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RESEARCH ARTICLE

The action of monoterpenoids with promising antiparkinsonian activity on fertility of *Drosophila melanogaster*

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ABSTRACT

Adequate treatment of Parkinson's disease is an important medical issue. Patients are suffered from numerous side effects of the widely used drugs. It has been found recently that monoterpenoid (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl) cyclohex-3-ene-1,2-diol and its diacetate demonstrate high antiparkinsonian activity in some animal models. At the same time, their genetic safety was not studied yet. The aim of this research was to investigate the effect of these compounds with antiparkinsonian activities on reproduction of *D. melanogaster.* Over 4000 of fruitflies cultivated under the action of these compounds were analyzed. It was found that the chemicals did not provoke genetic mutations or alter reproduction of *D. melanogaster.* The possible explanations for the detected phenomena are provided. It was found that the compounds do not affect fertility in *Drosophila*..

Key words: Parkinson's disease; fertility; drosophila; (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol; acetate; terpene.

1. Introduction

Parkinson's disease (PD) is a progressive neurological disorder which severely affects movement, speech, behavior and other human functions. Despite a wide range of palliative help, management of distorted physiological functions remains a serious difficulty [1], and up to now there is no new critical help to PD patients except for levodopa implemented in 1970s [2]. In this connection, development of new antiparkinsonian drugs, possessing both pathophysiological and symptomatic actions, is of a high priority. Several years ago, Russian scientists from Novosibirsk Institute of Organic Chemistry (Novosibirsk, Russia) reported the synthesis of a new monoterpenoid substance, (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (Diol), with antiparkinsonian activities that were proved in animal PD models, and special features of the compounds were also reported [3-6]. Both diol and its diacetate demonstrated high anti-PD activity *in vivo* [6]. A few years ago, we reported on genotoxicological safety of diol using *Allium cepa*-test system [7]. In this sttudy we demonstrated the absense of any chromosomal abberrations after application of diol. However, some drugs have fertility disturbing effect, and it is an important issue for PD patients who is at reproductive

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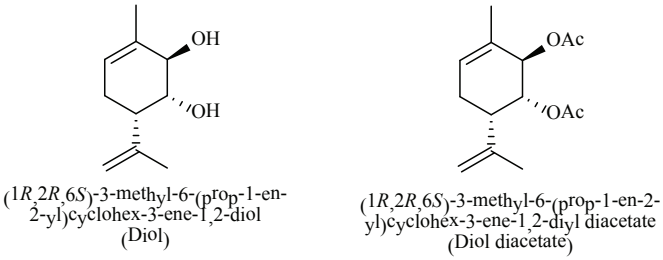
stage yet. The aim of this study was to investigate the action of diol and diol diacetate (Figure 1) on reproduction of *Drosophila melanogaster* to support these data on animal model.



(1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (Diol)

(1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diyl diacetate (Diol diacetate)

Figure 1. Structures of (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (diol) and (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diil diacetate (diol diacetate)

2. Results

D. melanogaster is widely used test-organism while investigating pathogenesis of Parkinson's disease [8, 9]. So, it is a good model to study effects of antiparkinsonian chemicals [10].

The total number of investigated flies was 2365 in F1 and 1764 in F2. It was detected that (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol did not affect the total fertility in F1 in comparison to control (difference is non-significant at the 0,05 level) (Table 1). At the same time, (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diil diacetate demonstrated about 50% reduction of total fertility in F1 (Table 1). Due to the specifics of gender fertility in F1; (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol application resulted in about 22% reduction of male's quantity (Table 1) whereas there was more the 50% reduction in the case of (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diil diacetate (Table 1). Concerning female quantity, we detected the 50% reduction in the case of (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diil diacetate (Table 1). Application of (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol did not affect significantly female in F1. Thus, only (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diil diacetate displayed a reduction in fertility (in all variants – about 50%) in F1. In the second generation (F2), we did not detect any differences between experimental and control variants (Table 1). We also compared the corresponding variants between F1 and F2. According to paired *t*-test, there was no differences in fertility between controls of F1 and F2. In all other variants, we also did not register differences between the corresponding F1 and F2 variants. Also, we did not detect changes in sex ratios in both generations (Table 2).

3. Discussion

In our study we did not detect any flies with morphological mutations reflecting in body changes. It is a very important observation since numerous results of other authors found various alterations in body structure due to the action of different compounds. For example, Delgado-Rodríguez and co-workers showed that complex mixtures extracted from air filters provoked wing mutations in *Drosophila* [11]. Frei *et al* reported on analogous wing mutations due to the action of mitoxantrone [12]. Other somatic mutations were described

Table 1. Action of Diol and Diol diacetate on drosophila fertility (mean±SE). Note: NS at $P<0.05$ – differences are non-significant at $P<0.05$.

Variants	Diol	Diol diacetate	Control
		F1	
Total	45.5±8.2 (NS at $P<0.05$)	29.3±8.6	60.1±10.5
Male	22.1±4.1	13.9±4.2	28.8±5.2
Female	23.4±4.1 (NS at $P<0.05$)	15.3±4.7	31.3±5.8
		F2	
Total	35.9±6.2 (NS at $P<0.05$)	31.1±5.5 (NS at $P<0.05$)	35.9±1.2
Male	16.8±3.5 (NS at $P<0.05$)	14.8±2.2 (NS at $P<0.05$)	18.4±1,0
Female	19.1±2.9 (NS at $P<0.05$)	16.3±3.4 (NS at $P<0.05$)	18.3±0.7

Table 2. Female / male ratios in F1 and F2 (action of Diol and Diol diacetate, mean±SE). Note: NS at $P<0.05$ – differences are non-significant at $P<0.05$.

Parameters	Variants		
	Diol	Diol diacetate	Control
		F1	
Female / male ratio	1.09±0,048 (NS at $P<0.05$)	1.17±0,233 (NS at $P<0.05$)	1.1±0,095 (NS at $P<0.05$)
		F2	
Female / male ratio	1.25±0.149 (NS at $P<0.05$)	1.06±0,127 (NS at $P<0.05$)	1.00±0,065 (NS at $P<0.05$)

under the action of anticancer drugs [13]. So, morphological changes may be considered as a significant marker of both mutagenic [14] and antimutagenic [15] effects.

Both compounds under study did not show any significant toxic effects on fertility of *Drosophila*. It was reported recently on the importace of fertility in drosophila studies [16]. The reduction of fertility in the case of diol diacetate is probably connected with differences in chemical structure of the chemicals. It is reasonable to propose that due to hydrolysis of diol diacetate *in vivo* there was a production of acetic acid that negatively affected insects: some suggestions on this issue were proposed before [17]. However, special experiments are warranted to check this assumption.

The ability to produce F1 and F2 offsprings suggests on the absence of embryotoxic effects of these compounds. It is possible to speculate that reproductive system was not affected with diol and diol diacetate: very likely that major players in the hormonal regulation of seminal protein production and insect male fertility - ecdysone receptors [18] –were not damaged by our chemicals. Moreover, the full restoration of fertility in F2 shows the ability of *Drosophila* flies to adapt to influences of diol and diol diacetate. The absence of changes in sex ratios in both generations seems an interesting fact too. So, no fertility damaging effect was detected: the similar results were reported in other model [19].

It is important to note that when using aversectin C (a broad-spectrum insecticide) as a positive control, we observed a 5-fold reduction of survival of flies of the first generation and 100% mortality in the second generation when using 1% of the substance, and the ratio of females to males was 0.364. A 10-fold decrease in the concentration of this insecticide led to the emergence of second generation larvae and pupae of fruit flies, but the development at this stage was stopped and the adults did not appear. The ratio of females to males was lower than the control (0.937). It is very interesting to note that inhibition of fertility of aversectin C was also reported in connection with its possible anticancer effects [20]. Germ cells like a cancer cells are under process of genome rejuvenation: therefore, if a compound reduces fertility, it also will demonstrate anticancer effect [21, 22]. It is a very important observation for theoretical biology.

4. Conclusion

Owing to a comprehensive analysis over 4000 of fruitflies, we obtained results suggesting on reproduction safety of the investigated chemicals toward *D. melanogaster.* It was found that diol and diol diacetate do not damage the fertility. Further research should include the vertebrate animals (mammals).

5. Materials and Methods

In this research we used a wild type of *Drosophila melanogaster*, which was obtained from Moscow State University (Department of Genetics), Moscow, Russian Federation. The flies were cultivated in 20 ml cylindrical glass tubes containing 5 ml of nutrient medium. The medium contained (per 1 L of medium): yeasts – 60 g, manna-croup – 40 g, agar – 10 g, propionic acid – 5 ml, bananas – 100 g, water – 1 L. Each tube contained 3 males and 2 females. As test chemicals, the following substances were taken (1%, w/w): (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diol (diol) and (1*R*,2*R*,6*S*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-3-ene-1,2-diil diacetate (diol diacetate) synthesized according to previously published methods [23, 24]. We added toxicants to growth medium during its preparation in the indicated concentration which was found to be active in previous animal studies in Parkinson models [3, 4]. We assessed fertility rate (a number of offsprings per each tube was detected), and female to male ratio in F1 and F2, respectively. Animals from F2 were cultivated on the same

nutrient medium. Control was common for both compounds. In general, over 4000 of fruitflies were analyzed. Results were analysed for goodness of fit to normal distributions by using the Shapiro-Wilk test. Pairwise comparisons between growth conditions were made for mean values of the different growth parameters using the one-sided Student's t-test. Calculations were made using the Origin 8.0 software for Windows.

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Authorship statement

Concept – M.V.T. K.P.V.; Design M.V.T.; Supervision – M.V.T. K.P.V.; Resource – O.V.A., N.F.S.; Materials – M.V.T. K.P.V., O.V.A., N.F.S.; Data Collection &/or Processing – M.V.T., R.G.K.; Analysis &/or Interpretation - ALL; Literature Search – M.V.T.; Writing – M.V.T. K.P.V.

Conflict of interest statement

No conflicts of interests were declared

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