

**Orijinal araştırma (Original article)**

## Biomonitoring of the genotoxic potentials of two edible insects species in vitro

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### Summary

In this study, we evaluated the genotoxic potentials of water soluble extracts of grasshoppers, *Saga ephippigera ephippigera* Fischer de Waldheim, 1846 and *Callimenus dilatatus* (Stal, 1876) (Orthoptera) on cultured human blood cells. The chromosome aberration (CA) and micronucleus (MN) tests were used to assess the DNA and chromosomal damage produced by aqueous extracts in vitro. The extracts were added to the cultures at eight different concentrations (1, 5, 10, 20, 25, 50, 75 and 100 mg/l). Our results indicated that these extracts did not show genotoxic effects at the tested concentrations. We conclude that this in vitro approach for biomonitoring genotoxicity assessment may be useful to compare the potential health risks of edible insects.

**Keywords:** Edible insects, genotoxicity, human blood culture, micronucleus, chromosome aberration

**Anahtar sözcükler:** Yenilebilir böcekler, genotoksisite, insan kan kültürü, mikroçekirdek, kromozom aberasyonu

### Introduction

The insects have been considered as an important food source in different regions of the world like Africa, Australia, Asia and the America due to their valuable nutritional contents. On the other hand, some of these edible insects such as *Hydrophilus piceus* Linnaeus, 1758 also used in alternative medicine (Rams–Elorduy, 1997; Jäch, 2003; Morris, 2004).

It was reported that about 1500 insect species has been consumed by humans (Food-Info, 2009). Of these, grasshopper species are more widely used for human consumption in many countries and are important as a source of protein as well as traditional cultural delicacies in various part of the world. Two grasshopper species *Saga ephippigera ephippigera* Fischer de Waldheim, 1846 (Orthoptera: Tettigoniidae) and *Callimenus dilatatus* (Stal, 1876) (Orthoptera: Tettigoniidae), treated here, are the largest members of the

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grasshoppers in Turkey. Both species, sometimes reaching the 25 cm body length in total, are widely distributed in Turkey (especially west, central and south parts).

On the other hand, edible insects constitute a very common and important food source in many developing countries although these insects may include contain vertebrate toxins (Akinawo et al., 2002). So eating of these insects may cause serious harmful effects on humans. At this context, the potential toxic effects of these popular edible insects needs further investigation. This research will also serve to improve the pharmaceuticals because it is well known that animal toxins may become important in curing diseases like cancer. And the genotoxic effects after exposure to extracts of edible insects have not yet been reported except Incekara and Turkez (2009). In their study, three aquatic edible insect species, *Hydrophilus piceus* (Linnaeus, 1758) (Coleoptera: Hydrophilidae), *Dytiscus marginalis* Linnaeus, 1758 (Coleoptera: Dytiscidae) and *Cybister* sp., (Coleoptera: Dytiscidae) were evaluated and found to be non genotoxic.

In this study we assessed the genotoxicity in human whole blood cultures treated with different concentrations of water soluble extracts of *S. ephippigera ephippigera* and *C. dilatatus* (Figs. 1a, b) for the first time by chromosome aberration (CA) and micronucleus (MN) tests. Genetic alterations, mainly CAs and formations of MN in cell cytoplasm, are the early biological effects of mutagenesis and/or carcinogenesis (Hagmar et al., 1998). In fact *in vitro* CA tests have come to play a central role in testing for mutagenic/carcinogenic potential of biological and chemical substances in most countries (Kirkland, 1992). The cytokinesis block MN test also offers the advantage by providing simultaneously information on both cell cycle progression and chromosome/genome mutations (Kirsch-Volders et al., 1997).

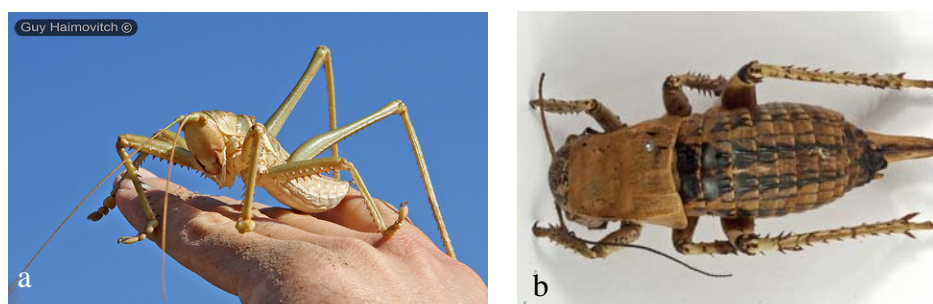


Figure 1. a: *Saga ephippigera ephippigera* Fischer de Waldheim, 1846, b: *Callimenus dilatatus* (Stal, 1876).

## Material and Methods

Grasshopper samples were collected from its natural habitats in Turkey, and killed in an anaerobic jar without any chemical treatment. Weights of insect samples were measured as a dry and determined that each *S. ephippigera*

*ephippigera* individuals was 17 g and *C. dilatatus* 15 g on average (five samples measured). The heparinized blood from three healthy non-smoking donors with no history of exposure to any genotoxic agent were used. Human peripheral blood lymphocyte cultures were set up according to a protocol (slightly modified in the content of the medium and the amount of the added heparinized blood samples) from Evans and O'Riordan (1975). The heparinized blood (0.5 ml) was cultured in 6 ml of culture medium (Chromosome Medium B, Biochrom<sup>®</sup>, Leonorenstr. 2-6.D-12247, Berlin) with 5  $\mu\text{g ml}^{-1}$  of phytohemagglutinin (Biochrom<sup>®</sup>). The aqueous extracts of *S. ephippigera* and *C. dilatatus* were added to the cultures. A stock solution of aqueous extract was prepared by mixing 1 g of dried insect powder with 1000 ml of water (boiled and cooled tap water) with constant stirring on a magnetic stirrer. The suspension of dried insect powder in water was left for 4 h, filtered through filter paper No.1 (Whatman) and the filtrate was stored in amber coloured air tight bottle at room temperature till use. Then, stock solutions were diluted and added to cell culture tubes. The final extract concentrations were 1, 5, 10, 20, 25, 50, 75 and 100 mg/l. The CA and MN tests were carried out on lymphocytes 72 h after treatment. Each individual lymphocyte culture without insect extract was studied as a control group.

The MN test was performed by adding cytochalasin B (Sigma<sup>®</sup>; final concentration of 6  $\mu\text{g ml}^{-1}$ ) after 44 h of culture. At the end of the 72 h incubation period, the lymphocytes were fixed with ice-cold methanol: acetic acid (1:1). The fixed cells were put directly on slides using a cytospin, and stained with May Grünwald-Giemsa (Sigma<sup>®</sup>). All slides were coded before scoring. The criteria for scoring micronuclei were as described by Fenech (1993). At least 2000 binucleated lymphocytes were examined per concentration for the presence of one, two or more micronuclei.

For analysis of structural chromosomal aberrations (chromatid or chromosome gap and chromatid or chromosome break) cultures were carried out for 72 h. Two hours prior to harvesting, 0.1 ml of colchicine (0.2  $\mu\text{g/ml}$ ) was added to the culture tube. To prepare slides, 3–5 drops of the fixed cell suspension were dropped on a clean slide and air-dried. The slides were stained in 3% Giemsa solution in phosphate buffer (pH 6.8) for 15 min. Twenty well-spread metaphases were analyzed for each concentration to detect the presence of chromosomal aberrations. Criteria to classify the different types of aberrations were in accordance with the recommendation of Environmental Health Criteria (EHC) 46 for environmental monitoring of human populations (IPCS 1985).

### **Statistics**

The statistical analysis of experimental values in the CA and MN tests which obtained from three replications was performed by one-way analysis of variance (ANOVA) and Duncan's test using the S.P.S.S. 13.0 software (SPSS,

Chicago, IL, USA) and the level of 0.05 was regarded as indicative of statistical significance for all tests.

## Results

Our results showed that the aqueous extracts of *S. ephippigera ephippigera* and *C. dilatatus* did not alter MN/1000 cell frequencies in human lymphocyte cell (Table 1, Figure 3). The mean  $\pm$  S.D. of the individual rates of CA values in treated and untreated groups are shown in Table 2 and Figure 2. The water soluble extracts of *S. ephippigera ephippigera* and *C. dilatatus* did not cause any statistically significant ( $p>0.05$ ) increases of CA rates dependent upon the number of concentrations tested. Nevertheless, the human blood cultures were found to be sterile after the applications of the extracts of *S. ephippigera ephippigera* and (at concentrations of 75 and 100 mg/l) and *C. dilatatus* (100 mg/l). The cytotoxic effects which were observed at increasing concentrations might cause the sterility.

Table 1. The effects of aqueous extracts from grasshoppers *Saga ephippigera ephippigera* Fischer de Waldheim, 1846 and *Callimenus dilatatus* (Stal, 1876) on MN frequencies in human blood cultures (Values are means  $\pm$  standart deviation, all p values were  $> 0.05$ , - symbol means cultures were found to be sterile)

Concentration	<i>Saga ephippigera ephippigera</i>	<i>Callimenus dilatatus</i>
Control	6.16 $\pm$ 0.92	6.16 $\pm$ 0.92
1 mg/l	6.62 $\pm$ 0.89	6.57 $\pm$ 1.09
5 mg/l	6.14 $\pm$ 1.13	6.85 $\pm$ 0.87
10 mg/l	5.98 $\pm$ 0.88	6.17 $\pm$ 1.15
20 mg/l	6.53 $\pm$ 0.77	6.44 $\pm$ 0.92
25 mg/l	6.74 $\pm$ 1.11	6.87 $\pm$ 1.42
50 mg/l	6.68 $\pm$ 1.07	6.42 $\pm$ 1.32
75 mg/l	--	6.83 $\pm$ 1.47
100 mg/l	--	--

Table 2. The effects of aqueous extracts from grasshoppers *Saga ephippigera ephippigera* Fischer de Waldheim, 1846 and *Callimenus dilatatus* (Stal, 1876) CA rates in human blood cultures (Values are means  $\pm$  standart deviation, all p values were  $> 0.05$ , - symbol means cultures were found to be sterile)

Concentration	<i>Saga ephippigera ephippigera</i>	<i>Callimenus dilatatus</i>
Control	0.39 $\pm$ 0.10	0.39 $\pm$ 0.10
1 mg/L	0.45 $\pm$ 0.10	0.45 $\pm$ 0.10
5 mg/L	0.42 $\pm$ 0.08	0.40 $\pm$ 0.08
10 mg/L	0.37 $\pm$ 0.04	0.45 $\pm$ 0.08
20 mg/L	0.48 $\pm$ 0.06	0.48 $\pm$ 0.10
25 mg/L	0.43 $\pm$ 0.09	0.34 $\pm$ 0.08
50 mg/L	0.50 $\pm$ 0.10	0.43 $\pm$ 0.09
75 mg/L	--	0.46 $\pm$ 0.12
100 mg/L	--	--



Figure 2. The sample metaphase of lymphocytes from the culture treated with 50 mg/l of *Saga ephippigera ephippigera* Fischer de Waldheim, 1846 extracts (x1000).

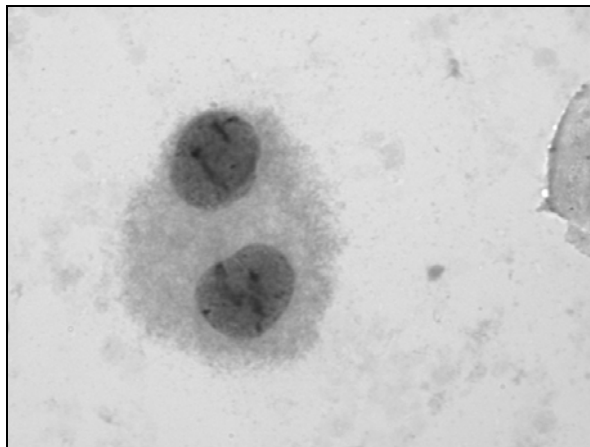


Figure 3. The sample binucleated lymphocyte cell photograph from the culture treated with 50 mg/l of *Callimenes dilatatus* (Stal, 1876) extracts (x1000).

## Discussion

The uses of many insect species as an important food source have become so widespread in many parts of the world, although very limited information is available concerning with their genotoxicity in the literature. Three different insect species (*Hydrophilus piceus* (Linnaeus, 1758), *Dytiscus marginalis* Linnaeus, 1758 & *Cybister* sp.) were tested for genotoxicity by using sister chromatid exchange (SCE) on human whole blood cultures by Incekara & Turkez (2009). In this study no *in vitro* adverse effects could be demonstrated and was revealed that three edible aquatic insects species, have no mutagenic potential. Our present results clearly indicated that water extracts of *S. ephippigera ephippigera* and *C. dilatatus* have no mutagenic potential. Basic toxicity information often provides a valuable perspective for predicting the

potential risk to humans. As a matter of fact, it was reported identification and subsequent lowering of exposure to genotoxic agents would remain one of the main goals for primary cancer prevention in man (Bartsch & Malaveille, 1989). According to the results of the present study, it is suggested that these insect species can be consumed safely, however it will also be useful to take into consideration the cytotoxicity at increasing doses. The safe concentrations of edible insect extracts in human blood as prescribed here, are valid only for *in vitro* conditions. In order to generalize this suggest, further *in vivo* studies are required on the absorption kinetics of these extracts from the gastrointestinal track into blood.

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 (Memorial University, 2010) and therefore further investigations on the potential genotoxic effects of these popular edible insects should be conducted.

We also offer that this *in vitro* approach which includes the collaborative use of two genetic endpoints such as CA and MN tests will serve to compare the potential health risks of edible insects related with mutagenesis or carcinogenesis.

## Özet

### Yenilebilir iki böcek türünün genotoksisite potansiyellerinin *in vitro* şartlarda biyolojik izlenmesi

Bu araştırmada, suda çözünür *Saga ephippigera ephippigera* Fischer de Waldheim, 1846 ve *Callimenus dilatatus* (Stal, 1876) (Orthoptera) ekstrelerinin insan kan kültürleri üzerine genotoksik potansiyelleri değerlendirilmiştir. *In vitro* şartlarda sulu ekstreler tarafından oluşturulan DNA ve kromozom hasarlarının tespitinde kromozom aberasyonu (KA) ve mikroçekirdek (MÇ) testleri kullanılmıştır. Ekstreler sekiz farklı konsantrasyonda (1, 5, 10, 20, 25, 50, 75 ve 100 mg/l) kültürlere uygulanmıştır. Sonuçlar, test edilen konsantrasyonlarda bu ekstrelerin genotoksik etkilerinin bulunmadığını ortaya koymuştur. Yenilebilir böceklerin mutajenite ve karsinojenite ile ilgili potansiyel sağlık risklerinin değerlendirilmesinde *in vitro* genotoksisitesinin biyolojik izlenmesinin faydalı olabileceği önerilmiştir.

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