VERTIMEC® MEDIATES PLASMA BIOCHEMICAL CHANGES AND HISTOPATHOLOGICAL DAMAGE IN THE KIDNEY OF RATS (*RATTUS NORVEGICUS*)

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Abstract

Abamectin (ABA) is a biopesticide extracted from the actinomycete soil microorganism Streptomyces avermitilis, and the active ingredient of vertimec®, a commercial formulation commonly used pesticide in Algeria. Vertimec® was administered orally to male and female rats at dose of 2,13 mg/kg/day. The duration of the treatment was 28 days and the duration of the study was 42 days. Plasma samples were used to determine the biochemical parameters (glucose, urea, creatinine, uric acid).The residual concentrations of abamectine B1a were estimated in plasma and kidney of rats using UPLC MS/MS method. Treatment with ABA affects the mean weight of most organs (kidney, liver, heart, thymus, and adrenal glands). Moreover significant change in glucose, urea, creatinine and uric acid levels intra plasma after 14 and 28 days were observed. All experimental animals showed time-dependent presence of B1a residues in the plasma samples at 14 and 28 days. B1a residues in the kidney were detected in male and female rats at the end of the experiment 42days. Light microscopic observations revealed variable signs of nephrotoxicity in the ABA group, which were represented by alteration of normal renal architecture, inflammatory cell infiltration congestion and dilation of proximal and distal tubules. In conclusion, our investigations show that exposure vertimec® affects the reproductive and kidney function in male and female rats.

Key words: *abamectin, biochemical parameters, kidney histopathology, rat*

1. INTRODUCTION

Biopesticides are made from naturally occurring substances that controls pests by non-toxic mechanisms and in eco-friendly manner (Kumar, 2015). They may be derived from animals, plants and microorganisms, and include living organisms, their products or by-products (Mazid et al., 2011).

Avermectins (AVMs) are fermentation products generated by the soil microorganism bacteria *Streptomyces avermitilis*. AVMs are applied as veterinary drugs for food-producing animals and as plant protection agents in the agricultural sector. These compounds are one of the most active insecticides include ivermectin (IVE), abamectin (ABA), emamectin (EMA), eprinomectin (EPRI) and doramectin (DORA). The specific mechanism of action of these compounds refers to blocks the transmission of nervous signal at the neuromuscular junctions leading to insect death (Kolar et *al*. 2008). It has been reported that AVM poisoning contributes to multi-organs damages, including kidney, liver, stomach, nervous system, and testes (Lankas and *al*., 1997; Hsu et *al*. 2001; Inoue and *al*., 2009; Khaldoun-Oularbi and *al*. 2015; Khaldoun-Oularbi and *al*. 2016).

Abamectin (ABA) has been used extensively all over the world and is still one of the most commonly used pesticides in Algeria. Abamectin is a mixture of avermectin B1a (>90%) and avermectin B1b (<10%). These two components, B1a and B1b, have very similar biological and toxicological properties (Hayes and Laws, 1990). It affects inhibitory synapses via a mode of action involving glutamate-sensitive chloride channels. Castanha Zanoli *et al.* (2012) have shown that ABM perturbs the mitochondrial bioenergetics. Al-Sarar et al. (2015), demonstrate that abamectine have a potential

genotoxic effects on CHOK1 cells under the experimental conditions. Furthermore, it has been reported that ABA accumulate at high levels in fatty tissue, e.g., liver and fat due to its high lipophilic nature (Gonzalez, 2009).In previous studies, we demonstrate that the B1a and B1b residues of emamectin benzoate, an avermectin insecticide, are found in the kidney but only B1a residues of abamectin are found in the liver of male and female rats using UPLC MSMS method (Khaldoun-Oularbi and *al*. 2013; Khaldoun-Oularbi and *al*. 2015). The central role of the liver and kidneys in drug metabolism predisposes them to toxic injury and drugs excreted from the kidneys may cause cellular damage leading to kidney dysfunction.

In the present study, we examined possible effects of abamectin on nephrotoxicity by using biochemical, histological and analytical techniques in male and female rat.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Abamectin (Vertimec® 1.8% EC) was obtained from Syngenta Agro Services AG, Algeria. A standard of ABA (>98.7%, purity, Syngenta Agrochemicals, Greensboro, USA) was obtained from the Ministry of Agriculture and Rural Development (Direction de la Protection des Végétaux et des Controles Techniques DPVCT, Algeria). All chemicals and solvents were of the highest available commercial grade and were purchased from standard commercial suppliers.

2.2. Animals and Study Design

For this study, twenty-eight (160 - 180 g) apparently healthy male and female albino rats (*Rattus norvegicus*) were obtained from the CRD-SAIDAL Medea, Algeria. The rats were acclimated to laboratory conditions [22±2 ◦C and 50-55% humidity under a light/dark cycle of 12h] for 8 days before treatment and had free access to a standard commercial pellet diet (ONAB: Office National des Aliments de Bétail, Alger, Algeria) and water *ad libitum*.

After acclimatization period under laboratory conditions, rats were randomly divided into 4 groups each containing 7 animals. Animal experiment was carried out during 28 days as follows:

Group 1: Control male rats, received distilled water.

Group 2: Abamectin-treated male rats received 2, 13 mg/kg/day of ABA.

Group 3: Control female rats, received distilled water.

Group 4: Abamectin-treated female rats received 2, 13 mg/kg/day of ABA.

ABA was prepared in distilled water.

The study protocol was performed in accordance with the OECD guideline 407 for repeated dose 28-dayoral toxicity studies in rodents. The tested dose and the length of treatments were chosen according to preliminary experiments on rats, as described previously (Elbetieha and Isa Daas, 2003). After 28 days of treatment, the rats were followed-up for an additional 14 day period to detect delayed occurrence or persistence of toxic effects, as well as potential recovery from toxic effects. The body weight was determined throughout the acclimation (8 days), experimental (28 days) and post-treatment (14 days) periods.

2.3. Evaluation of body weight and organs weights

At the end of the experiment (day 42), the rats were euthanized by cervical decapitation under light diethyl ether anesthesia and their organs were removed and weighed. The vital organs of male and female rats (liver, kidney, heart, thymus, adrenal, ovary and testicle) were weighed immediately after sacrifice and their relative weights (organ weight/100 g of BW) were calculated.

2.4. Biochemical Analysis

Blood samples were collected from the orbital sinus vein at 14, 28 and 42 days. After blood collection, plasma was separated by centrifugation at 4000 r/min for 15min, the following renal parameters uric acid and creatinine levels were assessed in plasma using a commercially available spectrophotometric kit (Biolabo, France) and analyzed by an auto-analyzer (Hitachi 912) instrument (Roche Diagnostics, Mannheim, Germany).

2.5. Histological Analysis

Kidneys were quickly removed and processed for histopathological investigation. Small pieces of all kidney samples were cut and kept in 10% buffered formalin phosphate solution, embedded in paraffin, and sectioned at 3µm. The serial sections were stained with hematoxylin-eosin (H&E).

2.6. Kidney ABA concentrations

An ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) method employing electro spray ionization (ESI) was developed for the determination of avermectin B1a in the kidneys of rats using methyl clonazepam as the internal standard (EI). Right kidney tissues of rats were cut into small pieces and placed in a glass tube; 1 000 µL of ACN were sequentially added to testes sample, then vortexed. The mixing and extraction steps were performed using a vortex for 2 min and ultrasonication for 15 min. After centrifugation at 10,800 rpm for 8 min, the supernatant was evaporated under nitrogen. The testes residues were reconstituted in 100 µL of the mobile phase [(A): (87%) ammonium formate solution and (B): (13%) ACN and formic acid]. After centrifugation at 4000 rpm for 10 min, 15 µL of the supernatant was injected into the UPLC—MS/MS.

2.7. Statistical analysis

Statistical analysis was performed using Statistica version 10.0 (Stat Soft Inc., Tulsa, USA). Data were calculated using one-way analysis of variance (ANOVA) followed by the Duncan's post-hoc tests. Data were expressed as the mean ±SD. $P < 0.05$ was considered as the level of significance.

3. RESULTS

3.1. Evaluation of body weight and organs weights

During the study period, there were no clinical signs of toxicity in any treatment group. However, there were significant decreases in body weights of rat's administrated ABA compared to control one after 28 days treatment. The reduction in body weights was more marked in male than in female rats compared to control (Table 1). Though, after 14 days post-treatment period ABA provokes an increase in body weights gain in male and female treated rats.

Table 1. Effects of 2.13 mg/Kg/ABA treatments on body weight (BW) in male (M) and female (F) rats at the acclimatation (Ac; 8 days), treatment (Tr; 28 days) and post-treatment (P-Tr; 42 days) periods.

Body weight				
Period	**Control M[c]**	**ABA M[a, c]**	**Control F[c]**	**ABA F[b, c]**
Ac	153.6±4.9	165.1±5.20	283.5±5.27	262.9±4.53
Tr	183.4±12.7	197.5±11.54	313.1±11.49	338.8±3.89
P-Tr	215.7±6.62	202.11±5.38*	276.41±12.24*	272.55±2.69*

[a]2.13 mg of ABA/kg/day; [b]2.13 mg of ABA/kg/day; [c]Results are given as a mean ± SD ($n = 7$).

*Statistically different from the control groups ($P < 0.05$).

As shown in table 2, there were insignificant changes in the relative weights of kidney (right and left), thymus and heart of male or female abamectin-treated rats. In contrast, treatment with 2.13 mg/Kg/ABA induced a significant reduction in relative liver, adrenal (right and left), testes (right and left) and ovary (right and left) weights of ABA-groups compared to respective control.

Table 2. Effects of 2, 13 mg/kg/day ABA on absolute and relative organs weights in male (M) and female (F) rats.

Organs	**Weight (g)**	**Control M[c]**	**ABA M[a, c]**	**Control F[c]**	**ABA F[b, c]**
Kidney (right)	Absolute	0,77 ± 0,14	0,70 ± 0,03	1,16 ± 0,09	0,98 ± 0,10
	Relative	0,35 ± 0,06	0,34 ± 0,01	0,34 ± 0,02	0,36 ± 0,01
Kidney (left)	Absolute	0,72 ± 0,13	0,70 ± 0,04	1,14 ± 0,09	0,96 ± 0,10
	Relative	0,36 ± 0,06	0,34 ± 0,02	0,33 ± 0,02	0,35 ± 0,01
Thymus	Absolute	0,54 ± 0,12	0,45 ± 0,08	0,45 ± 0,06	0,30 ± 0,10
	Relative	0,25 ± 0,05	0,22 ± 0,04	0,13 ± 0,01	0,11 ± 0,03
Heart	Absolute	0,64 ± 0,03	0,68 ± 0,04	0,93 ± 0,01	0,79 ± 0,07
	Relative	0,29 ± 0,01	0,34 ± 0,02	0,27 ± 0,03	0,29 ± 0,02
Liver	Absolute	14,2± 1,87	11,7± 2,73*	9,26 ± 0,65	9,7 ± 0,92*
	Relative	4,1 ± 0,38	4,3 ± 0,61*	4,19 ± 0,2	4,8± 0,32*
Adrenal (right)	Absolute	5,31 ± 0,2	4,31 ± 0,1*	4,98 ± 0,07	2,83 ± 0,05*
	Relative	0,46 ± 0,01	2,13 ± 0,05*	1,46 ± 0,02	1,08 ± 0,01*
Adrenal (left)	Absolute	5,43± 0,3	4,2 ± 0,7*	4,97 ± 0,05	2,95 ± 0,04*
	Relative	2,51 ± 0,01	2,07 ± 0,02*	1,46 ± 0,01	1,03 ± 0,01*
Testicle / Ovary (left)	Absolute	8,42 ± 0,04	6,61 ± 0,02*	1,69 ± 0,07	1,37 ± 0,3*
	Relative	3,90 ± 0,03	3,27 ± 0,03	0,50 ± 0,02	0,30 ± 0,01
Testicle / Ovary (left)	Absolute	8,35 ± 0,04	6,79 ± 0,01*	1,71 ± 0,1	1,32 ± 0,21
	Relative	3,87±0,01	3,36±0,06 *	0,51 ± 0,03	0,25 ± 0,01

[a]2, 13 mg of ABA/kg/day; [b]2, 13 mg of ABA/kg/day; [c]Results are given as a mean ± SD (n = 7).

*Statistically different from the control groups ($P < 0.05$).

3.2. Biochemical results

The effects of ABM on kidney biomarkers in male and female rats are given in Table 3. ABM exposure caused a significant($p < 0.05$) increase in creatinine and uric acid levels in ABM-treated rats at 14 and 28 days of treatment. However, after 14 days post-treatment period ABA did not affect plasma uric acid levels.

Table 3. Effects of 2,13mg of ABA/kg/day treatments on plasma biochemical parameters in rats

Period	Blood chemistry parameters		Rats (n = 7 per group)		
		Control M[c]	ABA M[a, c]	Control F[c]	ABA F[b, c]
Day 14	Creatinine (mg/dL)	5,00±0,28	6,20±0,25*	4,20±0,73	6,0 ±0,00*
	Uricacid (mg/dL)	5,36±0,36	4,60 ±0,40*	6,40 ±0,24	3,66 ±0,55*
Day 28	Creatinine (mg/dL)	5,59±0,28	5,72±0,25	5,25 ±0,13	5,78 ±0,05
	Uricacid (mg/dL)	6,35 ±0,45	5,00 ±0,54*	6,25 ±0,43	4,66 ±0,55*
Day 42	Creatinine (mg/dL)	5,24±0,28	8,26±0,25*	6,79 ±0,56	6,38 ±0,00*
	Uricacid (mg/dL)	5,80± 0,46	5,60 ±0,24	6,30±0,12	5,83±0,58

[a]2, 13 mg of ABA/kg/day; [b]2, 13 mg of ABA/kg/day; [c]Results are given as a mean ± SD (n = 7).

*Statistically different from the control groups ($P < 0.05$).

3.3. Histopathology of the kidney

Microscopic examinations showed pathological lesions induced by abamectin. There were histological alterations in the kidney of both male and female treated rats when compared to control. The main findings were a narrowed appearance of Bowman's space, a widened lumen, cellular in filtration including multiple foci of hemorrhage, tubular cell desquamation and cloudy swelling of tubules in the kidney (Figure 1). These histopathological changes were in agreement with biochemical parameters.

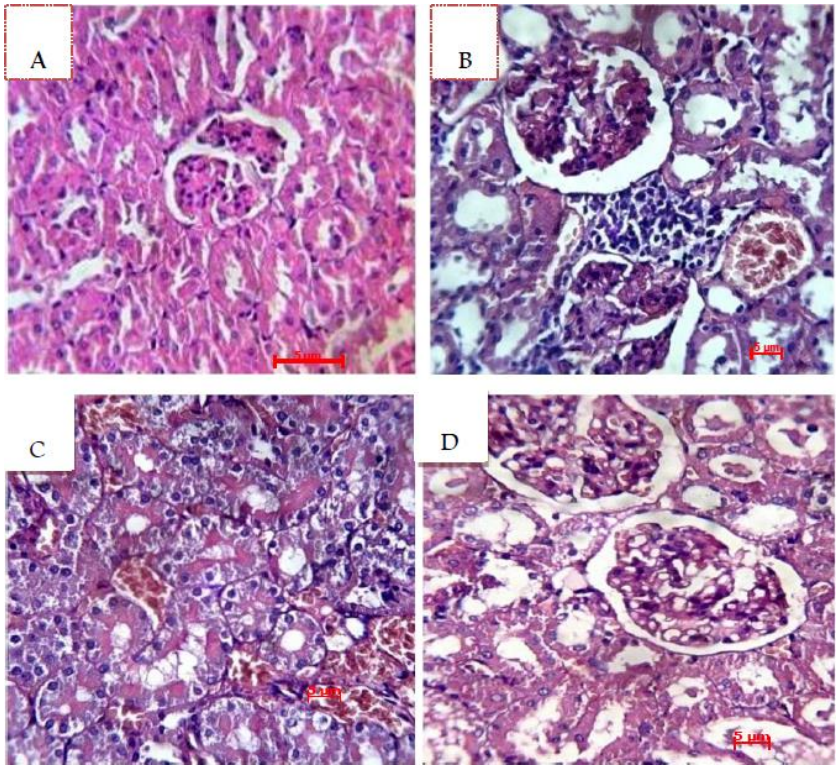

Figure 1. Effects of abamectin on the structure of rat kidney tissues. (A) Photograph of a kidney section of control rats showing normal histological structure architecture (H&E; (A) 100; (B) 400). Photographs, of a kidney section of ABA-treated group (B, C, D) after 42 days, showing inflammatory cell invasion, tissue congestion and vacuolization in tubular and glomerular cells H&E (400).

3.4. Plasma and liver abamectin concentrations

Abamectin was found in all kidney sections of male and female ABM-treated rats (Figure 2). Results in table 4 showed that higher avermectin B1a concentrations were recovered in the kidney of female ABM-treated rats (95ng/g) than those measured in male rats (81ng/g) at 42 days.

Table 4. Kidney abamectin B1a (ng/mg) concentrations at 42 days in male rats.

Kidney B1a Residues (ng/mg)				
Group	**Control M[c]**	**ABA M[a, c]**	**Control F[c]**	**ABA F[b, c]**
B1a (ng/mg)	0.00±0.00	81 ±1,24*	0.00±0.00	95± 2,18*

[a]2.13 mg of ABA/kg/day; [b]2.13 mg of ABA/kg/day; [c]Results are given as a mean ± SD (n = 7).

*Statistically different from the control groups (P < 0.05).

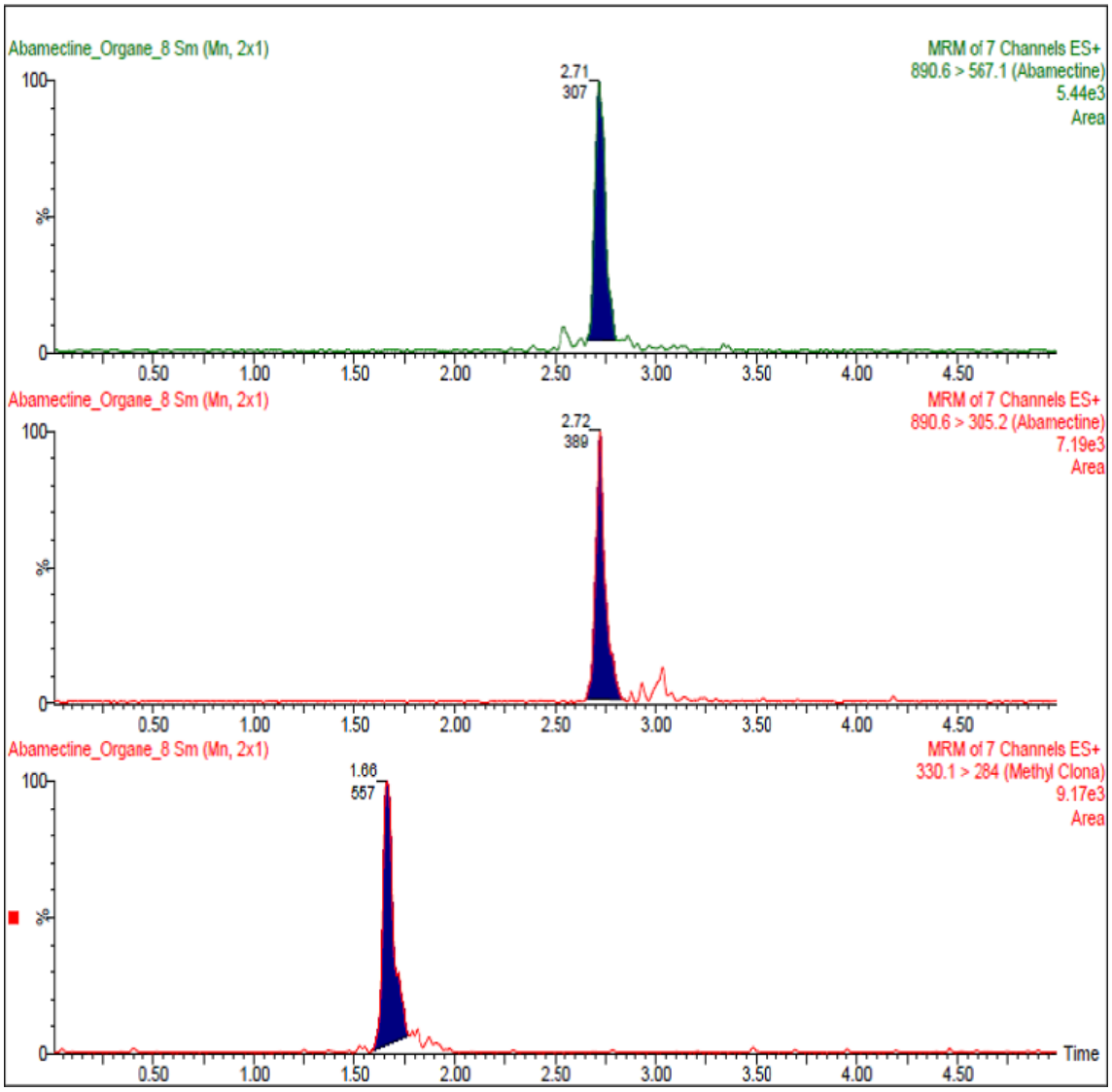

Figure 2. Chromatograms of the two MRM transitions of ABM B1a in the kidney. The mass transitions used are as follows: avermectin B1a: 890.6 > 567.1; 890.6 > 305.2 and EI: 330.1 > 284; 330.1255.1.

4. DISCUSSION

Xenobiotics and environmental pollutants such as pesticides are known to induce a broad spectrum of toxicological effects and biochemical dysfunctions constituting serious hazards to health (Fetoui and *al*., 2010). The kidney is the critical target organ for xenobiotic compounds which produce a variety of renal toxic effects involving tubular cells and glomerulus (Mohamed and *al*., 2003).

It is known that the variations in body weight are sensitive signs for the detection of potentially toxic chemicals (Bailey and al., 2004). During the experiment, the body weight was reduced in the ABA-treated rats after 28days. This result is consistent with our previous study showing that sub chronic exposure to emamectin benzoate an avermectin insecticide, significantly reduced body weight gain of rats (Khaldoun Oularbi and *al*., 2015).This is probably due to the toxic effect of the insecticide causing decreased intake and absorption of nutrients by the gastrointestinal tract and altered efficiency of food conversion (Sheriff and *al*., 2002). However, after the 14 days post-treatment period, the body weight of the ABA-treated rats remains higher than that of the control rats. Taken together, our results suggested that abamectin, like emamectin benzoate, exposure probably contribute to the development of obesity and diabetes (Khaldoun Oularbi and *al*., 2015).

The organ weights is important criteria for evaluation the toxicity of xenobiotics, in the present study absolute and relative organs weights of male and female rats were recorded. Results showed insignificant changes in the relative weights of kidney, thymus and heart of rats. But, treatment with 2.13 mg/Kg/day ABA induced a significant reduction in relative liver, adrenal, testes and ovary weights of male and female ABA-groups compared to respective control.

Also, results show that sub-acute oral administration of abamectin lead to systemic biochemical and histopathological damages of kidney. Plasma creatinine and uric acid increased in male and female ABA-treated groups, indicating that ABA disturbs the renal function. Similar results have been found in animals exposed to avermectin compounds (ivermectin, abamectin and emamectin benzoate) (Mossa and Abbassy 2012, Khaldoun and *al*., 2013; 2015).

The renal tissue showed in ABA treated group a severe glomerular and tubular lesions both in proximal and distal parts. The main findings were a narrowed appearance of Bowman's space, a widened lumen, cellular infiltration including multiple foci of hemorrhage, tubular cell desquamation and cloudy swelling of tubules in the kidney. Other studies reported histopathological alterations in kidney after exposure to ABA include necrosis, hemorrhage, degenerative changes and congestion (Abd-Elhady and Abou-Elghar 2013).

These morphological changes were confirmed by the increase in the plasma concentrations of urea and creatinine following ABA treatment. These results are in agreement with those performed by Eissa and Zidan (2010); Hamed and Abdel-Razik (2015) who showed that treatment of rats with insecticide causes an increase in plasma levels of creatinine and urea which is associated to renal dysfunction.

Liquid chromatography (LC) with fluorimetric detection has traditionally been used to determine avermectins in animal tissues (Danaher and *al*., 2006). Currently, liquid chromatography coupled to a triple quadru pole mass spectrometer (LC–QqQ–MS/MS) in the multiple reaction monitoring modes (MRM) is the technique of choice for confirm at or residue analysis (Hernando and *al*., 2007; Wang and *al*., 2011). In the present study, quantitative analysis of the abamectin B1a residue content in kidney was conducted using UHPLC-MSMS detection method. According to the literature, avermectin residues were detectable in various biological samples: fat, kidney, liver, muscle, testes, skin and milk (Hernando and *al*., 2007; Gianneti and *al*., 2011; Khaldoun Oularbi and *al*., 2016; 2015; 2014 and 2013; Turnipseed and *al*., 2005; Rübensam and *al*., 2013). Since ABA has high lipophilic properties and can accumulate at high concentration in biological samples including liver and kidney (Gonzalez 2009), maximum residue limits (MRL) have been established for some avermectins in specific tissues (Rúbies et *al*., 2015).

Significant levels of ABA residues were detected in kidney by a sensitive and rapid method using UPLC MS/MS, after 14-day post-exposure period. Only B1a isomer was detected in this study. Our results are consistent with the aforementioned findings.

In conclusion, this study demonstrates that abamectin provoke biochemical and histopathological alteration in the kidney of male and female rats.

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