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ALPHA-KETOGLUTARATE PARTIALLY PROTECTS FRUIT FLY *DROSOPHILA MELANOGASTER* FROM ETHANOL TOXICITY

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Abstract. Alpha-ketoglutarate (AKG) is an important intermediate in Krebs cycle and in metabolism of amino acids. Recently, it was proposed to apply as a dietary supplement to improve overall functional state of living organisms. In particular, AKG was supposed to use under exposure of animals and cell cultures to many toxic agents. In this context, this study aimed to elucidate the ability of dietary AKG to reduce toxic effects of ethanol on development of fruit fly *Drosophila melanogaster*, which is a popular model subject to research many aspects of biology of higher eukaryotes. For this aim, the effect of sodium salt of AKG on pupation speed of *D. melanogaster* w^{1118} on the medium supplemented with different concentrations of ethanol was studied. Ethanol at a low concentration (2%) did not affect the rate of larval pupation, whereas at higher concentrations it significantly delayed fly pupation and showed developmental toxicity reducing a number of total pupae formed. The most toxic developmental effects of ethanol were observed at its highest concentration (15%). The potential mechanisms of protective effects of AKG are discussed.

Keywords: rate of pupation; larvae; embryonic toxicity, dietary supplement.

Abbreviations: AKG, alpha-ketoglutarate; EthOH, ethanol.

1. INTRODUCTION

Currently, the substances which can improve adaptation of living organisms to various types of stresses are actively investigated. In this context, there is a growing interest in the study of alpha-ketoglutarate (AKG) as a dietary supplement to improve the overall functional state of organisms and increase their resistance to a number of stressors [3, 11]. Alpha-ketoglutarate is an organic ketoacid that is important for the transfer of cellular energy in the citric acid cycle and the proper metabolism of essential amino acids. Being a precursor for such amino acids as glutamate, glutamine and proline, AKG is involved in protein biosynthesis [11, 13]. It is considered also as one of the most important nitrogen transporters in metabolic pathways. The amino groups of amino acids are attached to it (by transamination) and carried to the liver where the urea cycle takes place to remove excessive ammonia from the body. One of the most important functions of AKG is to detoxify ammonia in tissues, especially in central nervous system. Alpha-ketoglutarate also scavenges ammonia released at catabolism of amino acids, thereby balancing the body's nitrogen homeostasis and preventing nitrogen overload in body tissues and fluids. In addition, it was proposed that AKG can display antioxidant

activity, in particular iron-chelating activity [22]. In contrast to oxaloacetate which cancels iron redox activity by forming inactive complexes, AKG can form active complexes with iron with potential pro-oxidant activity [22]. It was also shown that AKG prevented oxidative damages to lipids under ethanol administration in rats [26].

Ethanol is an important larval food resource and toxin for fruit fly *D. melanogaster* simultaneously [8]. The latter encounters ethanol in its natural habitat and possesses many adaptations that allow it to survive and thrive in ethanol-rich environments. Several assays to study ethanol-related behaviour in flies, ranging to have been developed in the past 20 years. These assays have provided the basis for studying the physiological and behavioural effects of ethanol and for identification of genes mediating these effects. In mammalian and insect models of ethanol intoxication, ethanol at low doses stimulated locomotor activity whereas at high doses was a sedative [5, 6]. *Drosophila* species which breed in fermenting fruits can encounter ethanol at concentrations up to 4–5% [9]. Some *Drosophila* species breed in wineries and breweries, where ethanol concentrations may be even higher [9, 17]. Ethanol can serve as a food resource at low concentrations, but at high concentrations it is toxic [20]. That ethanol has been an important selective agent for *Drosophila* which is supported by the results of interspecific comparisons: species which normally breed in fruit are more resistant to the toxic effects of ethanol, and have higher activity of the enzyme alcohol dehydrogenase [18]. Alcohol addiction is a common affliction with a strong genetic component [7]. Although mammalian studies have provided significant insight into the molecular mechanisms underlying ethanol consumption [4], other organisms like *D. melanogaster* are better suited for unbiased, forward genetic approaches to identify novel genes related. Behavioural responses to ethanol, such as hyperactivity, sedation, and tolerance, are conserved between flies and mammals [23, 27], as are the underlying molecular pathways [19].

Humans and flies share a large number of homologous genes: vertebrates have about four homologues for every gene found in *D. melannogaster*. Both, imago and larvae of *D. melanogaster*, have been used as classical tools for neuroscience and biology in general for over a century [2, 10]. Larvae have been workhorses for many aspects of behavioural neuroscience, including sensory research [12, 14], learning and memory studies [1, 21]. Recently, larvae have also been employed for drug discovery [24, 25]. In this study, we used *D. melanogaster* larvae as a model to study possible protective effects of alpha-ketoglutarate sodium salt (AKG) under exposure to high concentrations of ethanol. For this purpose, the effects of dietary AKG and ethanol at different concentrations either alone and in the mixture (AKG and ethanol) on pupation rate of fruit fly *D. melanogaster* were studied.

2. Materials and Methods

2.1. Drosophila Melanogaster Stocks and Media

The *D. melanogaster* strain w^{1118} flies were used in all experiments. Stock flies were reared on the standard yeast-corn-molasses food with 12:12 photoperiod at 25±1ºC. The experimental media consisted of 10% sucrose, 10% pressed yeast, 1% agar, and 0.2% nipagin (methyl-p-hydroxybenzoate, used to inhibit mold growth) (SY diet) were supplemented also with AKG and ethanol at different combinations. Fly pupation on different media was monitored.

2.2. Analysis of Pupation Rate

After egg laying for 5-6 h, eggs were transferred to vials containing SY diet either control or supplemented with different combinations of AKG and ethanol. About 100-150 eggs per vial containing 20 ml of food were placed. In these vials, eggs hatched and larvae developed until pupation. The number of pupated larvae was counted every day [15].

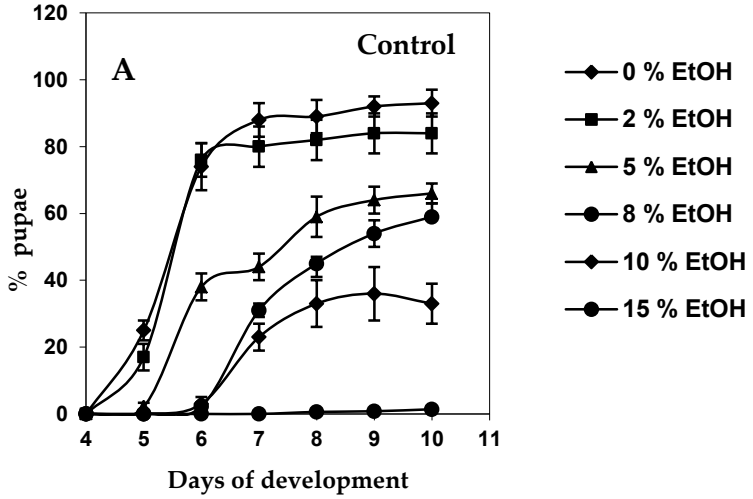
2.3. Statistical Analysis

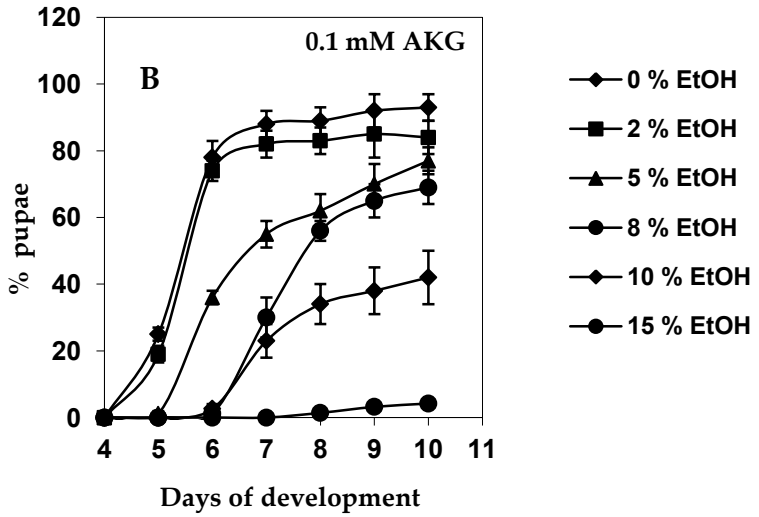
Experimental data are expressed as the mean value of 5 independent experiments ± the standard error of the mean (SEM), and statistical analysis was carried out using Dunnett's t-test.

3. Results and Discussion

Drosophila larvae stops feeding and initiate pupation at a very specific time after hatching and this can be used as a developmental transition point to assay growth pattern alterations [28]. In this study, the experiments regarding potential protective effects of AKG against ethanol toxicity were carried out with the w^{1118} fly strain. In each independent experiment, 100 eggs were added to sucrose-yeast medium containing different combinations of the used compounds and larvae were allowed to feed and develop to pupation. Percentage of pupae was calculated as the ratio between the numbers of pupae formed to the total number of eggs placed into the vial.

Fig. 1. (A) demonstrates the effect of ethanol at different concentrations on developmental pattern of w1118 flies rearing at control media. It is clear, ethanol delayed fly pupation in dose-dependent manner. At a concentration of 2%, ethanol virtually did not affect larvae pupation process. It is can be explained by evolutionary fitness of fruit fly to survive and live in fermented fruits where they can be exposed to ethanol at certain concentrations [9]. However, at higher concentrations ethanol delayed fly pupation. Finally, it demonstrated highest toxicity at highest concentration used (15%). These results are in good agreement with earlier data which demonstrated developmental toxicity of ethanol [16]. It was proposed that the developmental defects in *Drosophila* could be largely due to ethanol effects on insulin signaling. Supplementation of the medium with AKG at concentrations of 0.1 and 10 mM partly alleviated ethanol-induced developmental fly pattern (Fig. 1. (B)-(C)). Certainly, these effects were the most pronounced when we studied effects of 10 mM AKG under fly exposure to 15% ethanol.





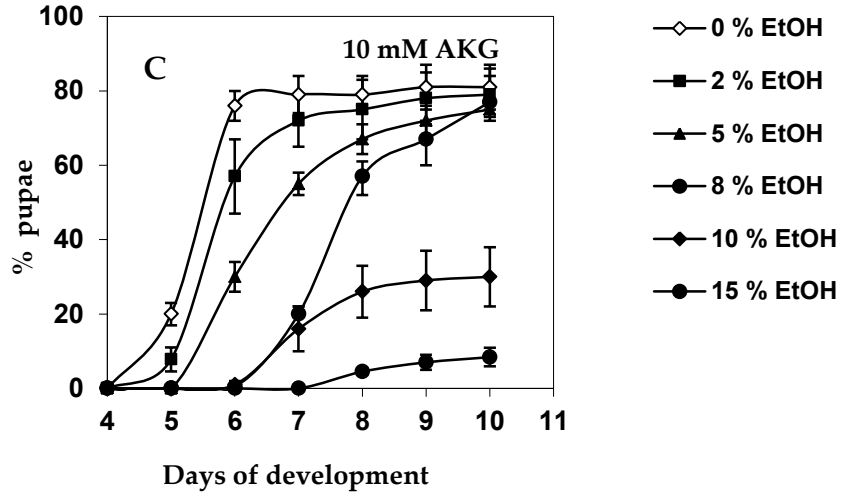


Fig. 1. Effects of different concentrations of ethanol on pupation pattern of D. melanogaster w^{1118} fed control SY food (A) or SY food supplemented with 0.1 mM (B) or 10 mM (C) AKG, n=5.

Fig. 2 shows total number of pupae formed when the larvae were reared on media supplemented with ethanol and AKG at different concentrations. Although in two groups, control and with ethanol at concentration 2%, virtually did not affect the number of pupated larvae, at concentrations 5, 8, and 15% it decreased the pupae number by 29, 40, and 98%, respectively. Supplementation of food with AKG did not affect the parameter in control and 2% ethanol fed groups, it influenced it in the rest ones, but to different extent. In the fly group maintained with 5% ethanol, AKG in both used concentrations, 0.1 and 10 mM, provided by 17 and 14% higher number of pupated larvae. Obviously, at highest concentrations used, 8 and 15%, negative ethanol effects were greatly prevented y AKG in concentration dependent manner, but the only at 10 mM AG the difference was statistically significant in 1.2 and 2.5-fold effects. This means that at high levels of ethanol, AKG partly diminished larval toxicity of ethanol, whereas at low ethanol levels AKG was not efficient.

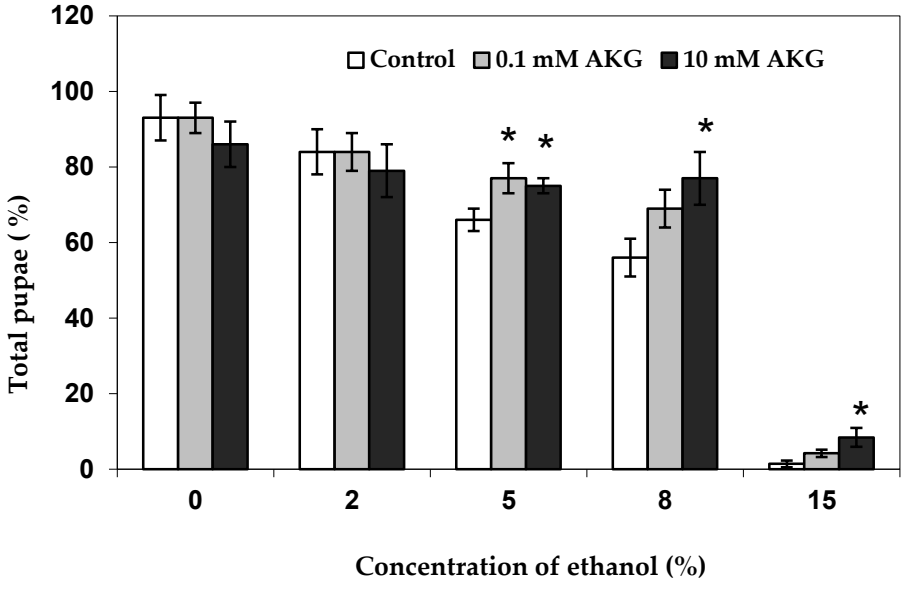


*Fig. 2. Total number of D. melanogaster w^{1118} pupae formed on the media supplemented with ethanol and AKG in different combinations. The number of eggs used was given as 100%. *Significantly different from respective control values with P < 0.05 using Dunnett's test, n=5.*

Thus, dietary AKG can partly alleviate developmental toxicity of ethanol at high concentrations on fruit fly *D. melanogaster*. It is possible that earlier found fact that AKG could prevent lipid peroxidation

in rats under chronic ethanol administration [26] may be one of the potential explanations of AKG effects. Therefore, it may be supposed that antioxidant AKG action can be potential mechanism in fruit fly against ethanol toxicity. The detailed mechanisms of AKG action need to be investigated further.

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Альфа-кетоглутарат (АКГ) – це важливий проміжний продукт циклу Кребса і метаболізму амінокислот. Нещодавно АКГ почали використовувати як харчову добавку для покращення загального функціонального стану живих організмів. Зокрема, було запропоновано використовувати АКГ при дії на тварин і клітинні культури токсичних речовин. У цьому контексті, метою даної роботи було з'ясувати здатність екзогенного АКГ зменшувати токсичну дію етанолу на розвиток плодової мушки *D. melanogaster*, яка є популярною моделлю для вивчення багатьох аспектів біології вищих

організмів. Нами досліджено вплив натрієвої солі АКГ на заляльковування плодової мушки *D. melanogaster*, в присутності етанолу за різних його концентрацій. Етанол за низької концентрації (2%) не впливав на швидкість лялькування плодової мушки, але за вищих концентрацій він сповільнював заляльковування. Найбільш токсичний вплив етанолу на розвиток мушок був знайдений при використанні його найвищої концентрації – 15 %. Обговорюються потенційні механізми захисної дії АКГ.

Ключові слова: швидкість заляльковування; лялечки; ембріональна токсичність, харчова добавка.