

TOXICOLOGICAL EFFECTS OF COCOA BEAN SHELL EXTRACT ON REACTIVE OXYGEN SPECIES PRODUCTION AND SURVIVAL OF *Drosophila melanogaster*

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INTRODUCTION: Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the organism's ability to neutralize them through antioxidant mechanisms. Excessive ROS production can lead to cellular damage and a reduction in antioxidant defenses. In this context, cocoa shell residues have emerged as a promising source of bioactive compounds with potent antioxidant activity. Notable among these molecules are flavonoids such as epicatechin, polyphenols, and alkaloids.

OBJECTIVE: To evaluate survival and ROS levels following exposure to different concentrations of cocoa bean shell extract in *Drosophila melanogaster*.

MATERIALS AND METHODS: Flies of the species *Drosophila melanogaster*, recognized by the OECD as an alternative model for preliminary toxicological screening, particularly for investigating toxic effects related to oxidative stress, were used. Flies of both sexes were maintained under controlled conditions of temperature (25 °C), humidity, and a 12-hour light/dark cycle, and were fed a standard diet. For the toxicological curve analysis, the extract was incorporated into the standard diet at concentrations of 0%, 1%, 2.5%, 5%, 7.5%, and 10%. Flies were exposed to the treatments for 7 days. Each group consisted of 50 flies, and mortality was monitored every 24 hours by counting dead flies. ROS levels were quantified using a fluorometric assay based on the reduction of dichlorofluorescein diacetate (DCFH-DA).

RESULTS AND CONCLUSION: An increase in ROS levels was observed in the groups treated with 3%, 5%, and 6% concentrations compared to the control group ($p < 0.0001$). Similarly, these same groups exhibited a significantly reduced survival rate relative to the control ($p = 0.0104$, $p < 0.0001$, and $p < 0.0001$, respectively). Thus, concentrations starting at 3% significantly promoted ROS generation and reduced survival rates, suggesting a threshold of cellular toxicity associated with the extract. These findings underscore the importance of evaluating the safety and potential risks of natural extracts. Further studies are needed to establish a therapeutic dose for future health-related applications.

Keywords: Cocoa; Toxicity; *Drosophila melanogaster*; Toxicological curve