

Evaluation of maternal and embryotoxic effects following the treatment of chloral hydrate in *Drosophila melanogaster*

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Abstract Chloral hydrate (CH) is commonly used as a sedative and a hypnotic in pediatric medicine. In this study, the effects of CH on various developmental stages of *Drosophila melanogaster* were investigated. Different concentrations of CH (0.1; 0.3; 0.5 and 1 mg/mL) were used during development of the flies. Maternal toxicity due to increasing the concentration of CH was observed as a large number of adult flies died. When the F₁ progeny of the control and application groups were compared, CH was found to extend the process of metamorphosis and to decrease the total number of offspring. The embryotoxic effects on the offspring and an increase in the number of malformed offspring was identified as depending on feeding. It was found that the difference between the groups was significantly important ($p < 0.05$).

Keywords *Drosophila melanogaster* · Chloral hydrate · Developmental stages · Maternal toxicity · Embryotoxic effect

Introduction

As reviewed by Gerretsen et al. (1979), chloral hydrate (CH) was synthesised by Liebig in 1832, and was introduced into clinical medicine as a hypnotic agent by Liebreich in 1869. CH is widely used in paediatric patients undergoing short procedures (e.g. diagnostic investigations), and is generally considered safe and effective in children (Kost and Roy 2010). However, adverse effects such as central nervous system depression, respiratory depression, cardiac arrhythmia, and gastric irritation can occur (Ludwigs et al. 1996; Rokicki 1996; Baselt 2004; Dogan-Duyar et al. 2010). Furthermore, there has been concern about the genotoxic effect of the prolonged use of CH based on studies conducted on rodents (Von Tungeln et al. 2002). In cases of overdose, management of this includes gastrointestinal decontamination, supportive care and eventually acute haemodialysis. Deaths occurring from a CH overdose have been reported (Graham et al. 1988; Englehart et al. 1998), and usually happen after a large dose of exposure; the cause has mainly been due to resistant cardiac dysrhythmia (Bowyer and Glasser 1980; Zahedi et al. 1999; Britton and Kosa 2010).

CH is widely used in both human and animal therapy; therefore, it is important to characterise any possible undesired effects in the different experimental models. *Drosophila melanogaster* and vertebrates show similarities such as peripheral nerves, suggesting that the mechanisms involved in the induction of

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developmental defects may be similar (Petersen 1990; Rodrigues et al. 2011). Therefore, *Drosophila* has been put forward as a useful, rapid and economical model for the preliminary screening of developmental toxicants (Schuler et al. 1982; Lynch et al. 1991; Rand 2010).

In this study, the possible toxic effects of CH on the developmental stages of *D. melanogaster* the model organism commonly used in toxicological research was investigated.

Materials and methods

Chemicals

CH and dimethyl sulphoxide (DMSO) were purchased from Fluka Chemical Corp. (Ronkonkoma, NY, USA). Diethyl ether, ethyl alcohol and Lacto Bacto Agar were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Flies

The flies used in the experiments were the Oregon R wild type strain of *D. melanogaster* Meigen (Diptera; Drosophilidae), which has been adapted to laboratory conditions by intermating it for a long time. This stock has a red eye colour, normal length wings, normal thoracic bristles and hair, and does not obtain any mutant genes. The flies were kept at a constant temperature of 25 ± 1 °C on a standard medium composed of maize-flour, agar, sucrose, dried yeast and propionic acid (standard *Drosophila* medium = SDM). The flies were kept in darkness, except during the transfers onto fresh medium (usually twice weekly). The humidity of the experimental chamber was 40–60 %.

The application of chloral hydrate to adult individuals

Four different single dose of CH (0.1; 0.3; 0.5 and 1 mg/mL) were added to 100 mL of SDM, and kept at room temperature for 24 h to diffuse the extracts to the medium. Flies with the same age were used for experiments, and ten male and ten female individuals were mated. All of these females used in the experiments were virgins. The culture vials containing only

SDM and SDM with DMSO were used as controls. In this experiment DMSO (1 %) was used as the solvent for CH. Then, developmental stages (metamorphosis stages) were followed daily. The dead parents were also counted. Parent flies were removed from the culture bottle when the first pupa appeared. The adult offspring (F_1) were examined according to their sexes and then the malformed individuals were recorded (Uysal and Aşkın 2007; Altun et al. 2011). The experiments were repeated three times for each group.

Statistical analyses

Statistical calculations were done by using SPSS 13.0 software. To be able to determine the statistical significance of the results, Duncan's one-way range test was applied. The differences between groups were considered significant at $p < 0.05$.

Results and discussion

In this study, maternal toxicity was observed at high CH concentration application groups (0.5 and 1 mg). Parental death was not noticed in the control groups and the first two application groups (0.1–0.3 mg). However, parental death was determined as a total of 14 females (46.67 %) and 18 males (60 %) in the 0.5 mg application group (since the fourth day of crossing), and as a total of 21 females (70 %) and 24 males (80 %) in the 1 mg application group (since the second day of crossing; Table 1).

Table 1 Frequency of mortality of *D. melanogaster* after exposure to CH concentrations of 0.1, 0.3, 0.5 and 1 mg/mL

Concentrations (mg/mL)	Total number of parent		Mortality (%)	
	♀♀	♂♂	♀♀	♂♂
Control	30	30	–	–
DMSO	30	30	–	–
0.1	30	30	–	–
0.3	30	30	–	–
0.5	30	30	14 (46.67)	18 (60)
1	30	30	21 (70)	24 (80)

SDM ('Control') and 'DMSO' were used as medium and solvent control, respectively

a,b,c,d Means with different letters are significantly different by Duncan's one way range test ($p < 0.05$)

Table 2 Effect of CH in the stages of development of *D. melanogaster*

Development stages	Treatment (mg/mL)											
	Control		DMSO		0.1	0.3	0.5	1	0.1	0.3	0.5	1
	♀♀	♂♂	♀♀	♂♂	♀♀				♀♀	♂♂		
Egg	1	1	1	1	1	1	1	2	1	1	1	2
1st L	2	2	2	2	2	3	3	3	2	3	3	3
2nd L	3	3	3	3	3	4	4	4	3	4	4	4
3rd L	4	4	4	4	4	6	6	6	4	6	6	6
Prepupa	5	5	5	5	7	8	8	7	7	8	8	8
Pupa	7	7	7	7	8	10	11	12	8	10	11	13
Adult	9	9	9	9	11	12	13	15	11	12	13	15
Σ F _I	555 ^a	539 ^a	542 ^a	535 ^a	345 ^b	268 ^c	249 ^c	85 ^d	333 ^b	262 ^c	243 ^c	76 ^d
Σ Malf. F _I	5 ^a	6 ^a	5 ^a	6 ^a	14 ^b	18 ^b	23 ^{bc}	41 ^d	13 ^b	22 ^c	23 ^c	42 ^d

Σ Malf.: the total number of malformed individuals, Σ F₁: the total number of individuals belonging to F₁ generation, L:larvae, DMSO: dimethyl sulphoxide

^{a,b,c,d} Means each column followed by the same letter are not different by Duncan's one way range test alpha =0.05

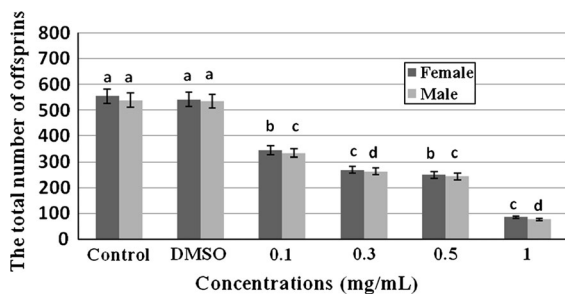


Fig. 1 The comparison of the total number of offspring belonging to the F₁ generation in controls and application groups with different concentrations of CH. SDM ('Control') and 'DMSO' were used as medium and solvent control, respectively. *a, b, c, d* means with different letters are significantly different by Duncan's one way range test ($p < 0.05$)

When the development stages of the offspring obtained from the application groups containing different concentrations of CH and those from the control groups were compared, it was observed that the development stages of the offspring from the DMSO control group were reached at the same time as the control group, whereas the development stages of the application groups took longer than those of the control groups (Table 2). The number of male and female offspring belonging to the F₁ progeny obtained from the application groups also substantially decreased (Table 2; Figs. 1, 2). According to our

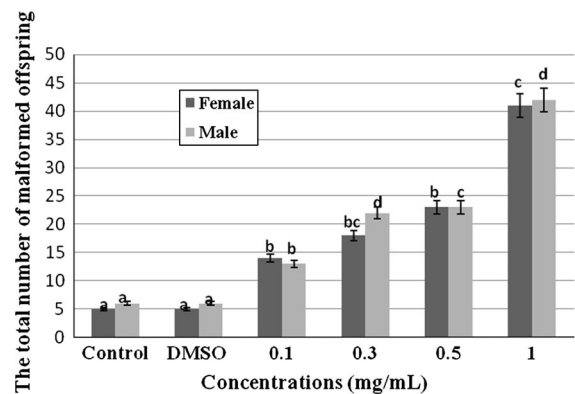


Fig. 2 The comparison of the number of malformed offspring belonging to the F₁ in controls and application groups with different concentrations of CH. SDM ('Control') and 'DMSO' were used as medium and solvent control, respectively. *a, b, c, d* means with different letters are significantly different by Duncan's one way range test ($p < 0.05$)

results, 555 ♀♀ and 539 ♂♂ offspring were counted for the control group, and 85 ♀♀ and 76 ♂♂ were counted for the highest application groups (1 mg). It was found that the difference between the groups was significantly important ($p < 0.05$).

For both male and female offspring belonging to the F₁ generation in the application groups, the developmental stages took longer to complete than in the control groups, and various malformations were

observed. However, it was found that the differences between the malformed male and female offspring obtained from the treated groups with the same concentrations was not significantly important ($p > 0.05$), except in the 0.3 mg application group. Depending on feeding in the F_1 progeny belonging to the observed treated groups, teratogenicity was also identified through the embryotoxic effects on the offspring, and an increase in the number of malformed offspring.

The malformed offspring were observed when crossed, although it was noted that the offspring could only survive for approximately 1 day, so the cross was not continued. As seen in Table 2, the malformations observed in the control groups indicate a positive correlation with increasing concentration. For both the control and application groups, the difference, statistically compared in terms of the number of malformed offspring, was significant ($p < 0.05$).

It was observed that the toxic effect increases with increasing CH amount in our study (Table 1; Fig. 1). In the literature there are many studies stating that even if CH is not toxic at low doses, it might be very toxic in overdose cases or when used for a long period of time (Laurent et al. 2006; Lin and Ma 2006; Wong et al. 2009). As a result of in vitro study using human lymphocyte cells, it was determined that CH causes aneuploidy, polyploidy and micronucleus formation and in addition results in sister chromatid exchange (Vian et al. 1995). In the study carried out by Zordan et al. (1994) on *D. melanogaster* with somatic mutation and recombination test (SMART) it was determined that CH has genotoxic effects. In studies related with the effects of CH on living things other than mice, rats and humans that were carried out on the TA100 and TA104 strains of the *Salmonella typhimurium* bacteria, it was determined that CH inhibited α -tocopherole and menadion that cleared the free radicals in the environment thereby causing mutations (Ni et al. 1994) and aneuploidy in some fungi species such as *Aspergillus nidulans* and *Saccharomyces cerevisiae* (Sora and Agostini Carbone 1987; Kappas 1989), mutations in somatic cells and gametes in *D. melanogaster* (Mason et al. 1992), changes of centrosomal material in sea urchins (*Strongylocentrotus purpuratus*) and the setting up of abnormal microtubules (Schatten and Chakrabarti 1998). In studies during which the effects of CH on the organs and tissues of mice were analysed histopathologically, it

was determined that the kidney and liver tissues of mice subject to high doses of CH enlarged and that microsomal protein (cytochrome b5, aminopyrine-*N*-demethylase, aniline hydroxylase) values increased (Sanders et al. 1982). As a result of a similar study, a statistically significant increase was determined in hepatocellular carcinoma and adenoma amounts in individuals subject to CH for long periods of time (Daniel et al. 1992). Merdink et al. (2008) has stated that the carcinogenic effect mechanism of CH may be in two ways either as *H-ras* proto-oncogen activation or peroxisome proliferation whereas Ni et al. (1996) stated that CH increases the formation of free oxygen radicals thus causing lipid peroxidation and displayed carcinogenic effects.

It was later understood that CH was reduced to trichloroethanol in the blood, and it was stated that hypnotic activity occurred as a result of this (Bringmann et al. 1999). In addition, it was concluded that CH was metabolised in the liver and that this biotransformation took place as either reduction or oxidation. So it was determined that CH was oxidized to trichloroacetic acid in the liver and the kidney and that it was reduced to trichloroethanol via alcohol dehydrogenase in the liver (Lipscomb et al. 1996). CH's pharmacology properties enhance the GABA receptor complex. It is believed that CH has general central nervous system depressant effects due to its active metabolite, trichloroethanol. It accomplishes this by rapid and extensive metabolism in the liver and erythrocytes by alcohol dehydrogenase. A small amount of CH and a larger portion of trichloroethanol are oxidized to a minor, less active metabolite, trichloroacetic acid, in one's liver and kidneys. This metabolite is excreted in the urine and bile together with trichloroethanol in free or conjugated form (Lu and Greco 2006).

According to the result of study the carried out with dichloroacetic acid and trichloroacetic acid, the two metabolites of CH, it was determined that these two metabolites caused liver tumours in mice (Pereira 1996). In another study, it was reported that trichloroacetic acid had toxic effects on the lung bronchial epithel cells of mice (Green et al. 1997). Whereas in literature there are many studies regarding the effect mechanisms of CH on mammals, studies related to insects and other organisms was minimal. Therefore, the effect mechanism of CH on insects should be researched with all details.

In the light of the results obtained in our study, embryotoxic effects with lower doses of CH were observed in *D. melanogaster*. It is thought that this effect may be explained with mechanisms published in the literature.

Conflict of interest The Authors declare that there is no conflict of interest.

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