

The genetic and oxidative effects of *Callimenus latipes* Stal (Tettigoniidae: Bradyporinae) extracts on human whole blood cultures

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Abstract

We aim to assess the *in vitro* genetic and oxidative effects of different *Callimenus latipes* Stal, 1875 (Orthoptera: Tettigoniidae: Bradyporinae) extracts (acetone, ethanol and diethyl ether) on human lymphocytes. We used the micronucleus (MN) test to monitor genetic damage. In addition total antioxidant capacity (TAC) of blood was measured to evaluate the oxidative status. For these aims, heparinized blood samples were obtained from two, non-smoking healthy men, with no history of exposure to any genotoxic agent. The extracts containing all body parts were sterilized and added to the cultures at different concentrations (1 to 160 ppm). Our results showed that the application of insect extracts at different concentrations did not elevate ($p < 0.05$) the number of MN formations. However, the extracts (except for ethanol) decreased TAC level at higher concentrations (80 and 160 ppm). Thus, these results suggest that *C. latipes* can be consumed safely, but it is necessary to take into consideration the cytotoxicity at increasing doses.

Key words: Edible Insects, Genotoxicity, Human Blood Culture, Micronucleus Test, Total antioxidant capacity

Introduction

Insects are important sources of human nutrition in many part of the world like Africa, Australia, Asia and the Americas. Over 1500 different species of insects have been reported as being consumed or edible around the world (Defoliart, 1995; Food-Info, 2009). Of these, grasshopper species are more widely used (directly or indirectly) for human consumption in many countries. Because this group of insects is important as a source of protein as well as being traditional cultural delicacies in various part of the world. *Callimenus latipes*, treated here, is one of the larger species. This species has not been reported as being consumed in any part of the world yet. But when its size is considered, it has as great a potential for human consumption as other grasshoppers (Defoliart, 1995). Although insects are widely consumed all over the world, very limited information is available concerning their toxic effects (Incekara & Türkez, 2009). To the best of our knowledge, no study has been carried out on *C. latipes* toxicity. Therefore, in the present study, we aimed to assess the *in vitro* genetic and oxidative effects of different *C. latipes* extracts (acetone, ethanol and diethyl ether) on human lymphocytes.

On the other hand, these insects can contain powerful pharmacologically active substances, which are known vertebrate toxins (Akinawo *et al.*, 2002). So eating of these insects may cause serious harmful effects on human. In this context the potential toxic effects of these popular edible insects need to be investigated in detail. Such studies will also be of use in biomedical production because it is well known that animal toxins may even become important in curing diseases like cancer. For this aim, we used sensitive and reliable short term genotoxicity (MN) and oxidative stress (TAC) screening tests (performed in two replicates) on human whole blood cultures.

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Specimens of, *Callimenus latipes* (Tettigoniidae: Bradyporinae) (Figure 1) were collected from the north of Iran, Polur, Mazandaran province and killed without any chemical treatment. The mean dry weight of specimens was calculated using 5 individuals. The heparinized blood from two healthy non-smoking donors with no history of exposure to any genotoxic agent was used. Human peripheral blood lymphocyte cultures were set up according to a slight modification of the protocol described by Evans and O’Riordan (1975). The heparinized blood (0.5 ml) was cultured in 6 ml of culture medium (Chromosome Medium B, Biochrom®, Leonorenstr. 2-6.D-12247, Berlin) with $5 \mu\text{g ml}^{-1}$ of phytohemagglutinin (Biochrom®). The acetone, ethanol and diethyl ether extracts of *C. latipes* (in concentrations of 1, 5, 10, 20, 40, 80 and 160 ppm) were added to the cultures. The cytogenetic (MN test for 72 h) and biochemical (TAC level for 1h) parameters were performed on blood cultures. Each /without insect extract was studied as a control group.


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Fig 1- *Callimenus latipes*, lateral

MN assay

The MN test was performed by adding cytochalasin B (Sigma®; 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. 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 (two cultures per concentration) for the presence of one, two or more micronuclei.

TAC level

The automated TAC (Trolox equivalent Antioxidant Capacity) assay was carried out by commercially available kits (Total Antioxidant Status, Rel Assay Diagnostics, Turkey) in plasma samples of cultures. In this assay, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) is incubated with metmyoglobin and H_2O_2 to produce ABTS radical cation. This species is blue-green. Antioxidants present in the sample cause a reduction in absorption proportional to their concentration (Prior and Cao 1999; Erel 2004).

Statistical analysis

Statistical analysis was performed using SPSS12.0 Software. The two-tailed Student’s *t*-test was used to compare MN rates and TAC levels between treated and control groups.

Results

The different extracts of *C. latipes*, prepared in different solvents like acetone, ethanol and diethyl ether, did not cause any significant important ($p < 0.05$) alterations of MN rates compared to the control value. Therefore, it is revealed that this insect species has no genotoxic potential (Figure 2). On the other hand, significant decreases ($P < 0.05$) of TAC levels were observed after the application of diethyl ether (at 160 ppm) and acetone (at 80 and 160 ppm) (Figure 3).

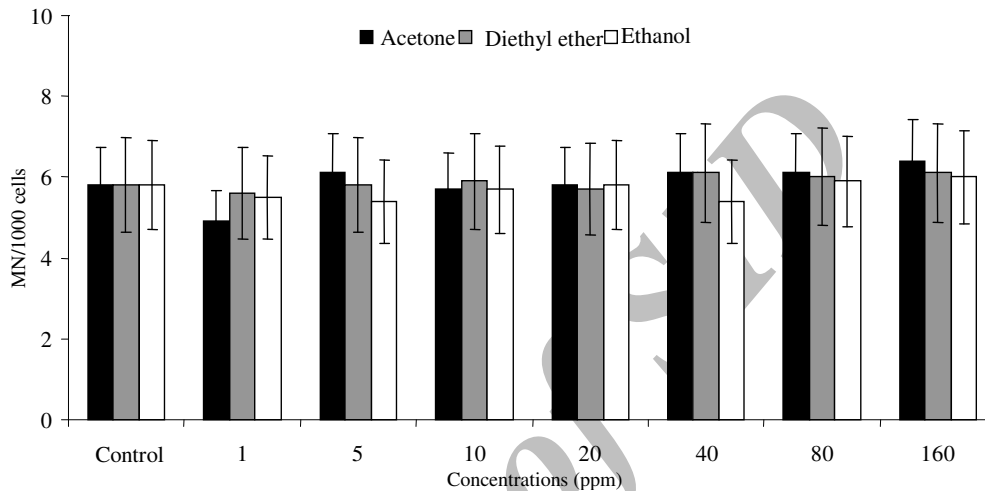


Fig. 2- The effects of different *C. latipes* extracts on MN rates *in vitro* for 72 h. (all values are similar to the control group at the significance level of 0.05)

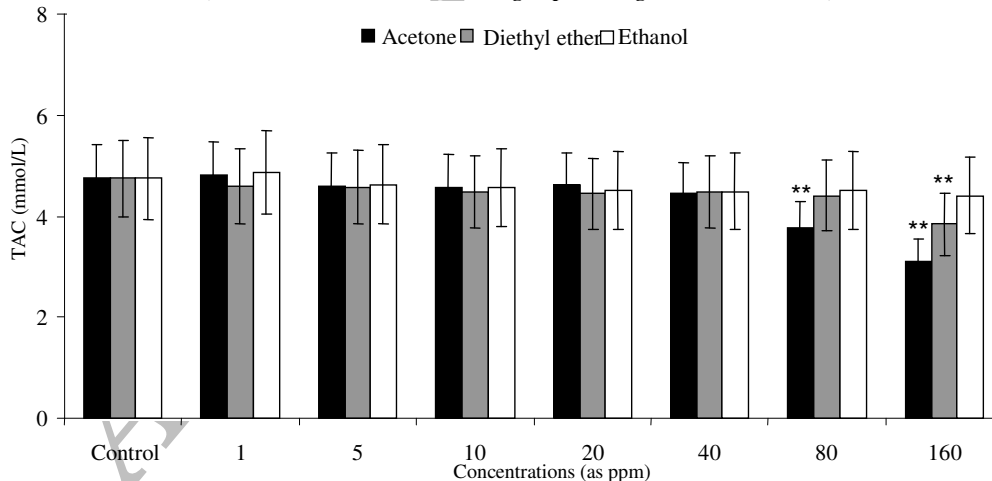


Fig. 3- The effects of different *C. latipes* extracts on plasma TAC level *in vitro* for 1h (** means statistically are different at the level of 0.05 from the control group)

Discussion

Our present results clearly indicated that acetone, ethanol and diethyl ether extracts of *C. latipes* have no mutagenic potential. Likewise, three different insect species (*Hydrophilus piceus* Linnéus 1758, *Dytiscus marginalis* Linnéus 1758 and *Cybister* sp.) were tested for genotoxicity by using sister chromatid exchange (SCE) on human whole blood cultures by İncekara & Türkez (2009). They found no *in vitro* adverse effects and revealed that the three edible aquatic insects species, had no mutagenic effect. Again, in other recent investigation, Türkez *et al.* (2010) reported non-mutagenic properties of *Saga ephippigera ephippigera* Fischer-Waldheim 1846, and *Callimenus dilatatus* Stål, 1875 (Orthoptera) on cultured human blood cells by using the chromosome aberration (CA) and micronucleus (MN) tests *in vitro*. In this study the safe concentration levels of different *C. latipes*

It is concluded that *C. latipes* can be consumed safely, but it is necessary to take into consideration the cytotoxicity at increasing doses. We also offer that this *in vitro* approach which includes the collaborative use of two different toxicity tests, MN and TAC, will serve to compare the potential health risks of edible insects related to mutagenesis or carcinogenesis.

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