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Antioxidant Activity of the Chitin Obtained from the Insect

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Abstract: The chitin can be obtained from an insect of the cuticle, trachea and peritrophic matrix of the insect during molting. For this reason, biotechnologically important chitin can be easily obtained from *Drosophila* and can be used in related sectors. The aim of this study was to investigate the antioxidant capacity (TAS) of the chitin obtained from *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) against total oxidation in 35-day old flies. Thus, the use of the chitin obtained from this insect in the basic science fields such as medicine, pharmacy, veterinary medicine has been determined in the aging process. Furthermore, in this study, the environmental and ecological impact of the release of products from natural biomolecules into the environment has been discussed.

Keywords: Drosophila melanogaster*, Nutrition, Chitin, Total Oxidant-Antioxidant Activity.*

Introduction

The chitin, a strong skeleton material in arthropods, is useful compound for biomolecular applications. The chitin can be obtained from natural various organisms such as fungi, crustaceans, nematodes, insects (Andersen, 1979; Kramer et al., 1995; Merzendorfer & Zimoch, 2003; Payre, 2004). The control of growth and development in insects depends on the triviality of constantly synthesized and reduced chitin structures (Merzendorfer & Zimoch, 2003). According to the free radical theory, which is one of the current theories of aging; living creatures are exposed to chemical substances or various foreign substances that cause environmental pollution, cause the formation of reactive oxygen species (ROS). The chitin is known to be effective in the prevention of oxidative stress by inhibiting protein oxidation and lipid peroxidation by eliminating the effect of free radicals in the presence of antioxidant activity (Jia *et al*., 2001; Hui et al., 2008).

It is known that 120000 species located in Diptera team of Insecta class. *Drosophila melanogaster* Meigen is one of these species; has a short life cycle can easily be cultured and used as a model organism for biological studies (Adams et al., 2000; Baker and Thummel, 2007; Saini et al., 2013). Enzymatic and non-enzymatic antioxidants are used to defend the oxidative action of all the tissues of the insects and the digestive tract. Enzymes such as catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione reductase (GR), Glutathione S-transferase (GST), disulfide reductase, methionine sulfoxide reductase (MSR), thioredoxin peroxidase (TRXP) are antioxidant enzymes found in *Drosophila* (Missirlis *et al*., 2003). Larval and adult stages fed with the same food in *Drosophila* morphological differences appear despite being quite a small body weight of 7.85% in the adults of this insect chitin, there is 141.4 kDa chitosan (Kaya et al., 2016a). Although there have been many studies on chitin in the literature, there is no information on model organisms and total oxidant (TOS) or antioxidant (TAS) activity due to aging in living nutrition. For this purpose, diets were coated with insect chitin and feed with fruit flies.

Material and Method

D. melanogaster (Wild type) stocks were cultured (25 ± 2ºC, 60-70% relative humidity, and 12/12 h L/D photoperiod) with an artificial diet (Rogina et al., 2000; Lesch et al., 2007). First, produced chitin from dead insects (10 days old adults) (Kaya & Baran, 2015; Kaya *et al*., 2015; Erdoğan and Kaya, 2016; Kaya et al., 2016a & b). Second, fruit flies larvae were feed with chitin, and insect mortality was calculated. After, individuals were fed with the determined concentrations (0.1-20 %) of chitin along with 35-day. Flies antioxidant capacity (TAS) and total oxidation activity (TOS) was

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determined 25 insects per replicate (Güneş, 2015). Experimental data were expressed as means ± S.E. The data were subjected to statistical analysis by one-way analysis of variance (ANOVA) was followed by least significant difference (LSD) test determine significant differences between means (Figure 1). A value of $p<0.05$ was considered significant.

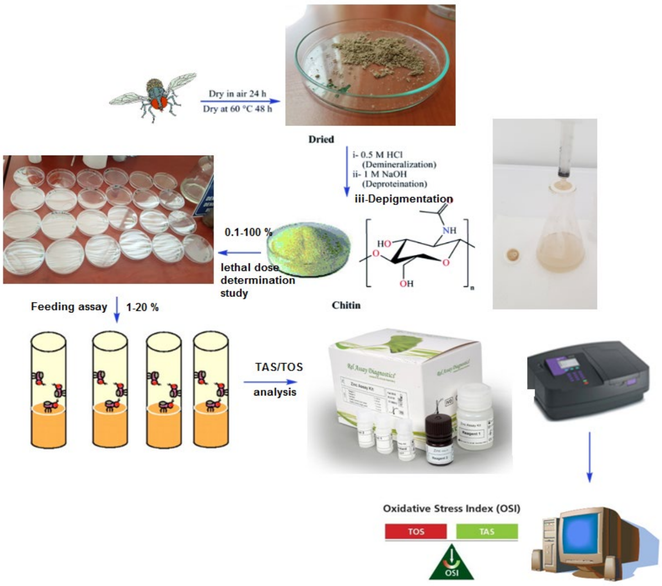

Figure 1. Experimental design

Results and Discussion

An antioxidant mechanism is introduced in order to perform normal functions in reactive oxygen species (ROS) exposed cells. Thus, ROS are removed by providing oxidant-antioxidant balance in healthy individuals. Oxidant and antioxidant molecules can be measured by various analytical methods (Tarpey et al., 2004; Çobanoğlu, 2011; Güneş, 2015). Recently measuring more practical total oxidant (TOS) or antioxidant status (TAS), since this is an example measured separately of the molecules is time consuming and difficult preferred (Ghiselli et al., 2000; Erel, 2004; Erel, 2005; Cobanoglu, 2011). They have developed an easy, durable and economical method for TAS and TOS measurement and have been able to calculate the oxidative index (Erel, 2005; Kosecik et al., 2005, Cobanoglu, 2011; Güneş, 2015). TOS, TAS and OSI levels in adults are shown in Figure 2.

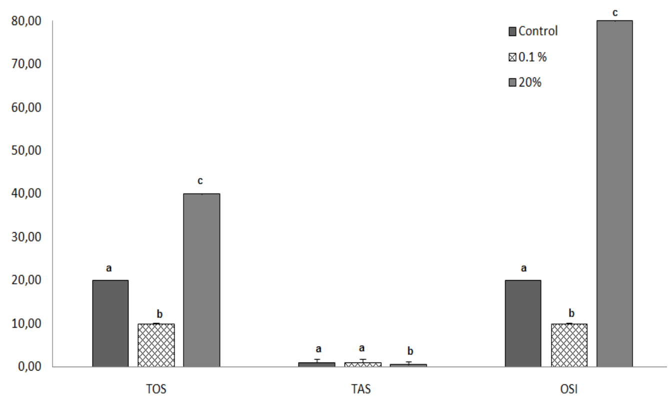

Figure 2. The effect of Chitin on TOS, TAS, OSI levels in the *D. melanogaster*. Four replicates with 25 insects per replicate, $p<0.05$ (LSD Test).

As is the study, the TOS and TAS levels of the control group were normal. Nutrient components and environmental factors induce ROS generation in insects interacting with each other or substances added to the nutrient (Güneş & Büyükgüzel, 2017). *Drosophila* protects themselves from the harmful effects of environmental stress depending on their detoxification capacities (Vasseur & Leguille, 2004). While low concentration did not make a difference in TAS level, it was determined that high concentration was higher than the control group ($p<0.05$). The results demonstrated that as the chitin concentration increases, the peroxidation increases in the insect, indicating that the amount of TOS increases.

Even nutritional carbohydrate changes can cause insecticidal oxidative stress (Jordens et al., 1999). Excessive carbohydrate intake or abundance of food triggers stress (Andersen et al., 2010). As in the study, the increased amount of TOS is thought to be due to the from the amount of carbohydrates. The chitin obtained from insect used also supports the change in peroxidation in our study of stress induction by inducing diabetes. Chitin can be used against oxidation at low concentrations.

Drosophila protects themselves from harmful effects depending on their detoxification capacities (Vasseur & Leguille, 2004). While many nutrients cause oxidative stress in *Drosophila*, but chitin also has antioxidant activity in *Musca domestica* (Jia *et al*., 2001; Hui et al., 2008; Zhang *et al*., 2011). It is observed that the insect is in the normal antioxidant and high oxidant level in 20% chitin. This situation can be against the material used; is able to be caused by many reasons such as egg formation, malnutrition, obesity, etc.

Conclusions

Drosophila is a recommended and used model for biological parameters, pigmentation, physiological, metabolic and morphological disorders in the toxicity studies. It is detected that adding chitin to nutritional compounds in high concentrations increases the level of oxidation and induced stress. It is emphasized that the chitin can be used at low concentrations. Thus, the use of chitin obtained from an insect in the basic science fields such as medicine, pharmacy, and veterinary medicine has been determined in the aging process. Our work will contribute to clarifying the mechanism of action of natural products taken with nutrition. For this reason, the important biotechnological chitin can be easily obtained from *Drosophila* and can be used in related sectors. But more detailed studies are needed to make definite inferences.

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