DETERMINATION OF LD_{50} AND ACUTE TOXICITY EFFECTS ON SOME BIOCHEMICAL PARAMETERS INDUCED BY AMITRAZ IN RATS

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ABSTRACT

The aim of the study is determine the toxic effects and some biochemical changes of amitraz insecticide in adult albino female rats. First the median lethal dose (LD_{50}) of oral amitraz was (717.125mg/kg)and were clear toxic signs of amitraz intoxication such as incoordination, dull, depression, salivation, bulging of eyes, recumbence and death. Then alpha $_2$ (α-$_2$) adrenoceptor antagonist atipamezole at (0.5 and 1mg/kg), and alkalinizing agents sodium bicarbonate at (50 and 100 mg/kg) were given by IP route, 15minutes before giving the oral administration of amitraz improved the prophylactic rates to 14.66 % and 24.45% for atepamizole, and to1.79 % and 17.38% for Sodium bicarbonate respectively, and the toxic signs of amitraz intoxication were reduced when atipamezole and sodium bicarbonate injected. After three hours of oral non-lethal amitraz dosing with (250 and 500mg/kg) increased serum glucose concentration (291.33±2.9 and 328.02±4.8mg/dL) respectively compared with the control group value (134.05±9.6 mg/dL), also the Amitraz at same doses were risen up the level of serum cortisol to (7.30±0.3 and 7.43±0.3 µg/dL) respectively as compared with the control value (2.39±0.26 µg/dL). Also it causes an increased serum level of transaminases enzymes (AST and ALT) compared with the control value, while amitraz at (500mg/kg, orally) increased enzyme ALP activity in the serum of rats when it is compared with control value. The results of this study refer to that amitraz may cause tissues damage and toxic effects in rats.

INTRODUCTION

Amitraz is a synthetic compound from formamidine pesticide family; it is used as an insecticide and acaricide in veterinary medicine to control many external parasites in different animal species. Amitraz works under a variety of product names as, Mitaban, Taktic, Kenaz and others (1). Main routes of amitraz exposure are ingestion by oral route, inhalation and dermal routes, causing toxicities in human and animals (2,3). Amitraz works by hyper stimulation of α_2 - adrenergic receptors in the central nervous system , The activity of α_2 is an autoreceptor presynaptic act as reduction of norepinephrine (4,5). Poisoning with amitraz illustrate many toxic signs which includes; CNS depression, unconsciousness, stiffness, deep coma and other signs as, bradycardia, hypopnea, hyperglycemia, polyuria, hypotension, hypothermia, emesis, delayed gastrointestinal transit , colic, bloat, nystagmus, mydriasis (6,7,8,9,10). Amitraz has a high lipophilic property that will rapidly absorb in the acidic media of the gastric compartment. The onset of toxic signs of amitraz is between 20 to 90 minutes after oral ingestion (11). The plasma level reaches its highest peak after two hours and the highest tissue level of amitraz is found in bile, liver, kidney eye, intestine, and muscle tissues (12). Peak urine level is reached after 6 to 23 hours of amitraz ingestion in dogs (13). The successful key of amitraz poisoning treatment start with the early diagnosis (14). In addition, yohimbine used in treating amitraz poisoning (15).

Regarding the wide spread of amitraz used the our aim in this study is to determine the median lethal dose of amitraz and the studying of some biochemical parameters and the prophylactic role of atipamezole and Sodium bicarbonate in reversing the toxic actions of amitraz in rats.

MATERIALS AND METHODS

Laboratory animals

Fifty six adult Albino female rats (150-300 g) were obtained from animal-house in College of Veterinary Medicine, Duhok University. They were housed under standard conditions at the temperature (18-24ºC) and 10/14 hours of the light/dark cycle with water and food *ad Libitum*. Animals were allowed to the new environment for 30 minutes prior to starting the experimental procedure. The committee ethics of Veterinary Medicine College at University of Duhok approved the present study.

Medications and chemical materials.

Amitraz doses were used (12.5% concentration, produced by Jordanian company; (VAPCOZIN), and Atipamezole (produced by Provet in Turkey) was also used and it was diluted in distilled water to obtain 5ml/ kg of body weight dosed intraperitoneal prepared freshly. All doses in this study were chosen depending on pilote study on adult female rats.

Experiment 1: Determination of the median lethal dose (LD_{50}) of amitraz by Oral administrations in rats by using up and down method.

In this study 6 healthy albino female rats weighing from (150-250 g) were used. The dose of amitraz at 500 mg/kg of body weight by oral route as initial dose was used. The final resulting of amitraz response was all alive-or-none (O for alive and X for dead) was assessed for each animal after 24 hours of administration. The increase (up) and decrease (down) of amitraz dose was in constant value (125 mg/kg), with repeating of this method is up and down for dose value in different rats, the estimation of the LD_{50} of amitraz in rats, used the following equation (16): **LD_{50}= Xf + Kd**

The symbols resemble the follows:

LD_{50}: Median lethal dose.

Xf: Last dose used.

K: From Dixon table.

d: Constant dose range (up and down dose).

Experiment 2: Effects of Atipamezole and Sodium bicarbonate by intraperitoneal administration on median lethal dose (LD_{50}) of amitraz in rats by using up and down method orally.

Group I:

In this group 11 albino female rats weighing (200-210 g) were used, divided in 2 sub grouping, the first subgroup dosing atipamezole at 0.5 mg/kg intraperitoneal (i.p) route and second sub group dosing atipamezole at 1 mg/kg i.p route before 15 minutes of oral (LD_{50}) amitraz administration (717.125 mg/kg) of each sub group . The final resulting of response was all alive-or-none was assessed for each animal after 24 hours of administration. up and down in amitraz dose at dose 150 mg/kg, with repeating of this method in up and down for dose value in each rat, we detected the LD_{50} of amitraz with the administration of atipamezole at dose 0.5 and 1 mg/kg by i.p route according to Dixon table, by using the equation mentioned earlier (16).

Group II:

In this group 11 albino female rats weighing (185-205 g) were used, divided in 2 sub grouping, the first sub group was treated with Sodium bicarbonate ($NaHCO_3$) at 50 mg/kg by i.p route and second sub group dosing sodium bicarbonate at (100 mg/kg), i.p route before 15 minutes of oral (LD_{50}) amitraz administration (717.125 mg/kg) of each sub group. The final resulting of response was all alive-or-none was assessed for each animal after 24 hours of administration. The increase and decrease in amitraz dose was in constant dose (150 mg/kg), with repeating of this method in up and down for dose value in each rat, we could estimate LD_{50} of amitraz with the administration of Sodium bicarbonate at dose (50 and 100 mg/kg)by i.p route according to Dixon table, by using the equation mentioned earlier.

After reaching the LD_{50} of amitraz by oral administration with atipamezole and Sodium bicarbonate by i.p route in rats in different doses , the prophylactic rate was measured from the following equation (17).

Prophylactic rate (mg/kg) = $\frac{LD_{50}\ of\ Amitraz\ with\ Atipamezole - LD_{50}\ Amitraz\ alone}{LD_{50}\ of\ Amitraz\ alone}$ X 100.

Experiment 3: Effect of non-lethal doses (acute toxicity) of amitraz in some biochemical parameters in serum of the rats.

The level of glucose, and activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and creatinine phosphokinase (CPK) were measured by spectrophotometer and the level of cortisol hormone was detected by enzyme linked immunosorbent assay (ELISA).

Twenty eight albino female rats weighing (200-250 g) were used. The rats were divided into 4 random groups; each group consisted of 7 rats as follows:

Group I: control group of rats administered with distilled water (5ml/kg) orally.

Group II: Administration of (100mg/kg) of amitraz to rats by the oral route.

Group III: Administration of (250mg/kg) of amitraz to rats by the oral route.

Group IV: Administration of (500mg/kg) of amitraz to rats by oral route.

After 3 hours of treatment, the blood was collected from the four groups from retro-orbital sinus to obtain the serum for measuring some biochemical parameters which included (glucose, cortisol, AST, ALT, ALP and CPK).

Statistical Analysis

The statistical analysis of data was conducted using SPSS program for window software. The data were expressed as mean ± standard error (SE), the difference between the experimental groups was statistically analyzed by one way analysis of variance (ANOVA) followed by the least significant difference test, The level of significant was at $p \leq 0.05$ (18).

RESULTS

The oral LD_{50} of amitraz was (717.125 mg/kg), and the ingested rats with amitraz showed toxicological signs during 6-12 minutes after the administration and the signs were incoordination, dull, depression, semi-closed eyes, recumbence, urination, defecation, and death (table 1).

The injection of (0.5 and 1 mg/kg) of atipamezole i.p in rats before 15 minutes of the median lethal dose (LD_{50}) of amitraz ingestion caused an increase in the LD_{50} of amitraz by oral route which it reached up to (822.275 mg/kg and 892.472 mg/kg) respectively. The prophylactic rate was to 14.66 % and 24.45% with the delay in the onset of toxicological signs on rats 8-12 and 15-25 minutes respectively (table 2). While injection of (50 and 100 mg/kg) of Sodium bicarbonate i.p in rats before 15 minutes of administration LD_{50} of amitraz caused an increase in the LD_{50} of amitraz orally which reached up to (729.125 and 841.775 mg/kg) respectively and the prophylactic rate was 1.76 % and 17.381% without delay the onset of the toxicological signs on rats 12-14 minutes in each one (table 3).

Table 1: **Determination of LD_{50} of amitraz in rats by oral route**

Measures	Result
Median lethal dose (LD_{50})	717.125mg/kg
Dose range used	500-750mg/kg
Initial dose	500mg/kg
Last dose	625mg/kg
Up and down dose	125mg/kg
Number of rats	6 (OOXOXO)
Onset of action	6-12 minutes
Toxicological signs	Incoordination, dull, depression semi-closed eyes, recumbence, urination, defecation, salivation and death

X: Death of the rat during 24 hours.
O: Surviving of the rat during 24 hours.

Table 2: **Effect 0.5 and 1 mg/ kg of atipamezole by i.p route on the LD_{50} of amitraz in rats orally using up and down method**

Measures	Result 0.5 mg/ kg	Result 1 mg/ kg *
Median lethal dose (LD_{50})	822.275mg/kg	892.472mg/kg
Dose range	717.125-867.125mg/kg	717.125-1017.125mg/kg
Initial dose	717.125mg/kg	717.125mg/kg
Last dose	717.125mg/kg	867.125mg/kg
Up and down dose	150mg/kg	150mg/kg
Number of rats	5 (OXOXO)	6 (OOXOXX)
Prophylactic rate	14.66%	24.45%
Onset of action	8-12 minutes	15-25 minutes
Toxicological signs	Incoordination, dull, depression, semi-closed eyes, eye bulging * , recumbence, urination, salivation and death	

X: Death of the rat during 24 hours.
O: Surviving of the rat during 24 hours.

Table 3: **Effect 50 and 100 mg/kg of sodium bicarbonate by i.p route on the LD_{50} of amitraz in rats orally using up and down method**

Measures	Result of 50 mg/kg	Result of 100 mg/kg *
Median lethal dose (LD_{50})	729.125 mg/kg	841.775 mg/kg
Dose range	717.125 – 867.125 mg/kg	717.125- 1017.125 mg/kg
Initial dose	717.125 mg/kg	717.125 mg/kg
Last dose	717.125 mg/kg	867.125 mg/kg
Up and down dose	150 mg/kg	150 mg/kg
Number of rats	5(OXOXX)	6 (OOXXOX)
Prophylactic rate	1.76 %	17.381 %
Onset of action	12- 14 minutes	12 –14 minutes
Toxicological signs	Incoordination, dull, depression, semi-closed eyes, eye bulging * recumbency, urination, defecation, salivation and death	

X: Death of the rat during 24 hours.
O: Surviving of the rat during 24 hours.

The level of biochemical values measured, the results were recorded as follows; 3 hours after amitraz administration at doses (250 and 500mg/kg),orally led to significant increase of glucose level in serum of rats when it is compared with the control group, while cortisol hormone level at doses (100, 250 and 500mg/kg),orally led to significant rise of hormone values in serum in comparison with the control group (table 4).

Amitraz ingestion orally at doses (100, 250 and 500mg/kg) , 3 hours prior to ingestion led to significant rising of AST level when it is compared with the control group , the doses (250 and 500 mg/kg),orally after 3 hours of giving amitraz manifested a significant elevation of ALT in comparison with the control (table 5). A significant *($p \leq 0.05$)* increase of ALP value was detected after 3 hours of rat taking amitraz dose (500 mg/kg) , orally when it is compared with the control group, while no difference in rat serum level of CPK was measured when doses (100, 250 and 500mg/kg) of amitraz was given orally 3 hours after ingestion in comparison with the control group (table 5).

Table 4: **Effect of amitraz administration at doses 100, 250 and 500mg/kg , orally on glucose concentration and cortisol level in serum of rats after 3 hours of administration.**

Treatments	Glucose level (mg/dL)	Cortisol level (µg/dL)
Group I: Control group	134.05±9.6	2.39±0.26
Group II: Amitraz 100 mg/kg.	203.08±2.4	7.22±0.5*
Group III: Amitraz 250mg/kg.	291.33±2.9 *a	7.30±0.3*
Group IV: Amitraz 500mg/kg.	328.02±4.8 *a	7.43±0.3*

Values represent means ± S.E of 7 rats/group.
*: significant with the control group at $p \leq 0.05$.
a: significant with group treated with 100mg/kg body weight (orally) at $p \leq 0.05$.

Table 5: **Effect of amitraz administration at doses 100, 250 and 500 mg/kg, orally on AST, ALT, ALP and CPK in serum of rats after 3 hours of administration**.

Treatments	AST level (IU/L)	ALT level (IU/L)	ALP level (IU/L)	CPK level (mg/dL)
Group I: Control group	13.09±0.54	6.35±0.43	213±26.40	0.75±0.03
Group II: Amitraz 100 mg/kg.	34.09 ± 3.55 *	7.15±0.62	227±16.20	0.94±0.13
Group III: Amitraz 250 mg/kg.	44.02±4.78 *a	13.20±1.97 *a	241±20.25	1.01±0.19
Group IV: Amitraz 500 mg/kg.	52.09±1.69 *a	16.59±0.75 *a b	280 ±19.82*	1.02±0.34

Values represent means ± S.E of 7 rats/group.
*: significant with the control group at $p \leq 0.05$
a: significant with group (II) treated with 100mg/kg body weight (orally) at $p \leq 0.05$
b: significant with group (III) treated with 250mg/kg body weight (orally) at $p \leq 0.05$

DISCUSSION

Acute insecticide poisoning is a significant reason for worldwide mortality and morbidity in animals and humans. Each year uncountable numbers of acute insecticide poisoning occur and most of these poisonings occur in the developing countries (1,2,3).

We have chosen amitraz in the present study; it is from formamidine family having both insecticide and acaricide properties used in veterinary medicine and agriculture for controlling large number of pests worldwide, such as mite, lice, ticks and other external parasites (19). Amitraz is used in its organic solvent at 12.5% - 20% and before application on animals and plants its diluted with water (20). The efficient role of amitraz is seen when its used to control Varroa disease in honeybees (21). Most cases of amitraz poisoning in humans are due to accidental or occupational exposures and some of them are due to suicidal attempts (22).

The aim of the current study is to induce amitraz poisoning in female rats by the oral route to in order to reach the median lethal dose of amitraz in rats by up and down method and also measuring the preventative role of atipamezole in reversing the toxic effects of amitraz induced to rats experimentally (16).

In the present study the median lethal dose LD_{50} of amitraz in rats was (717.125mg/kg) by oral route and this dose appears to be different when it is compared with previous studies in different animals exposed to amitraz poisoning, in dogs its 100mg/kg of body weight orally (23), in cats it is 75mg/kg of body weight (24), in mice its >1600mg/kg of body weight (25) , while the oral LD_{50} of amitraz in chicks showed very low dose when it is compared with other animals, its 53.03mg/kg of body weight orally (26). The difference between the LD_{50} among different animal species is due to the physiological, biological, anatomical and species variations between them (27).

Atipamezole is a potent and selective α_2-adrenergic antagonist (28) and alkalinizing agents Sodium bicarbonate it is used to reverse the toxic effect of amitraz. In the present study the effective prophylactic roles of atipamezole and Sodium bicarbonate ($NaHCO_3$) were observed when they have been used experimentally to reverse the toxic effects of oral LD_{50} of amitraz, giving atipamezole (0.5 and 1mg/kg) and Sodium bicarbonate at (50 and 100 mg/ kg) in rat by the intraperitoneal route before 15 minutes of amitraz administration led to increase of the LD_{50} of amitraz which it reached to (822.275 and 892.472mg/kg) in group treated with atipamezole and the Sodium bicarbonate group reached to (729.125 and 841.775) mg/kg in rats respectively; and the prophylactic rate of atipamezole in this study was up to 14.66% and 24.45% respectively but of Sodium bicarbonate 1.76 % and 17.381 % respectively. The reason of the increased LD_{50} of amitraz when atipamezole is given in advance due to the effective role of atipamezole in reversing amitraz intoxication with highly affinity of atipamezole to α_2-adrenergic receptors , our study is similar to previous articles done on atipamezole and yohimbine when they were used in treating amitraz intoxication in dogs and cats (14,28,29), but the reason of increased LD_{50} of amitraz when Sodium bicarbonate administered suspected for alkalizing urine to increase the excretion of the acidic toxicants (amitraz)and their metabolites.

In the present review, which was aimed to determine changes on some biochemical parameters induced by amitraz intoxication in female rats, the results of current study recorded an increase on most of the biochemical laboratory findings in female rats which included estimation of glucose level in serum, cortisol level , AST,

ALT, ALP and CPK. Giving of amitraz at doses (250 and 500 mg/kg) orally, displayed a significant increase in serum level of glucose in rats after three hours of administration when it is compared with the control group and previous authors have shown a similar increase in glucose value on different laboratory animals (30). The reason for hyperglycemia is due to the effect of amitraz to α_2-adrenergic receptor in the pancreas, which increase glucagon secretion with decrease of insulin secretion from pancreatic cells (7,10,30) .

After three hours of oral administration of amitraz in rats at doses (100,250 and 500mg/kg) orally, cortisol hormone level detected a significant elevation in its value when compared with control group, the current results agree with previous experiments done on cats regarding cortisol level (29,31), amitraz causes an increase in the level of cortisol hormone because of the stress and this stress may be stimulate the secretion of adrenocorticotropic hormone (ACTH) from hypothalamus to stimulate the adrenal gland for the secretion of cortisol from the adrenal cortex (32).

Giving amitraz at doses (100,250 and 500mg/kg) , orally resembled a significant increase in the laboratory finding of AST enzyme in the serum level of rats when it is compared with control group after three hours of amitraz administration and these measures corresponds with the previous author worked on amitraz (33,34), the elevation of AST enzyme is an indicator of the damage in the mitochondria of the hepatocellular tissues in mice (35). Elevation of ALT was observed in the present work when rats were given (250 and 500mg/kg),orally after three hours of amitraz administration in comparison to the control group and previous researchers (36) mentioned the same elevation of ALT and this elevation is due to generalized hepatocellular damage in rats.

The current observations for the detection of ALP level in serum of rats at dose 500mg/kg of body weight exhibited a significant increase in ALP serum level in comparison with control group and the present measures agrees with previous researchers (26,37) but our ALP value does not agree with other researchers regarding amitraz ingestion like in human (5) and in horse under prolonged use of amitraz by intravenous route (38).

The level of CPK when amitraz was given at doses (100, 250 and 500 mg/kg), orally in rats has not shown a significant difference of the enzyme when compared with control group thus; it indicates that amitraz has no effect on cardiac muscles and

our values comply with previously done report on cats (24) and it does not agree with former researcher that observed an increase in the CPK enzyme activity in different times (25).

Finally, we can summarize our results that there are many toxic effects of amitraz represented to affect the biochemical parameters in rats.

تحديد الجرعة المميتة الوسطية للأميتراز و تأثيراته السمية الحادة على بعض المتغيرات الكيموحيوية المحدثة في الجرذان

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الخلاصة

الهدف من هذه الدراسة هو تحديد التأثيرات السمية وبعض التغييرات الكيميوحيوية للمبيد الحشري الاميتراز في إنات الجرذان البالغة، حددت الجرعة المميتة الوسطية للاميترازوالمعطاة فمويا وكانت (717.125 ملغم / كغم من وزن الجسم) وكانت علامات التسمم واضحة والمتمثلة بعدم تناسق المشي ، الخمول ، جحوض العين والتدمع ،كثرة التبول ،زياده اللعاب، الرقود و الموت . أدى اعطاء مضاد مستقبلات الفا ٢ الادرينالية الاتيباميزول وبجرعة (0.5 و 1 ملغم / كغم) و كذلك العقار القلوي بيكاربونات الصوديوم بجرعة (50 و 100 ملغم/ كغم) عن طريق الخلب وقبل خمسة عشر دقيقة من اعطاء الاميتراز بالفم الى إحداث زيادة في نسبة الوقاية للحيوانات المعاملة بالاتيباميزول(14.66% و 24.45 %) على التوالي وفي الحيوانات المعاملة ببيكاربونات الصوديوم (1.79% و 17.38%) على التوالي ، كما قللا من شدة علامات التسمم المحدث مقارنة بالامتراز لوحده . أحدثت الجرعتان تحت السامة من الاميتراز(250 و 500 ملغم / كغم من وزن الجسم) بالفم وبعد ثلاث ساعات من المعاملة زيادة معنوية في تركيز الكلوكوزلمصل الجرذان (2.9± 291.33 و 4.8±328.02 ملغم/ 100 مل) على التوالي مقارنة مع مجموعه السيطرة(9.6 ± 134.05 ملغم/ 100 مل) وزيادة معنوية في مستوى كورتزول المصل (0.3±7.30 و 0.3 ± 7.43 مايكروغرام/ 100 مل) على التوالي مقارنة مع مجموعة السيطرة (0.26 ± 2.39 مايكرو غرام/ 100 مل) ، و سبب الاميتراز وبنفس الجرعتين الأنفة الذكر الى زيادة معنوية في نشاط و تركيز خميرة كل من ناقلة أمين الالنين وناقلة أمين الاسبارتيت مقارنة مع مجموعة السيطرة بينما سببت الجرعة (500 ملغم / كغم ،بالفم) الى زيادة معنوية في نشاط و تركيز خميرة الفوسفاتيز القلوية في المصل مقارنة مع مجموعة السيطرة. تشير نتائج دراستنا الى ان المبيد الحشري الاميتراز قد أدى الى احداث اذى لنسيج الكبد والعضلات بالاضافة الى تأثيراته السمية الاخرى للجرذان

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