EFFECTS POTENTIALS OF COMMERCIAL EDIBLE *HETEROMETRUS SPINIFER* IN VITRO

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ABSTRACT: In this investigation, the genotoxic and oxidative effects of water soluble extracts of *Heterometrus spinifer* (Malaysian Forest Scorpion) has been assessed on cultured human blood cells. The extract was added to the cultures at 12 different concentrations (0-2000 mg/L). Micronucleus (MN) test was used to monitor the DNA and chromosomal damage produced by scorpion extracts in vitro. In addition, to assess the oxidative effects, total antioxidant capacity (TAC) and total oxidant status (TOS) levels were measured. Our results indicated that these extracts did not show genotoxic effects at the tested concentrations. However, the extracts caused dose dependent alterations in both TAC and TOS levels. Based on the findings, it was concluded that the studied scorpion can be consumed safely, but it is necessary to consider the cellular damages which are likely to appear depending on oxidative stress at higher concentrations. It has been also suggested that this in vitro approach for oxidative and genotoxicity assessments may be useful to evaluate the potential health risks of edible scorpion.

KEY WORDS: Genotoxicity, *Heterometrus spinifer* scorpion, human blood culture, micronucleus test, oxidative status.

Scorpions and other arthropods are consumed as food supplements in many parts of the world. Scorpions are commonly eaten in China, Thailand, Cambodia and Bangkok. Scorpions same as insects are high in protein and apparently consist of important fatty acids and vitamins (Mayor, 2009). Having commercial importance, the edible scorpion *Heterometrus spinifer*, is quite large, averaging around 15 cm. As its name suggests the Malaysian Forest Scorpion is native to Malaysia though it is also seen in some other areas in Asia. For example, this species is common to Thailand and is one of a few species that is known to be edible. But many of the arthropods have venom and other defensive chemicals which are biologically active. So eating of these invertebrates may cause serious harmful effects on humans. In this context, the potential toxic effects of these popular arthropods need to be investigated in more detail. For this aim, we used sensitive and reliable short term genotoxicity (MN) and oxidative stress (TAC and TOS) screening tests (performed in five replicates) on human whole blood cultures. 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**.** The genotoxic and oxidative effects of edible scorpion extracts exposure have not yet been reported. In this study, we aimed to elucidate whether the water soluble extracts of *Heterometrus spinifer* scorpion have the genotoxic and oxidative effects in vitro or not.

MATERIAL AND METHODS

Scorpion extracts

Heterometrus spinifer scorpion was supplied from internet address (http://www.thailandunique.com/). A stock solution of aqueous extract was prepared by mixing 1,4 g of processed scorpion powder with 200 ml of water (boiled and cooled tap water) with constant stirring on a magnetic stirrer. The suspension of scorpion powder in water was left for 4 h, and filtered through filter paper No.1 (Whatman). The filtrate was stored in amber colored air tight bottle at room temperature till use. Then, stock solutions were diluted and added to cell culture tubes at different concentrations (0, 5, 10, 15, 25, 40, 75, 100, 200, 500, 1000 and 2000 ppm).

Cell cultures

The heparinized blood samples from five healthy male non-smoking donors with no history of exposure to any toxic agent were used in our experiments. From all volunteers involved in this study, hematological and biochemical parameters were analyzed, and no pathology was detected. Human peripheral blood lymphocyte cultures were set up according to a slight modification of the protocol described by Evans & O'Riordan (1975). A 0.5 mL aliquot of heparinized blood was cultured in 6 mL of culture medium (Chromosome Medium B; Biochrom, Berlin) with 5 mg/mL of phytohemagglutinin (Biochrom). The cultures were incubated in complete darkness for 72 h at 37°C. Experiments were conformed to the guidelines of the World Medical Assembly (Declaration of Helsinki). The MN test was carried out on lymphocytes 72 h after treatment. The TAC and TOS assays were carried out on plasma samples 2 h after treatment. Each individual lymphocyte culture without insect extract was studied as a control group.

MN assay

The MN test was performed by adding cytochalasin B (Sigma®; final concentration 6 mg/mL) 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, v/v). The fixed cells were put directly on slides, using a cytospin, and stained with Giemsa solution. All slides were coded before scoring. The criteria for scoring micronuclei were as described by Fenech (1993). At least 1000 binucleated lymphocytes were examined per concentration for the presence of one, two or more micronuclei.

TAC and TOS analysis

The automated Trolox equivalent antioxidant capacity (TAC) and total oxidant status (TOS) assays were carried out in plasma samples obtained from blood cultures for 2h by commercially available kits (Rel Assay Diagnostics®, Gaziantep, Turkey) (Erel, 2004).

Statistical analysis

Statistical analysis was performed using SPSS software (version 13.0, SPSS, Chicago, IL, USA). The Duncan's was used to determine whether any treatment significantly differed from controls or each other. Statistical decisions were made with a significance level of 0.05.

RESULTS

The results of the present study clearly indicated that the aqueous extracts of scorpion did not alter MN/1000 cell frequencies in cultured human lymphocytes (Figure 2). Nevertheless, the human blood cultures were found to be sterile after the applications of the extracts of scorpion at concentrations of 1000 and 2000 ppm. The cytotoxic effects observed at increasing concentrations might cause the sterility. Scorpion extract at 10 ppm caused significant increases of TAC level when compared to control value. However, different concentrations of scorpion (200, 500, 1000 and 2000 ppm) lead to significant decreases of TAC level when compared to control value (Figure 3). As shown from the results presented in Figure 4, the TOS levels increased at higher concentrations of scorpion (100, 200, 500, 1000 and 2000 ppm).

DISCUSSION

Edible arthropods are consumed as food supplements in many parts of the world. But many of them have venom and other defensive chemicals which are biologically active. So eating of these invertebrates may cause serious harmful effects on humans. Very few studies have been reported in relation to the genotoxic and oxidative effects of edible insect extracts but not scorpions. In the study by Incekara and Turkez (2009), 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 Incekara et al. (2010), 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 were investigated. In Turkez et al. (2010) 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 were evaluated and found to be non genotoxic. Again, recently Turkez et al. (2011) reported non-mutagenic properties of migratory locust (*Locusta migratoria*) on cultured human blood cells by using the chromosome aberration (CA) and MN tests in vitro. In addition, Adamolekun (1993) reported a seasonal ataxic syndrome associated with the consumption of the edible larva of *Anaphe venata* (Butler) in south-west Nigeria. Akinnawo et al. (2002) studied toxicity of the aqueous extracts of raw and processed larva of *Cirina forda* (Westwood) administered orally in white albino mice and albino rats. They suggested that the processed larva of *Cirina forda* (Westwood) is neither neurotoxic nor hepatotoxic to mice and rats; however, the neurotoxic nature of the raw extract needs further investigation. MacEvilly (2000) suggested that insects should not be eaten with nuts or shellfish as both have been shown to trigger allergic responses in hypersensitive individuals. There are also very few reports of human death caused by eating insect (Blum, 1994; Steyn, 1962).

Our present results clearly indicated that water extracts of *Heterometrus spinifer* scorpion 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 scorpion can be consumed safely; however, it

will also be useful to take into consideration the cytotoxicity at increasing doses. The safe concentrations of edible scorpion 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. The results of the present study revealed that the scorpion extract caused significant increases of TAC levels at 10 ppm in vitro. Our results also revealed that aqueous extracts of scorpion caused decreases of TAC levels, at higher concentrations than 200 ppm. And the scorpion extracts caused increases of oxidative stress at the concentrations higher than these concentrations. The cytotoxic effects of overdoses applications of scorpion extracts could be explained by the increases of TOS levels. Eating arthropods have become more popular day by day around the world (Memorial University, 2010) and therefore further investigations on the potential genotoxic effects of these commercial arthropods should be conducted. We also offer that this in vitro approach which includes the collaborative use of genetic endpoints and oxidative stress markers will serve to compare the potential health risks of edible insects related with mutagenesis or carcinogenesis.

Based on the findings, it was concluded that the scorpion can be consumed safely, but it is necessary to consider the cellular damages which are likely to appear depending on oxidative stress. It has been also suggested that this in vitro approach for oxidative and genotoxicity assessments may be useful to evaluate the potential health risks of edible arthropods.

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LITERATURE CITED

Adamolekun, B. 1993. Anaphe venata entomology and seasonal ataxic syndrome in South West Nigeria. Lancet 341: 629.

Akinnawo, O. O., Abatan, M. O. & Ketiku, A. O. 2002. Toxicological study on the edible larva of *Cirina forda* (Westwood). African Journal of Biomedical Research, 5: 43-46.

Bartsch, H. & Malaveille, C. 1989. The relation between carcinogenesis and mutagenesis: re-evaluation. Bulletin de l'Académie Nationale de Médecine, 173 (8): 1005-1012.

Blum, M. S. 1994. The limits of entomophagy: a discretionary gourmand in a world of toxic Insects. The Food Insects Newsletter, 7 (1): 6-11.

Erel, O. 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical Biochemistry, 37: 277-285.

Evans, H. J. & O'Riordan, M. L. 1975. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. Mutation Research, 31: 135-148.

Fenech, M. 1993. The cytokinesis blocks micronucleus technique. A detailed description on the method and its application to genotoxicity studies in human population. Mutation Research, 285: 35-44.

Incekara, U. & Turkez, H. 2009. The genotoxic effects of some edible insects on human whole blood cultures. Munis Entomology & Zoology, 4 (2): 531-535.

Incekara, U., Turkez, H., Memiş, E., Güner, A. & Vafei, R. 2010. The in vitro cytogenetic and oxidative effects of *Bradyporus latipes* (Tettigoniidae: Bradyporinae) extracts. Journal of Entomological Researh of Islamic Azad University, 2: 1-5.

MacEvilly, C. 2000. Bugs in the system. Nutrition Bulletin, 25: 267-268.

Mayor, V. 2009. 15 Strange Meals of the World. Available from: http://villageofjoy.com/15-strange-meals-of-the-world-part-i/.

Memorial University, 2010. Edible Insects. URL: http://www.mun.ca/ biology/ bpromoters/edibleinsects.php. March, 2010.

Steyn, D. G. 1962. Grasshopper (*Phymateus leprosus* Fabric.) poisoning in a Bantu child. South African Medical Journal, 36: 822-823.

Turkez, H., Incekara, U & Erman, O. 2010. Biomonitoring of the genotoxic potentials of two edible insect species in vitro. Turkish Journal of Entomology, 34: 411-417.

Turkez, H., Incekara, U., Güner, A., Aydın, E., Dirican, E. & Togar, B. 2011. The cytogenetic effects of the aqueous extracts of migratory locust (*Locusta migratoria* l.) in vitro. African Entomology (in press).

Figure 1. Commercial *Heterometrus spinifer* scorpion in sale.

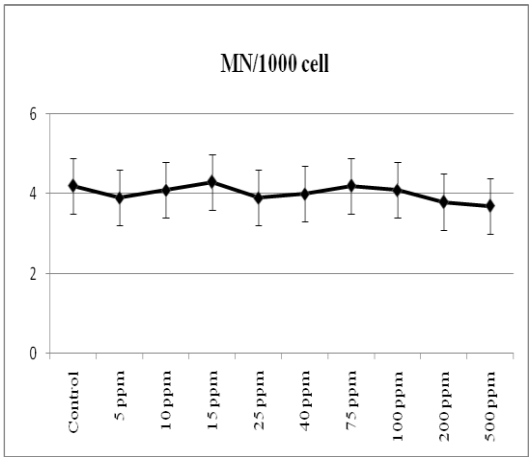

Figure 2. The effects of aqueous extracts from *Heterometrus spinifer* scorpion on MN/1000 cell values in human blood cultures.

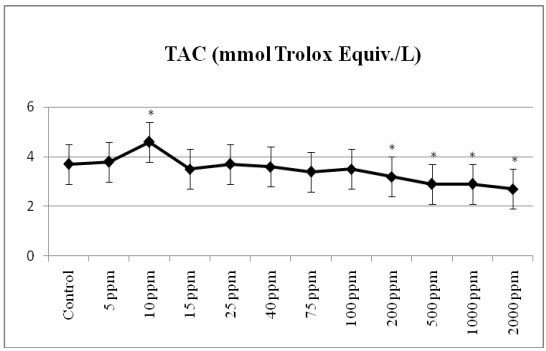

Figure 3. The effects of aqueous extracts from *Heterometrus spinifer* scorpion on TAC levels in human blood cultures (Values are means ± standart deviation, * symbol means statistically significant differences from control).

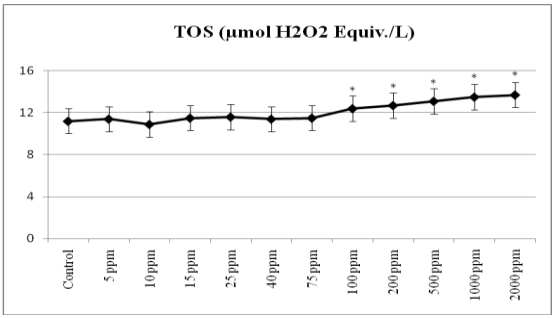

Figure 4. The effects of aqueous extracts from *Heterometrus spinifer* scorpion on TOS levels in human blood cultures (Values are means ± standart deviation, * symbol means statistically significant differences from control).