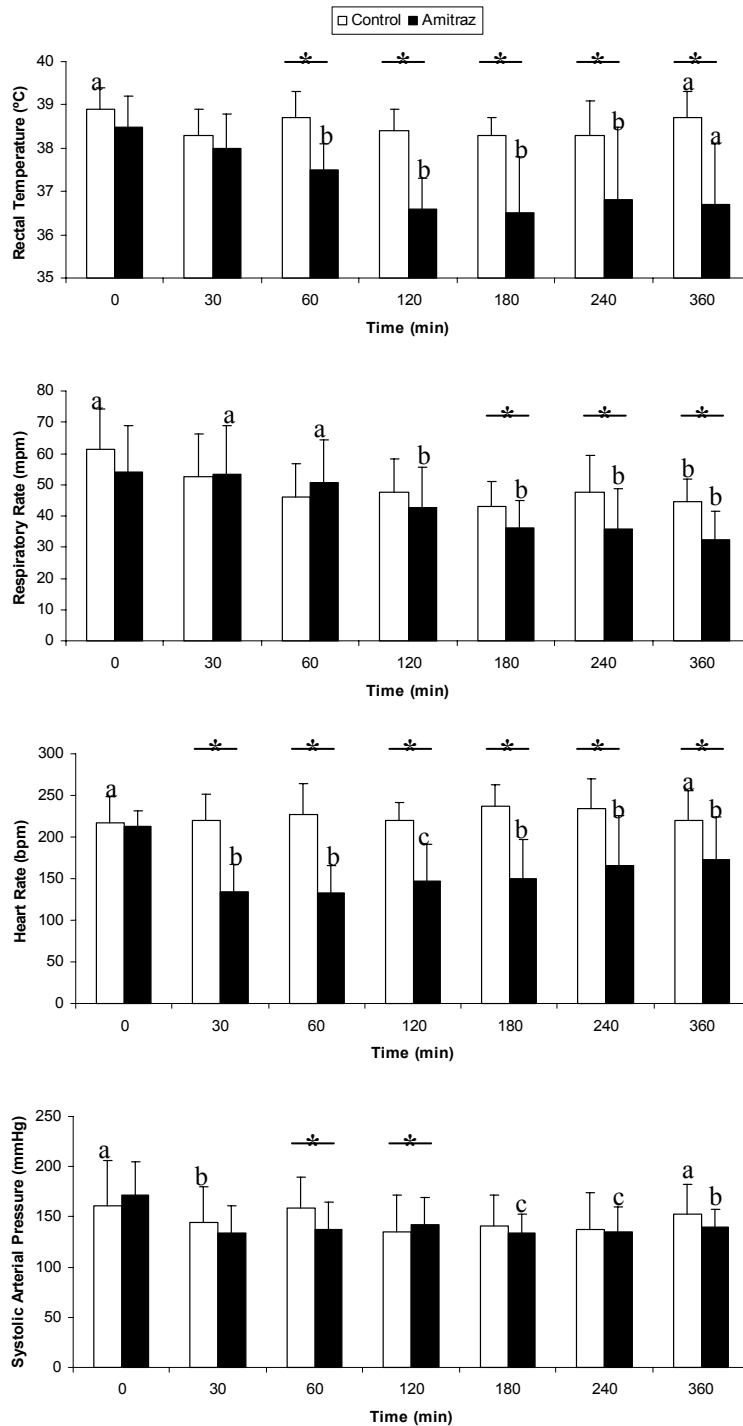


Figure 1. Effects of experimental amitraz intoxication on rectal temperature and cardiorespiratory parameters in cats. Values as mean (\pm sd) from eight cats each condition. Different lower-case letters above the mean indicate significant difference (Friedman, $P < 0.05$). Asterisk indicates statistical difference between groups (Kruskal-Wallis, $P < 0.05$).

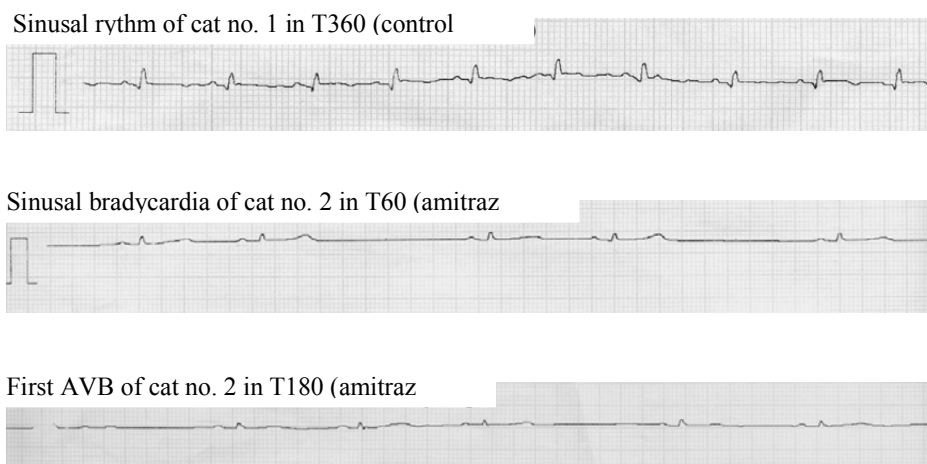


Figure 2. Examples of cardiac patterns in cats. ECGs from lead II (paper speed = 50mm/sec; 1cm = 1mv; 35 Hz; 60 Hz).

This study describes the first report of sinus bradycardia and 1st degree A-V block induced by amitraz in cats. Prolongation of PR interval indicates 1st degree A-V block (1st AVB) (Goodwin, 2002). The variation of the PR interval may occur in the case of vagal tonus alteration or secondarily to ectopic focus, causing dissociation of the atrial and ventricular activities (Zipes, 1992). The α_2 -adrenergics agonists reduce CNS sympathetic tonus, increase parasympathetic activity, and induce sinus bradycardia or 1st or 2nd degree A-V block; yet, complete 3rd degree A-V block with escape pulsations rarely occurs (Zipes, 1992; Goodwin, 2002).

Amitraz provoked mydriasis until 240min (Fig. 3). In the amitraz group, cats showed high sedation at 30min, a response significantly different compared to time zero ($P>0.05$) until 240min (Fig. 3). Amitraz caused dose-dependent mydriasis and sedation mediated by post-synaptic α_2 adrenoceptors (Hsu and Kakuk, 1984). The MISR after amitraz administration was 175.0 ± 70.7 min.

Amitraz increased plasma glucose levels only from 60min, both to time zero and the 60-min control (Fig. 4). This represented a 95.3% increase compared to time zero. The amitraz-increased serum glucose reached a maximum at 60min and then gradually decreased until

360min. In some cats, amitraz caused hyperglycemia (above 200mg/dl) at 60min: cats No. 3 (225.5mg/dl), No. 5 (222.8mg/dl) and No. 8 (209.4mg/dl). The transitory hyperglycemia observed in cats intoxicated by amitraz was very similar to that reported in dogs by Andrade et al. (2005). The hyperglycemia occurs by the action of amitraz and its active metabolite, BTS 27271, by α_2 -adrenergic-receptor-mediated inhibition of insulin secretion, possibly by adenylcyclase inhibition mediated by G PTX-sensitive proteins (Chen and Hsu, 1994). The α_2 -adrenergic receptor subtype involved in this action is α_{2D} , which is located within the pancreatic islets (Abu-Basha et al., 1999).

There are interests in human medicine for the possible role of α_2 -adrenergic receptors to decrease the activity of pancreatic cells in patients with diabetes type 2 (Ortiz-Alonso et al., 1991). It is quite evident that these patients are more sensitive to amitraz intoxication than normal individuals, thus requiring more studies on this topic (Abu-Basha et al., 1999).

Insulin significantly decreased at 180min and 360min in the control cats, but within the basal range for cats. Amitraz decreased insulin with respect to control cats at 60min, though subsequently insulin increased at 180min and 360min.

Control cats showed serum cortisol levels slightly increased over all times quantified and no time effect was observed. Amitraz decreased plasma cortisol only at 60min. Cats administered amitraz showed that cortisol serum level increased at time zero. Plasma cortisol, insulin and glucose concentrations are altered in stressed animals (Noble, 2002). In the present study, serum cortisol levels in the control cats were slightly increased at some times (0, 180 and 360min), and cats from the amitraz group slightly increased plasma cortisol at time zero.

However, such slight increase in cortisol may result from handling stress, as has been described in the literature (Oppermann and Baken, 1997). Amitraz-induced cortisol decrease may be a consequence of CNS depression caused by α_2 -adrenergic central receptor stimulation, resulting mainly in decreased sympathetic CNS efflux, catecholamine, and other substances related to stress (Noble, 2002; Miller and O'Callaghan, 2002).

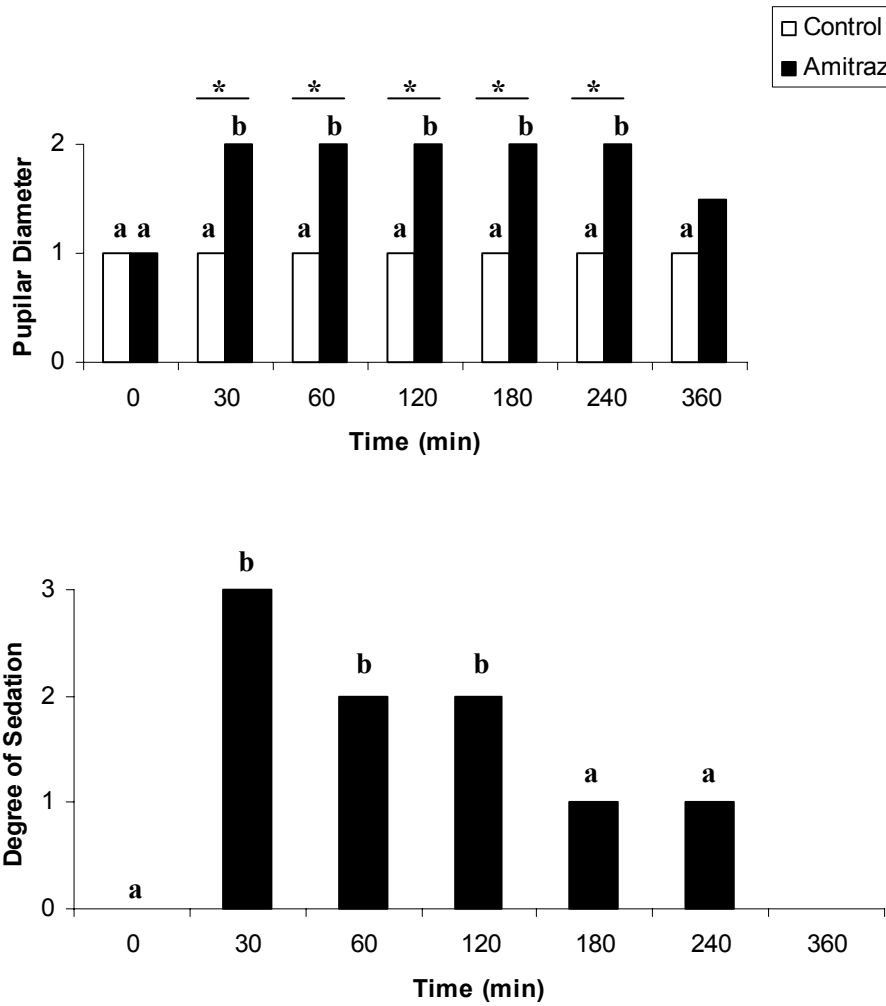


Figure 3. Effects of experimental amitraz intoxication on medians of pupular diameter and degree of sedation in cats. Pupular diameter was scored as normal (1), mydriasis (2) or myosis (3). Degrees of sedation are shown only for the Amitraz group and responses were scored as absent (0), mild (1), moderate (2) or high (3). Values from eight cats each condition. Different lower-case letters above medians indicate significant difference among moments and within a group (Friedman, $P < 0.05$). Asterisk indicates statistical difference between groups (Kruskal-Wallis, $P < 0.05$).

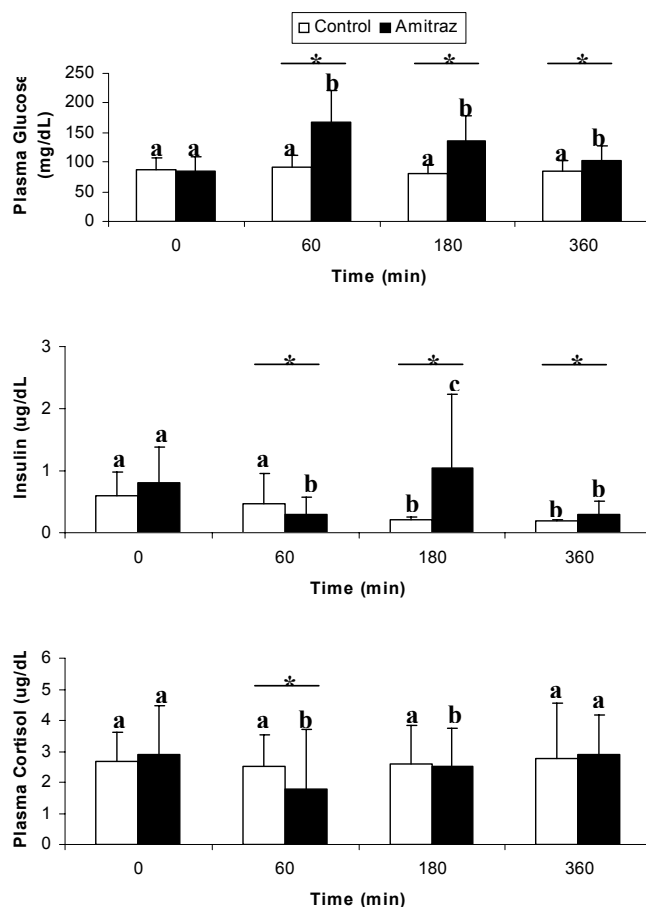


Figure 4. Effects of experimental amitraz intoxication on metabolism (metabolite and hormones) in cats. Mean (\pm sd) values from eight cats in each condition. Values as mean (\pm sd) from eight cats each condition. Different lower-case letters above the mean indicate significant difference (Profile analysis, $P < 0.05$). Asterisk indicates statistical difference between groups (Profile analysis, $P < 0.05$).

RBC, WBC, urea, creatinine, ALT and AST values were not affected by amitraz administration ($P > 0.05$). These observations have also been described by other authors in cats (Gunaratnan et al., 1983), in humans (Atabek et al., 2002), in horses under prolonged use of amitraz by intravenous route (Queiroz-Neto et al., 2002) and in mice (Filazi et al., 2003). Other clinical signs which occurred only in cats intoxicated with amitraz were vomit (62.5%), sialorrhea (50.0%), diuresis increase (25.0%), vocalization (25.0%) and ataxia (62.5%). An interesting aspect was the increase in appetite (50.0%) after the end of the experiment in cats intoxicated by amitraz, a formamidine pesticide. Formamidines are a new class of appetite stimulants in mice (Pfister et al., 1978) and furthermore, the α_2 -adrenergic agonist in low

dosages can stimulate appetite (Hall and Taylor, 1994). The present study is the first demonstration of such an action in cats.

The clinical signs observed in cats intoxicated by amitraz were: sedation, loss of reflexes, hypothermia, bradycardia, bradyarrhythmia, hypotension, bradypnea and mydriasis. Some cats also presented vomiting, diuresis, sialorrhea, vocalization and increased appetite. The bradyarrhythmias observed were sinus bradycardia and 1st degree A-V block. Metabolic and hormonal alterations were transitory hyperglycemia and hipoinsulinemia and transitory decrease of plasma levels of cortisol. Amitraz intoxication in cats did not change RBC, WBC, urea, creatinine, ALT or AST concentrations.

These results demonstrate that amitraz intoxication in cats is very similar to the same reported of this intoxication in others species.

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