International Journal of Biological and Agricultral Reasearch

(IJBAR)

Journal home page: www http://www.univ-eloued.dz/ijbar/

ISSN: 2661-7056

Evaluation of the effect of spinosad on lipid peroxidation and antioxidant markers in female mice

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Received 4 August 2019; Accepted14 September; Available online 1 December 2019

Abstract

A Like any synthetic substance, or even natural, pesticides can produce harmful effects on human and animal health. The purpose of the present study is to evaluate the oxidative profile following the exposure of female mice to 9.2 mg/kg/day of spinosad. This is to examine the relationship between insecticide exposure and the risk of developing oxidative stress. Adult mice of the Naval Medical Research Institute were given by gavage daily for 18 days with spinosad, at rate of 9.2 mg/kg/day. Short-term exposure caused lipid auto-oxidation, expressed as an increase in malondialdehyde concentration in treated mice. Lipid peroxidation is associated with high level of plasma total anti-oxidative power and low level of plasma vitamin C. These results support the toxicity of the bioinsecticide witch induce oxidative stress in female mice.

Key words: Bioinsecticide, Spinosad, Toxicity, Oxidative stress, Female mice.

Introduction

Phytosanitary products, whether natural or synthetic, are used to increase agricultural yield [1] and protect plants and humans from various diseases [2]. These products are biologically active and therefore intentionally toxic to target organisms. Because of their intrinsic danger, unexpected contact of these substances with undesignated targets may cause serious disturbances to these targets. Man is one of those involuntary targets because he is the applicator of these substances but also, consumer of contaminated food resources by residues [3, 4].

During these two decades, toxicological research focused on the induction of oxidative stress after exposure to pesticides as a possible mechanism of toxicity. Oxidative stress has been reported to play an important role in the toxicity of synthetic pesticides including organophosphorus, N-methylcarbamates, organochlorines, pyrethroids, triazines, neonicotinoids, dithiocarbamates and paraquat [5, 6]. Maiza et *al*. [7] showed the involvement of bioinsecticides such as spinosad, neurotoxic molecule derived from the fermentation of a bacterium (*Saccharopolyspora spinosa*), in the induction of oxidative stress and lipid peroxidation of cell membranes in *Blattella germanica*. This bioinsecticide has low toxicity for human [8] and mammals [9], also preserves the environment [10] due to its rapid degradation [7].

According to several studies, pesticides that have the property of inducing oxidative stress resulting in the denaturation of biomolecules: proteins, membrane lipids, sugars and DNA [11, 12]. Indeed, oxidative stress is the result of imbalance between oxidants (malonidialdehyde (MDA), cause overproduction of reactive oxygen species in the intra- and extracellular spaces, hydroperoxides (ROOH), carbonylated proteins), and antioxidants (which neutralize ROS such as superoxide dismutase, glutathione peroxidase, reduced glutathione, and catalase [13].

The expression of a lipid peroxidation by a high level of malondialdehyde, an oxidative decomposition product of unsaturated lipids is used as a biomarker of oxidative stress [14] reflects the oxidative status of individuals exposed to xenobiotics [15]. This lipid peroxidation can be inhibited by an active antioxidant defense process based on enzymatic molecules but also with a non-enzymatic antioxidant system that includes vitamins A, C and E [7]. The primary objective of this study was to determine the effect of gavage of female mice by spinosad at a dose of 9.2 mg/kg/day (1/1000 LD50) on three status markers oxidant/antioxidant: malondialdehyde, the total antioxidant power of plasma and vitamin C.

Materials and methods

Animals

The study was carried out on female mice of the *Naval Medical Research Institute* strain weighing on average 21.71g ± 2.56g. After a period of acclimatization, the animals are divided into two groups, control and treated with spinosad. They have free access to water and food. The temperature of the animal house is maintained at 22°C, with a hygrometry of 65% and a photoperiod of 12 hours on 24°C.

Treatment

Eighteen mice are divided into two batches:

The first group comprises nine female control mice which are force-fed daily by distilled water at a rate of 10 ml/kg. The second batch comprises nine mice fed daily with spinosad at a rate of 9.2 mg/kg/day dissolved in 10 ml/kg of distilled water. At the end of the administration period, the mice are sacrificed; the blood is recovered in EDTA tubes. After centrifugation at 3000 rpm for 15 minutes, the plasma is used to perform the different dosages.

Dosage of plasma malondialdehyde

Malondialdehyde is the most used marker of lipid peroxidation especially by the simplicity and sensitivity of the technique. This assay is performed according to the method of Nourooz-Zadeh et *al*. [16]. By a hot acid treatment, the aldehydes react with thiobarbituric acid (TBA) to form a chromogenic condensation product consisting of two TBA molecules and one MDA molecule. The intense absorption of this chromogen is at 532 nm. The plasma MDA concentration is calculated using the extinction coefficient of the MDA-TBA complex ($\varepsilon=1.56\times10^5$mol-1.L.cm-1).s.

Determination of the total antioxidant power of the plasma

The total antioxidant power of plasma or the ability of plasma to absorb free oxygen radicals (ORAC) is determined by a method based on the anointing of the time of hemolysis of red blood cells induced by a generator of free radicals [17]. The principle of this method is based on the monitoring of hemolysis of red blood cells, as a function of time, induced by an oxidant (hydrogen peroxide H_2O_2). The ORAC of plasma is calculated by measuring the net protective area under the kinetic curve of hemolysis. The optical densities are obtained by reading at 450nm.

Dose of vitamin C

L-ascorbic acid, or vitamin C, is considered to be the most important antioxidant in extracellular fluids. The purpose of the assay is to determine the antioxidant defense capacity. Plasma vitamin C is assayed according to the method of Jagota and Dani using the Folin reagent and a standard range of ascorbic acid [18]. After precipitation of plasma proteins by trichloroacetic acid and centrifugation, the supernatant is mixed with distilled water and Folin's reagent. Vitamin C present in the supernatant reduces the Folin reagent giving a yellow color. The intensity of the coloration obtained is proportional to the concentration of vitamin C present in the sample and the reading of the absorbance is at 520nm. The concentration is determined from a standard curve obtained with an ascorbic acid solution.

Statistical calculation

The results are presented as mean ± standard deviation. The normality of the sample distribution is verified by a *Shapiro-Wilk* test using the statistical software S.P.S.S. (*Statistic Package for Social Science*). Intra

and intergroup comparisons are made by the *t-test* (*student test*) after the application of the *Ficher test*. The difference is judged statistically as when 0.05> p> 0.02.

Results and discussion

Plasma malondialdehyde content

As shown in Figure 1, Plasma MDA contents expressed in µmol/l show an increase statistically significant 9.2 mg/kg/day of spinosad (2.47 µmol/l ± 0.27 µmol/l) compared to the control mice (1.89 µmol/l ± 0.14 µmol/l).

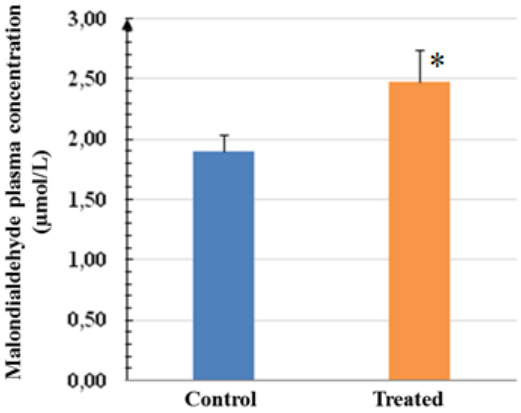

Figure 1. Plasma malondialdehyde contents in female control adult mice treated with spinosad (SPD). Statistically significant difference (P = 0.029).

The malondialdehyde assay results, the most potent marker of lipid peroxidation, revealed a significant increase in plasma MDA in SPD-treated mice. Similar results were found by Aboul-Enein et *al*.[19]in male mice exposed to SPD at 367.5 mg/kg/day, equivalent to 1/20 of the LD50, for two weeks.

Total antioxidant plasma power (ORAC)

The dosage of oxidative stress parameters show a non-significant variation in ORAC between the two groups of control mice (0.48 ± 0.10 IU) and treated (0.73 ± 0.14IU) (Figure 2).

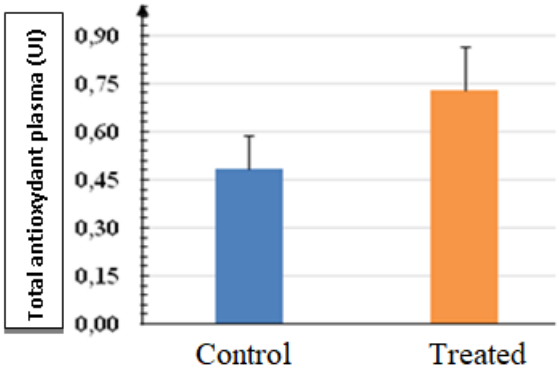

Figure 2. Total antioxidant plasma power (ORAC) in female mice adults control and treated with spinosad (SPD). No statistically significant difference (P = 0.06).

The total antioxidant power test of plasma shows a non-significant increase in SPD-treated mice compared to control mice. These results are similar to those recorded by Raina et *al*. [20]who found that the antioxidant activity increased by the increase of catalase activity, superoxide dismutase (SOD) and glutathione S-transferase (GST) in rats treated with cypermethrin, a synthetic insecticide belonging to the pyrethroid family [20, 21].

Content of plasma vitamin C

The results show a non-significant decrease in the plasma levels of vitamin C, an antioxidant molecule, in mice treated with SPD (Figure 3).

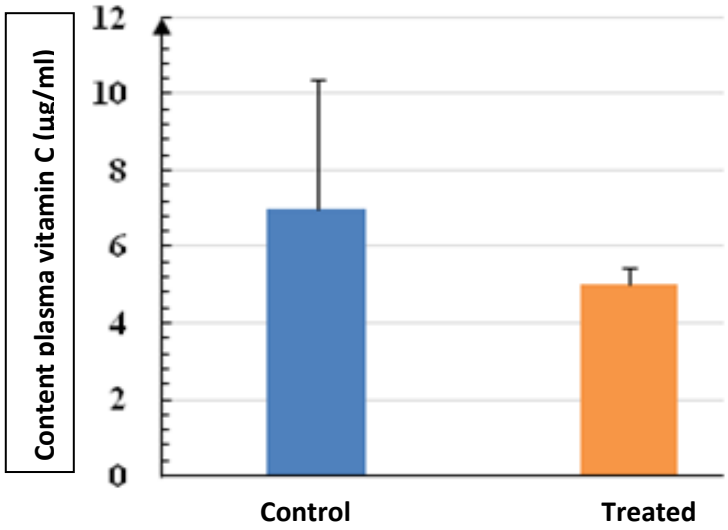

Figure 3: Content of plasma vitamin C (µg/ml) in control adult female mice and treated with spinosad (SPD). No statistically significant difference (P = 0.34).

These results are in agreement with a study which showed a decrease in the level of ascorbic acid in adult mice after treatment by the Manebe, a synthetic fungicide belonging to the dithiocarbamate family [22], used at a rate of 1/6, 1/4 and 1/2 of the LD50 for 7 days [23].

Conclusion

The present study shows results that support the bioinsecticide toxicity. This toxicity is related to lipid peroxidation induction in female mice.

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