

THE GENOTOXIC EFFECTS OF SOME EDIBLE INSECTS ON HUMAN WHOLE BLOOD CULTURES

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ABSTRACT: In this study, we aimed to determine the effects of different aquatic insect extracts on SCE frequency of cultured human blood lymphocytes. With this aim, the heparinized blood samples obtained from two non-smoking individuals with no history of exposure to any toxic agent. The water soluble extracts containing all body parts were sterilized and added to the culture tubes at different concentrations (10-100 mg/L). SCEs were visualized by a combination of Fluorescent and Giemsa (FPG) techniques. In comparison to control sets, the treatments of different concentrations of water soluble insect extracts did not elevate ($p < 0.05$) the frequencies of SCEs. Furthermore, the present findings revealed that human blood cultures may be a useful in vitro system for evaluating the mutagenic potential of edible insect species.

KEY WORDS: Edible Insects, Genotoxicity, Human Blood Culture, SCE Test.

Insects have played an important part in the history of human nutrition in Africa, Australia, Asia and the Americas. Insects often contain more protein, fat, and carbohydrates than equal amounts of beef or fish, and a higher energy value than soybeans, maize, beef, fish, lentils, or other beans. As over 1500 different species of insects have been reported as being consumed or edible around the world (Defoliart, 1995; Food-Info, 2009). Of these, *Hydrophilus piceus*, *Dytiscus marginalis* and *Cybister* sp., treated here, are widely used for human consumption in many countries. *Hydrophilus piceus* is also used in alternative medicine in South-East Asia countries due to anti-diuretic aspects (Jäch, 2003; Rams-Elorduy, 1997; Morris, 2004).

The sister chromatid exchange (SCE) test in peripheral blood lymphocytes is a very sensitive cytogenetic technique and widely used for the evaluating the genotoxicity of many suspected organic and inorganic substances (Perry and Evans 1975). On the other hand, edible insects constitute a very common and important food source in many developing countries although these insects contain powerful pharmacologically active substances, which are known vertebrate toxins (Akinnavo et al. 2002). So eating of these insects may cause as serious harmful effects on humans. In this context the potential toxic effects of these popular edible insects needs to be investigated in more detail. These toxicity researches will also serve to biomedical productions because it is well known that animal toxins may even become important in curing diseases such as cancer. And the genotoxic effects after exposure to extracts of edible insects have not yet been reported. In this study we assessed the genotoxicity in human whole blood cultures treated with six different concentrations (10, 20, 30, 50, 75 and 100 mg/L) of water soluble extracts of *H. piceus*, *D. marginalis* and *Cybister* sp. (Figs. 1a,b,c) for the first time by SCE test.

According to our knowledge, no investigation has been carried out on the genetic effects of these edible insects on humans.

MATERIALS AND METHODS

Beetle samples were collected from its natural aquatic habitats in Erzurum province and surroundings (East Anatolia), and killed without any chemical treatment.

Blood samples were obtained by veinpuncture from two non-smoking individuals at the ages of 25 and 29 with no history of exposure to any toxic agent. The extracts of three different insect species were *H. piceus*, *D. marginalis* and *Cybister* sp. studied and the sterilized extracts were added to the cultures just before incubation for cytogenetic analysis. Treatments of water soluble insect extracts with all part of the body with various concentrations (0, 10, 20, 30, 50, 75 and 100 mg/L) were applied to human blood cultures. With the aim of providing successive visualization of SCEs, 5-bromo-2'-deoxyuridine (Sigma) was added at culture initiation. The cultures were incubated in complete darkness for 72 h at 37 °C. Exactly 70 h and 30 min after beginning the incubations, demecolcine (N-Diacetyl-N-methylcolchicine, Sigma) was added to the cultures. After hypotonic treatment (0.075 M KCl), followed by three repetitive cycles of fixation in methanol/acetic acid solution (3:1, v/v), centrifugation, and resuspension, the cell suspension was dropped onto chilled, grease-free microscopic slides, air-dried, aged for three days, and then differentially stained for the inspection of the SCE rate according to fluorescence plus Giemsa (FPG) procedure (Perry and Wolff, 1974). For each treatment condition, well-spread twenty five second division metaphases containing 42 - 46 chromosomes in each cell were scored by one observer, and the values obtained were calculated as SCEs per cell.

Statistical analysis

Statistical analysis was performed using SPSS Software (version 12.0, SPSS, Chicago, IL, USA). The two-tailed Student's *t*-test was used to compare SCE frequencies between treated and control groups.

RESULTS AND DISCUSSION

Twenty metaphases from each culture were evaluated for SCE. The mean±S.D. of the individual frequencies of SCE values in treated and untreated groups are shown in figures 2, 3 and 4. The water soluble extracts of *H. piceus*, *Cybister* sp. and *D. marginalis* did not cause any statistically important ($p < 0.05$) alterations of SCE frequencies dependent upon the number of doses treated.

According to these results, it is revealed that the edible aquatic insect species, treated here, have no mutagenic potential.

Ordinarily, insects are not used as emergency food to ward off starvation, but are included as a normal part of the diet throughout the year or when seasonally available. Eating insects have become more popular day by day around the world, and therefore further investigations on the potential toxic effects of these popular edible insects should be conducted.

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Figure 1. A) *H. piceus*; B) *D. marginalis*; C) *Cybister* sp.

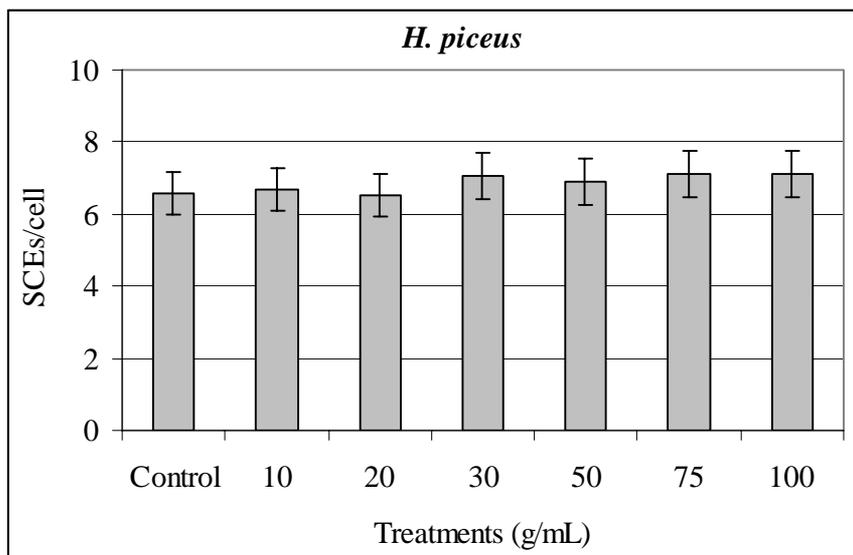


Figure 2. The effects of *H. piceus* extracts on SCE frequency *in vitro*.

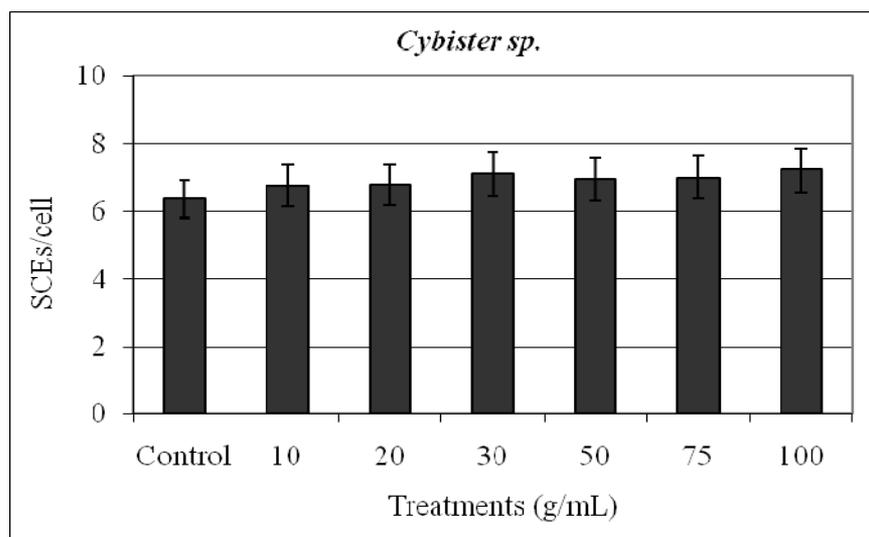


Figure 3. The effects of *Cybister sp.* extracts on SCE frequency *in vitro*.

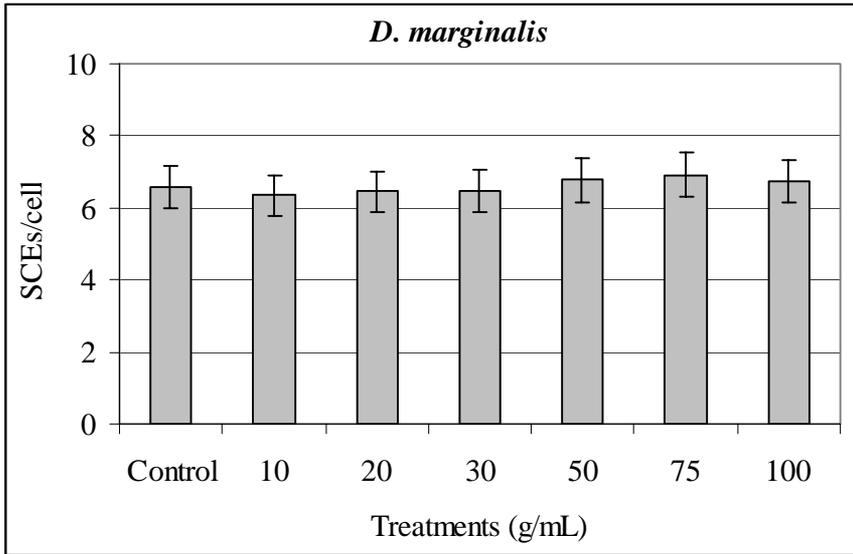


Figure 4. The effects of *D. marginalis* extracts on SCE frequency *in vitro*.