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Original Article

Determination of Dichlorvos Effect on Uterine Contractility Using Wavelet Transform

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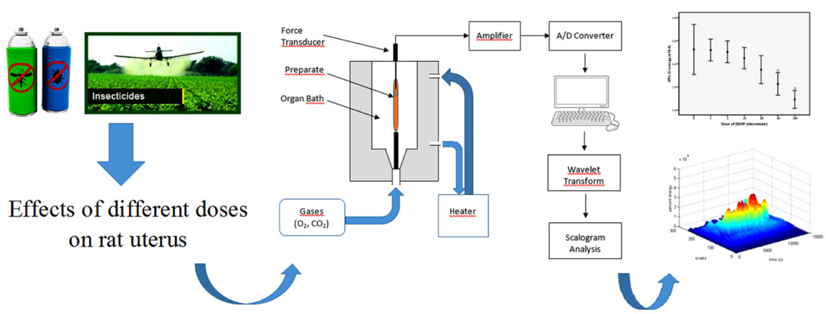
Graphical abstract



Abstract

Purpose: The use of organic synthetic pesticides has increased heavily in recent years and organophosphate insecticides constitute 20–38% of the total pesticides used worldwide. Dichlorvos, an organic phosphate insecticide, is widely used for the control of the agricultural, industrial and domestic pest in most of the agricultural countries like Turkey and it is known that many chemical toxins are capable of producing changes in uterine contractions. In this study, the effects of different doses of dichlorvos on isolated uterus muscle contractions are evaluated.

Methods: Wavelet scalogram analysis is preferred in the study because of its simultaneous time, frequency and energy screening capability.

Results: Obtained results show that 50 μM and 100 μM doses of DDVP significantly decreased the energy of contractions.

Conclusions: The study is important in that it shows toxic effects of DDVP on uterus which is responsible for reproductive function of mammalians and that it draws attention to an important public health problem. Furthermore, this is the first study showing that the wavelet transform can be readily utilized to determine the effects of insecticides and similar chemicals on the uterus spontaneous activity.

Keywords: Dichlorvos; Uterus; Wavelet scalogram; Spontaneous activity; Contraction

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1. Introduction

The use of organic synthetic pesticides has increased about 40-fold in 21st century. Pesticides are used to kill or control pests. Organophosphates, organochlorines, carbamates and pyrethroids are various groups of pesticides used worldwide. Currently, among the various groups of pesticides, organophosphates are in a major and most widely used group. The first organophosphate, tetraethyl pyrophosphate, was developed and used in 1937. In a quick time, several other organophosphorus pesticides were industrialized and commercialized. Globally, organophosphate insecticides constitute 20–38% of the total pesticides used [1]. Insecticides can damage various organs of human beings, including nervous, endocrine, reproductive, cardiovascular, respiratory and gastrointestinal systems, by different routes of exposure [2–7]. The organophosphate insecticide dichlorvos (2,2-dimethyl-dichlorovinyl phosphate, DDVP) is widely used in agriculture and at home for the control of pest. Like all organophosphate insecticides, dichlorvos inhibits activity of acetyl-cholinesterase, an enzyme responsible for the hydrolysis of acetylcholine. In addition to neurotoxicity, dichlorvos leads to nephrotoxicity, hepatotoxicity, genotoxicity and reproduction toxicity in non-target organisms [8–11].

Uterine contractions are important in many different reproductive functions including sperm and embryo transport, implantation, menstruation, gestation and parturition. Irregular uterine activity may underlie common and important disorders such as infertility, implantation failure, dysmenorrhea, endometriosis, spontaneous miscarriage or preterm birth [12]. Many chemical toxins are capable of producing changes in uterine contractions [13–17]. Wrobel et al. [13] examined effects of DDT and its metabolite (0.1, 1, 10 or 100 ng/ml) on the function of epithelial cells and muscle strips of bovine oviducts from 1 to 5 day of the estrous cycle. They concluded that oviductal secretion of prostaglandins is affected by DDT and DDE. In the study of Mlynarczuk et al. [14], luteal cell and myometrial stripts from a cow at early pregnancy were treated with the chloro-organic compounds (1 or 10 ng/ml). They suggested that chloro-organic compounds can influence uterine contractions and increase the risk of abortions in pregnant females. Criswell et al. [15] showed the lindane-induced inhibition of spontaneous activity in pregnant rat uterus. The effects of the essential oil of Mentha pulegium that is used in folk medicine as an abortifaceant, were assessed on the isolated rat myometrium by Soares et al. [16], and they showed the inhibitory effects of the essential oil of the *Mentha pulegium* on the isolated rat myometrium. Wrobel et al. [17], investigated the adverse effects of aldrin and dieldrin on both myometrial contractions and secretory functions of bovine ovaries and uterus in vitro. Obtained results showed that aldrin and dieldrin inhibit myometrial contractions.

In recent years, some mathematical approaches such as Fourier transform, wavelet transform, and neural networks have been applied to analyze uterine spontaneous signals [18–20]. Wavelet transform has an important place among them with its superior performance in analyzing nonstationary biological signals [21]. Because of that, there exist wide range of studies such as on the analysis of uterine signals [22,23], cardiac health and electrocardiography (ECG) [24–26], and electroencephalography (EEG) and electromyography (EMG) signals [27–29] in literature, in all of which the wavelet transform is utilized in analysis of the signals. In this study, the effects of different doses of DDVP on isolated uterus muscle contractions are evaluated by using wavelet scalogram analysis. Although there are studies which investigate the effects of various chemicals on uterine contractility, to the best of our knowledge, wavelet analysis has not previously been applied to test the effects of insecticides on the uterine contractions.

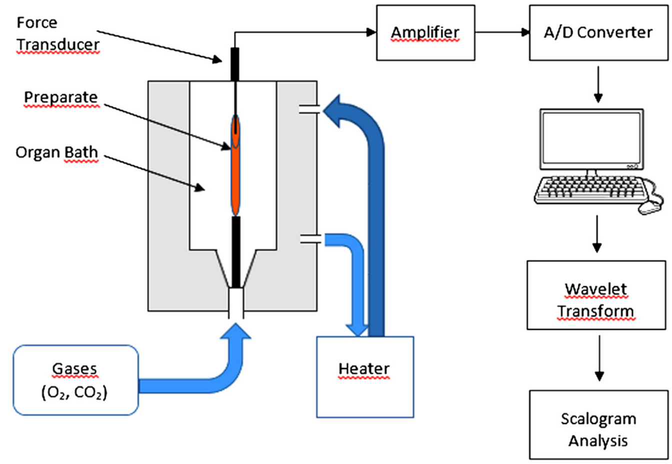

Fig. 1. Schematic diagram of organ bath and experimental setup.

2. Materials and methods

2.1. Animals and the data acquisition

Ten Sprague-Dawley albino female, young adult, non-pregnant rats (8–10 weeks of age, with average body weight of 200–250 g) were used in this study. Rats were obtained from the Experimental Animal Center, University of Mersin, Turkey. The rats were housed polycarbonate boxes with steel wire tops and rice husk bedding. They were maintained at the cycle of 12-h light and 12-h dark (at $25 \pm 2\,°C$ temperature with 55% humidity). Also they were given a standard pellet feed and tap water until the experiment time. The study was approved by the research and ethical committee of the Mersin University.

DDVP-containing solutions were prepared immediately before performing each experiment. Concentrations used in the present study ranged from 1–100 µM dichlorvos, which was selected according to our preliminary experiments. Rats were killed by inhalation of high dose of ether/ethyl alcohol (ethanol -C_2HO). Ventral incision was carried out and immediately uterus was dissected. Uterine horns (1.5–2 cm) were removed and freed from fat and the loosely bound connective tissue. The uterine was then placed in a 20 mL organ bath filled with Krebs solution having the composition of (mM) NaCl 118.0, KCl 4.55, $MgSO_4$ 5.7, KH_2PO_4 1.1, $CaCl_2$ 2.52, $NaHCO_3$ 25.0 and glucose 11.0, with pH adjusted to 7.4 (Fig. 1). The muscle tension was recorded by an isometric force transducer (Grass

FT.03) coupled to an amplifier (2 Channel Bridge 20-200 Internal Adjustable: X1(XS) Hugo Sachs Electronic) and then digitized by an A/D converter with a rate of 1 sample/s and finally stored on a computer for offline analysis. Throughout the experiments, the bath solution was gassed with a pressure tube having a mixture of 95% O_2 and 5% CO_2. All experiments were conducted at 37 °C.

Uterine horns placed in organ bath were then allowed to equilibrate at a resting muscle tension of 0.25 g for about 60 min. When the contractions became regular, data acquisition process was conducted for each preparation according to the following protocol: Spontaneous activity was recorded for 45 min while the preparation was in normal Krebs solution and this record is regarded as control data. Then DDVP was added cumulatively to the solution at the doses of 1 µM, 5 µM, 10 µM, 20 µM, 50 µM and 100 µM with intervals of 45 min between the doses.

2.2. Data analysis methods

In this study Wavelet Transform is used to analyze the recorded data. The continuous wavelet transform (CWT) can decompose a signal into a set of finite basis functions [30]. Wavelet coefficients $C_x(a,\tau)$ are produced through the convolution of a mother wavelet function $\Psi(t)$ with the analyzed signal $x(t)$ as

$$C_x(a,\tau) = \frac{1}{\sqrt{a}} \int x(t)\Psi^*\left(\frac{t-\tau}{a}\right)dt \tag{1}$$

where a and τ denote the scale and translation parameters respectively; $*$ denotes complex conjugation. By adjusting the scale a, which is inversely related with the frequency, a series of different frequency components in the signal can be extracted. The $\sqrt{a}$ is for energy normalization across the different scales. The wavelet transforms, thus projects the information of the time series $x(t)$ on the two dimensional time–frequency space (translation τ and scale a). In this study, the complex Morlet wavelet function, given in (2), is used as mother wavelet.

$$\phi_0(t) = \pi^{-1/4} e^{jw_0 t} e^{-(1/2)t^2} \tag{2}$$

Here, w_0 is the wavelet central pulsation and $w_0 = 2\pi$ is used in this study. Morlet wavelet is a Gaussian-windowed complex sinusoid and it is known that the Gaussian's second order exponential decay of the Morlet function gives a good time localization in the time domain [31], since it shows a great similarity to the shape of recorded uterine contraction signals. The scalogram of x represents the energy of x for each time instant and frequency component and defined by the function

$$S(a,\tau) = \left|C_x(a,\tau)\right|^2 \tag{3}$$

Clearly, $S(a,\tau) \geq 0$ for all time and scale values, and if $S(a,\tau) > 0$, the signal x is said to have details, i.e. energy components, at scale a and time τ. Therefore, the scalogram enables to detect the most representative scales (or frequencies) or time instants of a signal, i.e. the scales or time instants that contribute the most to the total energy of the signal. In this study, wavelet coefficients of the recordings were calculated using (1) at 256 discrete scales which span a frequency interval of 3 mHz to 1 Hz, and then the scalogram was obtained at these coefficients using (3). Finally, energy values at each coefficient is normalized using (4) and percent energy distribution of the recordings for each time and frequency values were obtained:

$$SC(a,\tau) = 100 * S(a,\tau) \Big/ \sum_{\substack{a=1\\ \tau=0}}^{\substack{T\\ 256}} S(a,\tau) \tag{4}$$

where T is the total signal length under analysis. For quantification and comparison purpose, mean energy values of each DDVP concentration intervals were calculated from $SC(a,\tau)$ for each recording. Then the results were involved in statistical evaluation.

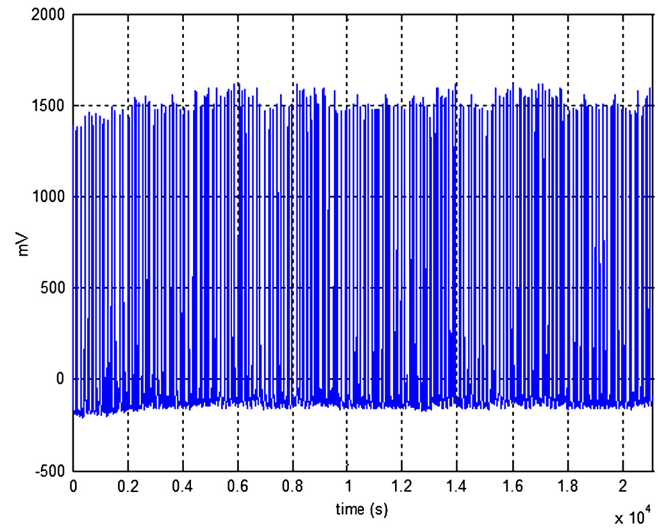

Fig. 2. 6 hours recording when there is no addition of DDVP.

2.3. Statistical evaluation

Statistical analysis was performed using a statistical packet program. After testing for normality of distribution with Kolmogorov–Smirnov test, data of mean contraction energy values calculated for each DDVP concentrations were analyzed with repeated measures analysis of variance. The results were considered statistically significant when $p < 0.05$.

3. Results

In the study, total data acquisition time was about five and a half hour and a single and continuous signal which contains records of control and all DDVP concentrations was recorded from each of ten preparations. In order to determine whether there is a change in normal uterus activity or not for an experiment duration, a six hours long activity signal was recorded from 3 uterus preparations without addition of DDVP. A sample of this control recording is given in Fig. 2. The results show that the activity continues at about 8–10 mHz contraction frequency for 6 hours when there is no intervention to the preparation.

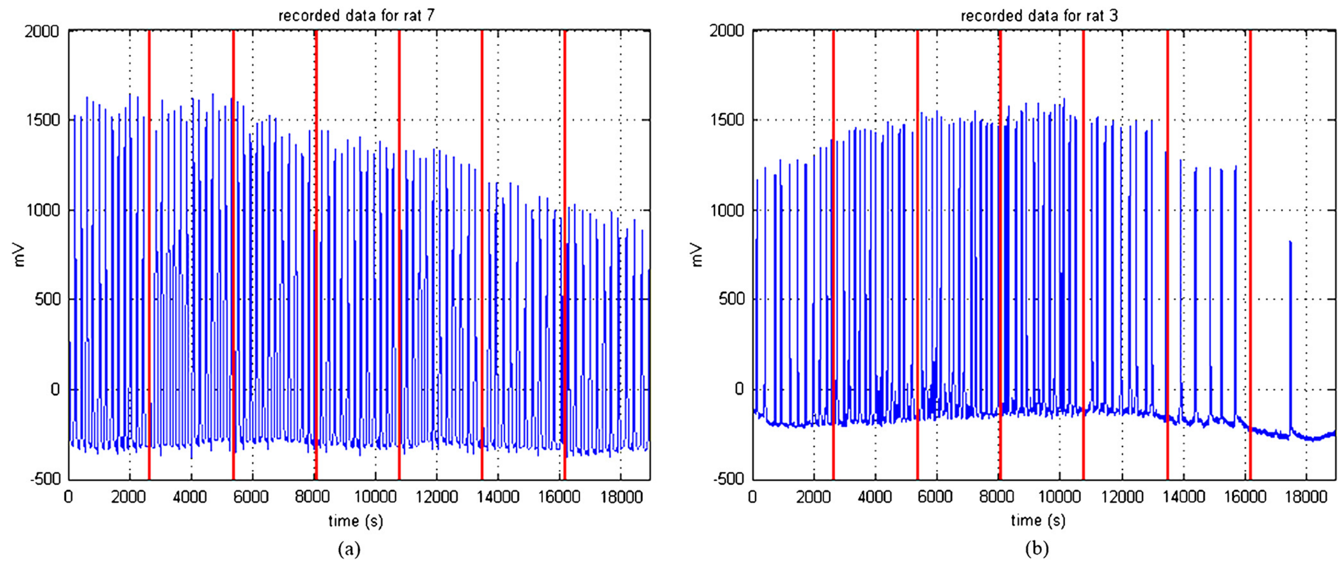

Fig. 3. Spontaneous activity signals recorded from the two of the preparations. Red vertical lines show the DDVP concentration changes.

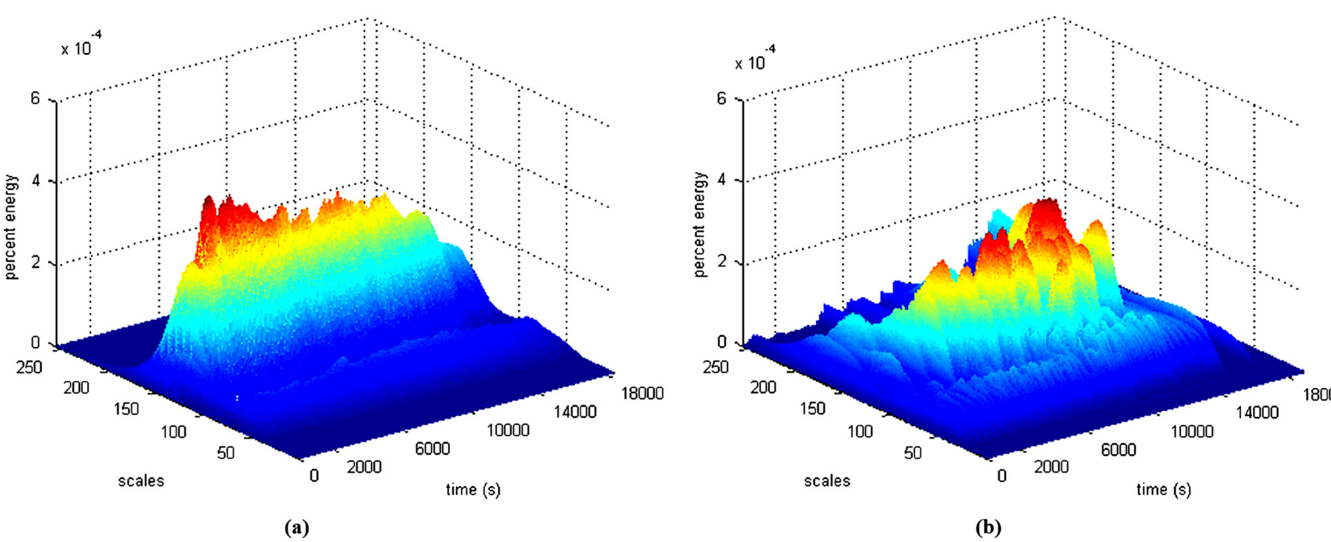

Fig. 4. 3D percent energy distribution plots of the recordings given in Fig. 3.

Plots of recorded signals measured from two of ten preparations are given in Fig. 3. Here, Fig. 3-a shows a typical record which resembles 7 of the recordings, whereas the plot in Fig. 3-b shows an example of excursive record resembled by remaining 2 recordings. It is important to note that all of ten measurements were included in further analysis. Red vertical lines in the figure show the DDVP concentration changes at 1 μM, 5 μM, 10 μM, 20 μM, 50 μM and 100 μM, respectively.

Contraction recordings are analyzed in order to reveal the effect of DDVP on uterine contractions. To do this, scalogram of the recordings were calculated from wavelet coefficients using (1) to (3) first, and then percent energy values of the recordings are calculated using (4). Fig. 4 shows the 3D percent energy distribution plots of the recordings given in Fig. 3 for all scale and time values. In this figure, x axis shows time, y axis shows scales and the z axis shows the corresponding percent energy values for the time and the scale. In order to increase the comprehensibility and ease the analysis, top view of these plots are also given in Fig. 5. Note that, red vertical lines used in Fig. 2 are also used in this figure to indicate concentration changes.

In accordance with the definition of the wavelet transform, wavelet coefficients and percent energy distributions of the recordings are obtained according to the scale values. But it is possible to convert these scales to pseudo frequency values using the formula

$$f_a = \frac{f_c}{a \cdot \Delta} \tag{5}$$

Here, f_c is the center frequency of the wavelet used in hertz, a is the scale value, Δ is the sampling period and f_a is the pseudo frequency corresponding to the scale a, in hertz. Obtained pseudo frequency correspondence table for the scales in Figs. 3 and 4 is given as plot in Fig. 6-a. Also it is given in logarithmic scale in Fig. 6-b.

Analyzing the last three figures together will give insight about the characteristics of uterus contractions under investiga-

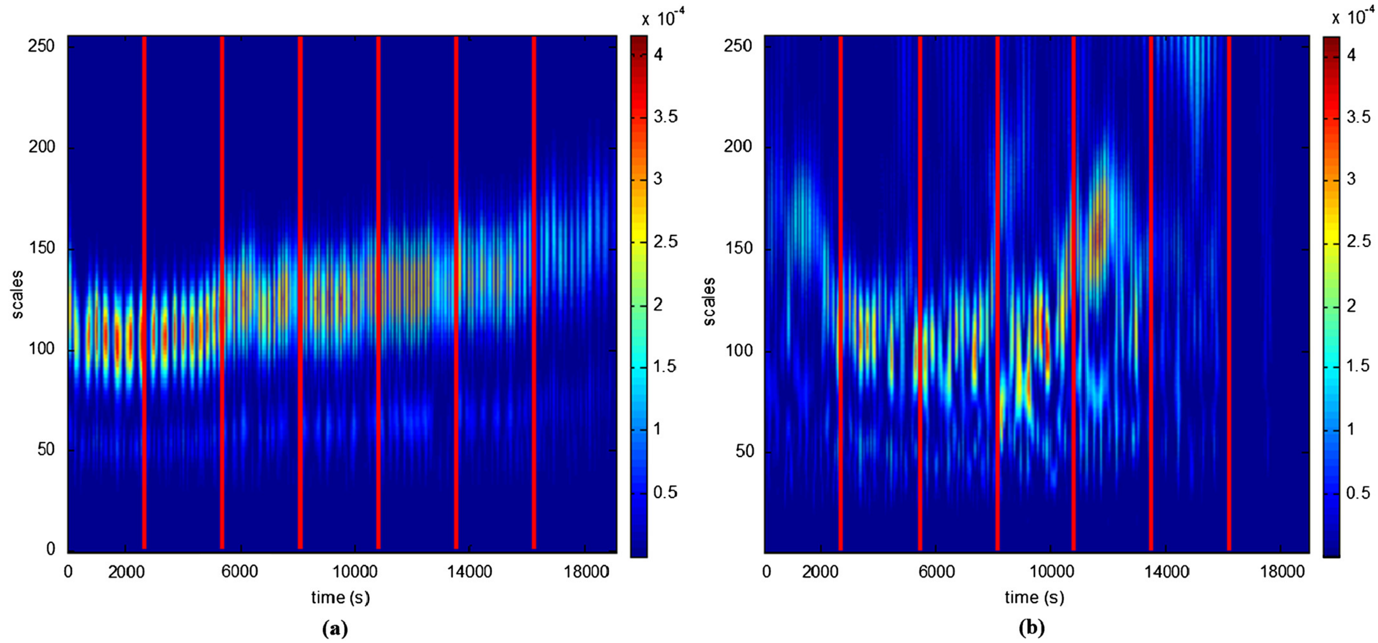

Fig. 5. Top view of percent energy distribution plots given in Fig. 4.

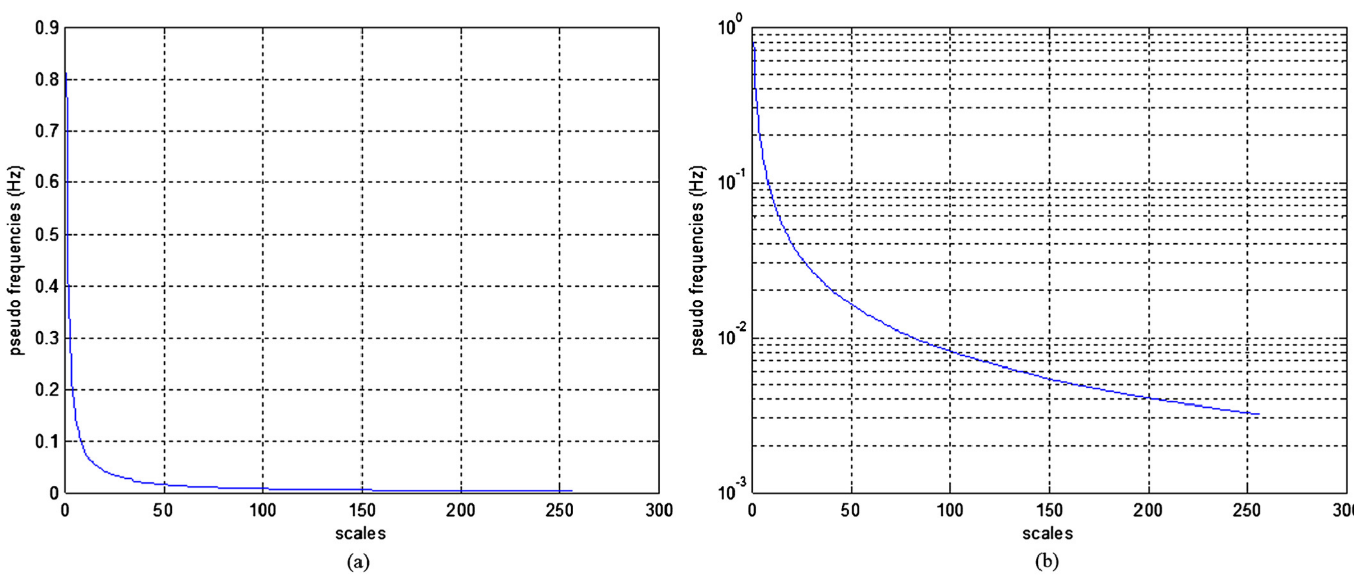

Fig. 6. Correspondence plots of scales and pseudo frequencies: (a) in normal plot, (b) in logarithmic plot.

tion. From Fig. 3-a, 4-a, and 5-a, it can be said that increasing DDVP concentration decreases the energy of contractions while decreasing their contraction frequency. Mathematically, if the percent energy distribution data is analyzed at different concentration values and a mean percent energy value is calculated from the percent energy values having a magnitude of greater than 1×10^{-4} for each concentration, it is seen that, at control and at 1 μM DDVP concentration, contractions have about the 3.8% of the total energy of recorded signal and the dominant frequency for this concentration is obtained at 105. scale which corresponds 8.3 mHz. When the concentration is increased to 5 μM, 10 μM and 20 μM, contractions become having about the 3.5% of the total signal energy and the dominant frequency becomes 6.5 mHz. Finally, for 50 μM and 100 μM DDVP concentrations, contractions lose their energy substantially and frequency of the contractions decreases to almost half of the frequency observed in control period. On the other hand, when the results of excursive data shown in Fig. 3-b, 4-b and 5-b are analyzed, it is seen that they show a similar behavior in the sense of decreasing energy and frequency while increasing DDVP concentration. The main difference of these recordings are the earlier die-outs of the contractions. To find a more generalized result from all ten recorded data, statistical evaluation of the results were performed. Obtained results are given in the Fig. 7.

Fig. 7 also shows that for all experiments, the increasing DDVP treatment dose decreases the energy of uterus contrac-

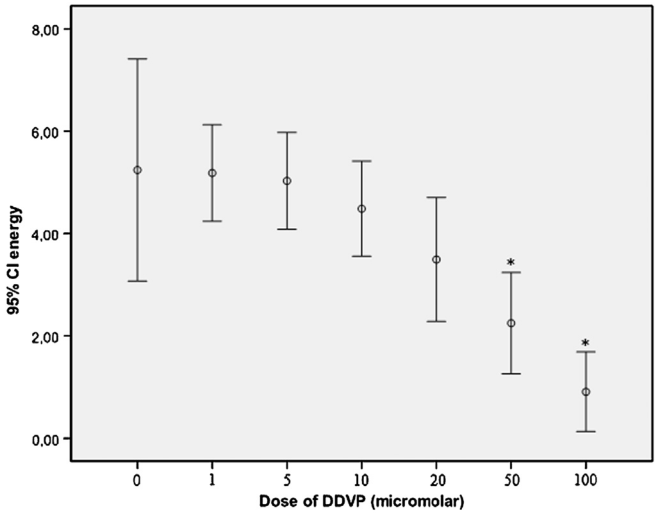

Fig. 7. Mean energy values in control and different doses. CI = confidence interval. Bars represent mean + standard deviation values. ∗ Significant difference from control (0) at $P < 0.05$.

tions. This decrease is significant in concentrations of 50 and 100 μM ($p < 0.05$).

4. Discussions

Myometrial contraction may be of vital importance in physiological processes such as sperm and embryo transport and implantation, and in disorders such as dysmenorrhea and endometriosis. In the present study, the effects of different doses of dichlorvos, an organic phosphate insecticide, on the spontaneous activity of rat uterus were investigated by using wavelet transform. The results showed that 50 and 100 μM dichlorvos concentrations decrease the energy of contractions significantly. Several studies in literature have examined the effect of insecticides on contractile activity of uterus. Loch-Caruso et al. [32] used 10 μM and 100 μM lindane and investigated the peak force and frequency of uterine contraction. They found decreased peak force and frequency for one hour exposure. Itthipanichpong et al. [33] studied 0.2 mg/mL 6-deoxyclitoriacetal on isolated rat uterus and found decreased spontaneous contraction. Similarly, Gravina et al. [34] studied rotenone on mouse uterus and they found decreased contraction force. Wrobel et al. [17] investigated the effect of 0.1, 1 or 10 ng/ml aldrin and dieldrin on myometrial strips from non-pregnant cows and they observed inhibited myometrial contractions. In the current study, DDVP is used at the doses of 1, 5, 10, 20, 50 and 100 μM and significantly decreased contraction energy was found at doses of 50 and 100 μM. Contraction mechanism of uterine is based on the phosphorylation of myosin light chains. Phosphorylation, by the enzyme myosin light chain kinase, is regulated by intracellular calcium concentration and binding of calcium to calmodulin. Phosphorylation of myosin light chains promotes interaction of actin and myosin cross bridges. Magnitude of contractions is related with the number of cross bridges and frequency of them is related with the attaching and detaching speed of cross bridges to the active region of actin. Therefore, from the results of the current study it could be concluded that DDVP might be taking effect by decreasing both the number and the attaching/detaching speeds of cross bridges.

In literature, various methods have been used to analyze spontaneous activity records and to conduct effect analysis studies. Temporal domain parameters such as the amplitude of the contraction, the area under the contraction curve and the time between contractions, as well as frequency domain parameters such as the amplitude of the fundamental frequency and the value of the fundamental frequency are widely used in most of these studies. But for temporal domain analysis, generally an operator dependent analysis, i.e. selecting definite number of contraction curves manually, marking the start and finish points of the curves manually, is required and these manual processes both take more time to analyze and use less number of results in analysis. Similarly, in case of frequency domain analysis, only one fundamental frequency value (magnitude & frequency) is obtained for a record and it becomes necessary to split the records in order to reveal more frequency information, which is also a time consuming process. On the other hand, the wavelet transform used in this study enables us to analyze the recordings in time and frequency domains, simultaneously. More clearly, since the analysis results in time frequency axis, it is possible to find the fundamental frequency of a contraction occurring in a time instant in the recording. Moreover, the area under the contraction curve can be approximated by the value of scalogram result (3rd dimension of Fig. 4), which can be combined with the time and frequency values. Therefore, wavelet analysis is thought as a more suitable and faster tool in analysis of recordings used in this study.

5. Conclusions

In this study, the effect of DDVP, an organic phosphorus insecticides which is widely used in agricultural pest control, on uterus spontaneous activity is analyzed using wavelet transform and revealed that 50 μM and 100 μM doses of DDVP significantly decrease the energy of contractions. The study is important in that it shows toxic effects of DDVP on uterus which is responsible for reproductive function of mammalians and that it draws attention to an important public health problem. Furthermore, this is the first study showing that the wavelet transform can be readily utilized to determine the effects of insecticides and similar chemicals on the uterus spontaneous activity.

Acknowledgements

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Competing interests: None declared.

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Ethical approval: Mersin University Experimental Animals Ethics Committee.

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