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Research paper

Comparative endocrine disrupting effects of abamectin and indoxacarb insecticides

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Abstract

Abamectin and indoxacarb are relatively new insecticides with different mode of action and are applied to control a wide range of plant pests. However, their side effects to mammals are not fully studied. Accordingly, current study aimed to compare the adverse effects of both insecticides against the endocrine biomarkers: triiodothyronine (T3), tetraiodothyronine (T4), follicular-stimulating hormone (FSH), progesterone, and testosterone. These parameters were measured after orally-injecting rats with 1/20 LD_{50} doses of each of abamectin and indoxacarb for 60 days. Results showed that indoxacarb had pronounced reduction in the contents of T3 and FSH hormones compared to control and abamectin. Also, indoxacarb increased testosterone level compared to abamectin. T4 level was reduced by abamectin treatment compared to indoxacarb. Progesterone content was significantly increased after the abamectin treatment, while it was decreased after the indoxacarb treatment. However, the tested insecticides belong to avermectins and oxadiazine chemical groups that have different mode of action. They showed some similarity in their effect on T3, T4, and FSH, except for the progesterone hormone that showed a contradicting response. These two insecticides were marked as safe but current study highlight the need for caution during their application in the integrated pest management program.

Keywords: *Insecticides; Avermectins; Oxadiazine; White Albino Rats; Endocrine Biomarkers.*

1. Introduction

Pesticide development is an ongoing process. Production of new pesticides with different modes of action is a successful strategy to cope with a) the increasing demand for new products for pest management and b) the development of resistance to existing products. Abamectin and indoxacarb are relatively new insecticides with different modes of action and are used extensively to control many pests on different crops. Abamectin is derived from *Streptomyces avermitilis*, comprises at least 80% of avermectin B_{1a} and 20% of avermectin B_{1b} and is currently used as an insecticide, acaricide, and anthelmintic (Tomlin, 2004; Kolar et al., 2008).

Abamectin was introduced in 1985 as the lead member of the avermectins. It kills pests by interfering with the function of γ-aminobutyric acid (GABA) receptor in the peripheral nervous system and blocking the electrical activity in nerves and muscles (Clark et al., 1995). Specifically, it was reported that the target site for avermectins in insects and nematodes were glutamate-gated chloride channel, γ-aminobutyric acid (GABA)-gated chloride channel, and histamine-gated chloride channel (Turner and Schaeffer, 1989; Korystov et al., 1999; McCavera et al., 2007; Abd-Elhady and Abou-Elghar, 2013).

Abamectin is being used to controls leaf miners and mites on vegetable and fruit crops in the US, Brazil, Mexico, Italy, Egypt, France, Spain, Indonesia and Argentina (BASF, 2013). Although it was reported as toxic pesticide to fish, mammals and aquatic organisms (Lankas and Gordon, 1989; Abd-Elhady, 2012; BASF, 2013; IRAC, 2016). Moreover, abamectin showed maternal toxicity after a two-generation reproductive toxicity study on mice and rats (Lankas and Gordon, 1989; Pacqué et al., 1990; Abd-Elhady, 2012). Reproductive toxicity was reported for abamectin but no side effect was noticed on the testosterone hormone content after orally-injecting rats with doses of 1 and 4 mg kg^{-1} daily in sub-acute and sub-chronic studies (Celik-Ozenci et al., 2011). Also, the fertility was significantly reduced in male rats ingested abamectin at 10 mg kg^{-1} bw (1/30 LD_{50}) for 210 days once a week (Abd-Elhady and Abou-Elghar, 2013).

In parallel to abamectin, indoxacarb (methyl (S)-N-[7-chloro-2, 3, 4a, 5-tetrahydro-4a-(methoxycarbonyl)indeno[1, 2-e] [1], [3], [4]oxadiazin-2-ylcarbonyl]-4'-(trifluoro methoxy) carbanilate) was the first commercial broad-spectrum insecticide from the oxadiazine group (Tomlin, 2004). It belongs to the IRAC toxicity group number 22A, where it exerts its mode of action (insecticidal activity) through blocking of the voltage-dependent sodium channel (IRAC, 2016). It's been used on vegetables mainly against the insects of the Lepidoptera, Coleoptera, Hemiptera, Diptera, Orthoptera, and Hymenoptera (Wing et al., 1998; Liu et al., 2002; BASF, 2013). It has a selective action with no effects on non-target insects and it blocks the movement of sodium ion and stops insects feeding and paralysis (Dinter and Wiles, 2000).

However, indoxacarb was reported to be bioactivated inside the insects by amidases and esterases but catabolized in mammals (BASF, 2013; IRAC, 2016). Studies showed delayed toxicity on mice and rats after 14 days of administration of a single dose equal to the maximum tolerated oral dose (MTD = 500 mg kg^{-1}) (Shit et al., 2008). Also, chronic exposure (30 day) to indoxacarb caused deleterious oxidative stress effects as appeared on the lipid, protein, and glutathione content and glutathione-S-transferase, superoxide dismutase, and catalase enzyme activities of mice administered-orally with 6, 8 12 and 24 mg kg^{-1} d^{-1} (Mudaraddi et al., 2012). Studies on the adverse effects of these two insecticides on the endocrine system of mammals are limited. Therefore, current

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study was designed to compare the sub-chronic side effects of abamectin and indoxacarb on steroid and reproductive hormones of male rats.

2. Materials and methods

2.1. Chemicals and pesticides

Commercial kits of follicle stimulating hormone (FSH), progesterone, testosterone, triiodothyronine (T3), and thyroxine (T4) hormones were provided from reputed local chemical company. The commercial formulation of abamectin (Vertemic®, 1.8% EC) and indoxacarb (Avaunt®, 15% SC) were supplied by Syngenta Agro Services AG, Egypt.

2.2. Animals and treatments

Male albino rats (*Rattus norvegicus*) (110±10 g) were obtained from the Research Institute of Ophthalmology, Giza, Egypt. Animals were housed in small groups (5 each) inside propylene cages (25 X 50 cm) at 25±2 °C, 12 h dark/light photoperiod, and 70±10 % RH. Rats were fed with commercial pelleted rodent feed and water ad libitum. The animals were acclimatized to laboratory conditions for two weeks, and then divided into three groups of five adult males each. Rats in group-1 were administered 15 mg kg^{-1} b.w. (1/20 LD_{50}) of abamectin, group-2 rats were orally-injected with 86.6 mg kg^{-1} b.w. (1/20 LD_{50}) of indoxacarb. The third group of rats were orally-given equal volume of the solvent (distilled water) that was used to prepare the insecticide doses. Rats were given oral doses for 60 days. Handling of the experimental animals was consistent with the international principles on the care and use of experimental animals (National Research Council, 2011).

2.3. Blood collection

The animals from the three groups were sacrificed 24 hours after the last treatment. Rats were anesthetized using diethyl ether for 10 s before the neck vessels were aseptically severed. Blood was collected in 15 mL screw cap test tubes. Serum was separated by centrifugation of blood samples at 4,000 rpm for 10 min (Universal 32R, Hettich Zentrifugen model D-78532, Germany). Serum was stored in 200 µL portions in Eppendorf tubes at -20ºC until analysis.

2.4. Estimation of hormones content

Serum samples were used to estimate their contents of FSH, T3, T4, testosterone, and progesterone hormones. Follicle-stimulation hormone (FSH). Determination was carried out according to the method reported by Marshall (1975) and Rebar et al. (1982) using International Immuno Diagnostics kits. Concentrations of FSH in mlU mL^{-1} was estimated from a standard curve. Testosterone hormone determination was carried out according to the method reported by Granoff and Abraham (1979) and Tietz (1995) using the International Immuno Diagnostics kits and was expressed as ng mL^{-1}. Triiodothyronine (T3) hormone determination was carried out according to the method reported by Burke and Eastman (1974) using the International Immuno Diagnostics kits and expressed as ng mL^{-1}. Determination of the T4 hormone was carried out according to the method reported by Skelley et al. (1973) using the International Immuno Diagnostics kits and expressed as ng mL^{-1} from a standard curve. Progesterone content was determined according to the method reported by Cameron and Scarisbrik (1973) and Kakabakos and Khosravi (1992) and expressed in ng mL^{-1}.

3. Method validation

3.1. Accuracy and precision

Accuracy and precision were evaluated using five concentrations (0.5, 1, 2.5, 5, and 7.5 ng mL^{-1} of T3, 2, 5, 10, 15, and 25 µg dL^{-1} of T4, 5, 15, 50, 100, and 200 mlU mL^{-1} of FSH, 0.5, 3, 10, 25, and 50 ng mL^{-1} of progesterone, and 0.1, 0.5, 2, 6, and 18 ng mL^{-1} of testosterone) within the same day to obtain repeatability (intra-assay precision) and over 5 consecutive days to obtain intermediate precision (inter-assay precision). The accuracy and precision were calculated and expressed in terms of percent recovery and coefficient of variation (CV %), respectively (Ermer, 2005).

3.2. Statistical analysis

The present study was designed as an oral sub-chronic toxicity study. Results were analyzed using the General Linear Model (GLM) procedure of statistical analysis system as one way analysis of variance (one-way-ANOVA) (SAS version 9.3). Significant means were compared using Tukey-Kramer Honestly Significant Difference (HSD) post-hoc multiple comparison test ($P<0.05$) (SAS, 2013).

4. Results and discussions

The reproducibility of the analysis methods of T3, T4, FSH, testosterone, and progesterone were presented in Table 1. The inter-assay values (n = 25) ranged from 6.58 - 8.64% and intra-assay (n = 5) values were between 7.04 and 10.54%. These results were within the acceptable range and confirm that the analytical techniques were reliable.

Table 1: Summary of statistical analysis and percentages of the inter- and intra-assay values of tested hormones.

Variable	Mean	R^2	F value	Pr>F	Inter-Assay	Intra-Assay
Triiodothyronine (T3)	0.60	0.94	75.70	<0.0001	7.15	10.05
Tetraiodothyronine (T4)	6.83	0.94	62.21	<0.0001	6.97	9.24
Follicle-Stimulating Hormone (FSH)	10.16	0.93	60.15	<0.0001	8.64	10.54
Progesterone	0.41	0.88	31.77	<0.0001	7.83	8.71
Testosterone	3.52	0.67	9.09	0.0069	8.06	9.16

4.1. Effects of abamectin and indoxacarb on hormones adverse effects on thyroid hormones

Hormones are required for normal growth, development and metabolism of cells (Yen, 2001; Puri, 2011). Hormones are produced in the blood via several glands for example the thyroid gland, is the largest gland in humans, produces two principal hormones: thyroxine (tetra-iodothyronine; T4) and tri-iodothyronine (T3) (Puri, 2011). Both T3 and T4 are bio-indicators of the hypothalamus and pituitary glands activities (Kirsten, 2000; Mebis et al., 2008). These hormones have important function in the body, specifically the stimulation of metabolism (Puri, 2011; Quraishi et al., 2015). T3 and T4 help acquiring the element iodine and convert it into the form that is biologically available (Granner, 2003). Abamectin significantly decrease the content of both T4 and T3 hormones compared to control (Figure 1). Percentages of reduction of T4 and T3 compared to control were -50 and -45%, respectively. Also, indoxacarb reduced the concentration of both T4 and T3 hormones by -39 and -60%, respectively compared to the control group (Figure 2).

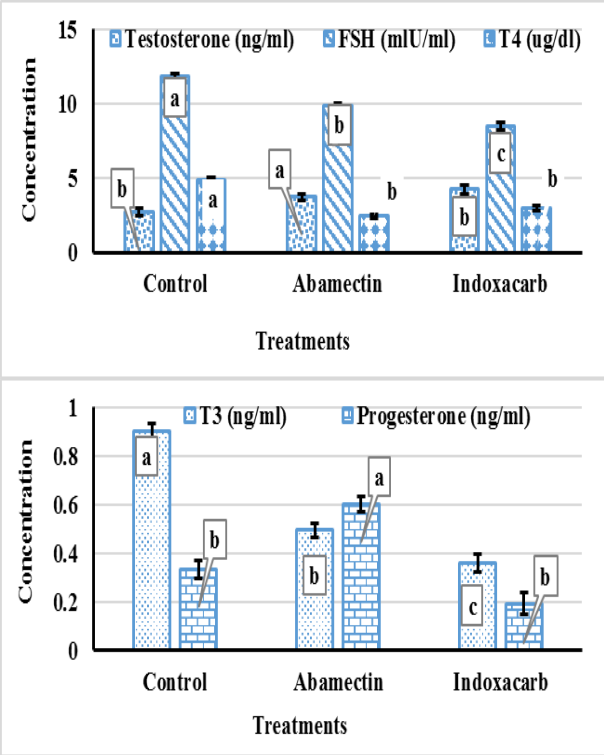


Figure 1. LS Mean values of content of (A) testosterone (ng mL^{-1}), follicle-stimulating hormone (FSH) (mIU mL^{-1}), and tetraiodothyronine (T4) (µg dL^{-1}) and (B) contents of triiodothyronine (T3) (ng mL^{-1}) and progesterone (ng mL^{-1}). Significance letters were based on Tukey-Kramer Honestly Significant Difference (HSD) *post-hoc* multiple comparison test ($P<0.05$). Charts were plotted in single graph based on similarity of the vertical scale value.

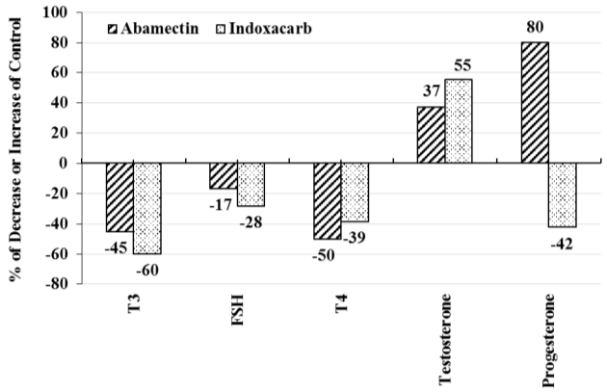


Figure 2. Percentages of decrease or increase of the contents of triiodothyronine (T3) (ng mL^{-1}), follicle-stimulating hormone (FSH) ($mIUml^{-1}$), tetraiodothyronine (T4) (µg dL^{-1}), testosterone (ng mL^{-1}), and progesterone (ng mL^{-1}).

4.2. Adverse effects on pituitary gland hormones

Follicular-stimulating hormone (FSH) is released from the gonadotropes in the pituitary gland (Puri, 2011). In women, FSH stimulates the aromatization of androgens to estrogens in the ovary, while in men, it works with testosterone in Sertoli cells in the testes to support spermatogenesis (Langouche and Berghe, 2008; Puri, 2011). Pesticides interfere with the normal function of FSH, where abamectin and indoxacarb significantly reduced the content of FSH hormone (Figure 1). Reduction were of -17 and -28% for abamectin and indoxacarb, respectively compared to control (Figure 2). So, these two insecticides would change FSH function in testes, which might affect the sperm formation and/or viability.

4.3. Adverse effects on steroid hormones

Testosterone works in association with FSH for normal spermatogenesis (Puri, 2011). FSH promotes the secretion of androgen binding protein, which permits high concentration of testosterone. It is necessary for spermatogenesis in seminiferous tubules (Puri, 2011). Both abamectin and indoxacarb increased the levels of testosterone (Figure 1) by 37 and 55%, respectively compared to control (Figure 2).

Progesterone is produced and secreted by the corpus luteum (Granner, 2003; Puri, 2011). Difference effects were found for abamectin and indoxacarb on progesterone. Abamectin significantly increased, while indoxacarb decreased the content on progesterone (Figure 1). Percentages of increase and decrease compared to control were 80 and -42% for abamectin and indoxacarb, respectively (Figure 2). These results were not in line with that of Celik-Ozenci et al. (2011). They reported that abamectin pose no adverse effects on testosterone hormone after orally injecting rats daily with doses of 1 and 4 mg kg^{-1} in subacute and sub-chronic studies. On the other hand, fertility of rats was significantly reduced after ingesting abamectin at 10 mg kg^{-1} b.w. (1/30 LD_{50}) for 210 days, once a week (Abd-Elhady and Abou-Elghar, 2013).

5. Conclusions

Indoxacarb showed pronounced reduction effect in T3 and FSH and an increase in the testosterone content compared to abamectin. Only T4, which was affected (reduced) after the abamectin treatment. Almost all of the tested biochemical parameters were affected similarly after both treatments of abamectin and indoxacarb except for the progesterone hormone, which showed different response to both insecticides. Progesterone was significantly increased after the abamectin treatment, while it was decreased after the indoxacarb treatment. The tested insecticides belong to avermectins and oxadiazine and exert their insecticidal effect with different mode of action blockers of GABA and sodium channel, respectively. They showed similarity in affecting T3, T4, and FSH except for progesterone hormone that showed contradicting response.

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