IN VITRO ASSESSMENT OF GENOTOXIC AND OXIDATIVE EFFECTS POTENTIALS OF EDIBLE BAMBOO WORMS AND WEAVER ANTS

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ABSTRACT: In the present study we have assessed the genotoxic and oxidative effects of water soluble extracts of Bamboo Worms, *Omphisa fuscidentalis* (Lepidoptera: Pyralidae), and Weaver Ants, *Oecophylla smaragdina* (Hymenoptera: Formicidae), on cultured human blood cells. The extracts were added to the cultures at 12 different concentrations (0-2000 ppm). Micronucleus (MN) test was used to monitor the DNA and chromosomal damage produced by aqueous extracts *in vitro*. In addition, to assess the oxidative effects, total antioxidant capacity (TAC) and total oxidant status (TOS) levels were also 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 insects 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 insects.

KEY WORDS: Edible insects, genotoxicity, oxidative status

Insects have been consumed as an important food resource for thousands of years. Nowadays, an estimated 2086 insect species are consumed by 3071 ethnic groups (Ramos-Elorduy, 2009). 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. Some of the commonly eaten species of insects include grasshoppers, crickets, termites, ants, beetle larvae, moth caterpillars, and pupae (Capinera, 2004). Insects are rich in protein and amino acids, fat and carbohydrates, nutritive elements such as iron, calcium and vitamins A, B1, B2 and D (DeFoliart, 1989; Ramos-Elorduy, 2005). Some experts are of the opinion that edible insects may be an inexhaustible protein source for humans in the future. Therefore edible insects offer an important nutritional resource for humans. Besides the nutrient content, insects have medicinal properties because they include important substances such as antimicrobial proteins and peptides, enzymes and hormones (Yamakawa, 1998). In various parts of the world, many species of insects have been used in traditional and folk medicine (Paoletti, 2005). 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 (Akinnawo, et al., 2002). Very limited information is available concerning the genotoxic and oxidative effects of edible insects. Their potentially toxic effects should be investigated in more detail because eating insects may cause serious harmful effects on humans.

In order to investigate this further we used sensitive and reliable short-term genotoxicity and oxidative stress screening tests (performed in five replicates) on human whole blood cultures. Genetic alterations, mainly MN in cell cytoplasm, are the early biological effects of mutagenesis and/or carcinogenesis (Hagmar, et al., 1998). 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). The important oxidative parameters including TAC and TOS are used to monitor the development and extent of damage due to oxidative stress by insects in human blood.

The aim of the current study is to elucidate whether the water soluble extracts of Bamboo Worms, *Omphisa fuscidentalis* (Lepidoptera: Pyralidae), and Weaver Ants, *Oecophylla smaragdina* (Hymenoptera: Formicidae), have genotoxic and oxidative effects *in vitro*. Both insects are commonly consumed and have commercial importance. These are commonly consumed insects.

MATERIALS AND METHODS

Insect extracts

Bamboo Worms and Weaver Ants were supplied from Unique Foods Ltd., Thailand. Processed insects were triturated in a mortar. A stock solution of aqueous extract was prepared by mixing 1.4 g of processed insect powder with 200 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, and filtered through filter paper No.1 (Whatman). The filtrate was stored in an amber-colored air-tight bottle at room temperature until 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

Heparinized blood samples from five healthy male non-smoking donors with no history of exposure to any toxic agent were used in our experiments. Hematological and biochemical parameters were analyzed from all the volunteers, 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 conformed to the guidelines of the World Medical Assembly (Declaration of Helsinki). 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 2 h using 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). Duncan's test was used to determine whether any treatment significantly differed from controls or each other ($P<0.05$).

RESULTS

The results indicated that the aqueous extracts of Bamboo Worms did not alter MN/1000 cell frequencies in cultured human lymphocytes (Fig. 1). Nevertheless, the human blood cultures were found to be sterile after the application of the extracts of Bamboo Worms at concentrations of 1000 and 2000 ppm. The cytotoxic effects observed at increasing concentrations might cause the sterility. Different concentrations of Bamboo Worms (1000 and 2000 ppm) lead to significant decreases of TAC level when compared to the control values (Fig. 2). As shown from the results presented in Fig. 3, the TOS levels increased at higher concentrations of Bamboo Worms (500, 1000 and 2000 ppm).

The water soluble extracts of Weaver Ants did not cause any statistically significant difference MN/1000 cell frequencies upon concentrations tested (Fig. 1). Nevertheless, the human blood cultures were found to be sterile after the application of the extracts of Weaver Ants at a concentration of 2000 ppm. There was only one increase seen in antioxidant or "TAC" levels (at 40 ppm) for the weaver ant extract. All other TAC levels were either not significantly different from the controls (which would indicate no change in antioxidant levels) or decreased as compared to the controls. As shown from the results presented in Fig. 3, the TOS levels increased at higher concentrations of Weaver Ant (500, 1000 and 2000 ppm).

DISCUSSION AND CONCLUSION

Although the eating of insects has become widespread in parts of the world, very few studies have investigated whether this has harmful effects on humans. 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. (2005) studied toxicity of aqueous extracts of raw and processed larva of *Cirina forda* administered orally in mice and rats. They suggested that the processed larva of *Cirina forda* (Westwood) is neither neurotoxic nor hepatotoxic to these animals; however, the neurotoxic nature of the raw extract needs further investigation. Also, Akinnawo, et al. (2005) studied the effects of oral administration of extracts of raw and processed larvae of *Cirina forda* on morphometry and histopathology in rats. Their results indicated that the raw edible larva of *Cirina forda* was toxic to rats. The liver, kidney and to a lesser extent the heart appear to be the target organs. However, processing the larvae by boiling and sun-drying reduced the toxicity to the liver and heart but not the kidney. 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. Recently, three aquatic edible insect species, *Hydrophilus piceus*, *Dytiscus marginalis* and *Cybister* sp., were evaluated and found to be non-

genotoxic (İncekara & Türkez, 2009). The *in vitro* genetic and oxidative effects of *Callimenus latipes* extracts (acetone, ethanol and diethyl ether) on human lymphocytes were investigated and the results suggested that C. *latipes* can be consumed safely, but it is necessary to take into consideration the cytotoxicity at increasing doses (İncekara, et al., 2010). Water-soluble extracts of grasshoppers, *Saga ephippigera ephippigera* and *Callimenus dilatatus,* were evaluated using cultured human blood cells; they were found to be non-genotoxic (Türkez, et al., 2010).

Our present findings demonstrate that water extracts of *Omphisa fuscidentalis* and *Oecophylla smaragdina* have no mutagenic potential. This suggests that these insect species can be consumed safely; however, it is advisable to take into consideration the cytotoxicity of insect extracts at increasing doses. The safe concentrations of edible insect extracts on human blood as described here are valid only for *in vitro* conditions. In order to gain greater insight *in vivo* studies are required on the absorption kinetics of these extracts from the gastrointestinal tract. The results of the present study reveal that *Oecophylla smaragdina* extract causes significantly increased levels of TAC at 40 ppm *in vitro*. The results of this study may encourage people to consume these insects as supplements of vitamins A, B_2 and C. In fact, it has been found that vitamin A, C, E and carotenoids, besides previously recognized functions of preventing particular lipido- and avitaminosis, significantly participate in the protection of the human body against oxidation stress that is characterized by balance disturbance between speed of free radical creation and reactive oxygen forms with pace of their neutralization by enzymes and antioxidants (Rutkowski, et al., 2010). And vitamin C was reported to be (together with glutathione) a major component of the non-enzymatic antioxidant system in the water-soluble compartment. Vitamin B_2 was found to be acting mainly as cofactor for glutathione reductase which keeps glutathione in the reduced state. It can therefore be considered an indirect antioxidative vitamin (Böhles, 1997). The relationship between strong antioxidant defences and the content of minerals such as Ca, Mg, Fe and P has also been reported (Kharb & Singh, 2000). Our results also revealed that aqueous extracts of *Omphisa fuscidentalis* and *Oecophylla smaragdina* lead to decreases of TAC levels at concentrations 1000 and 2000 ppm. Each extract augmented oxidative stress with an increase in its concentration. The cytotoxic effects of high levels of insect extracts could be explained by the increased levels of TOS. Therefore, we think that the similar damage may also occur in the human tissues as related over consumption. Taking all this into account, we suggest that insects can be consumed as a source of human nutrition but their appropriate amount must also be determined for human diet.

Eating insects has become more popular around the world; however, further researches are needed in order to prove their potential genotoxic effects. We suggest that the *in vitro* approach used here which includes the collaborative use of genetic endpoints and oxidative stress markers is a valuable technique for comparing the possible health risks of edible insects in relation to mutagenesis and carcinogenesis. This toxicity research may also be of much value in the formulation of novel biomedical products because it is well known that animal toxins may be effective in the treatment of diseases such as cancer.

Based on the findings, it was concluded that the Bamboo Worms and Weaver Ants can be consumed safely, but it is necessary to consider the cellular damage which is likely to appear. This depends on the extent of oxidative stress. It has been also suggested that this *in vitro* approach for oxidative and genotoxicity

assessment would be very helpful for evaluating the potential health risks of edible insects.

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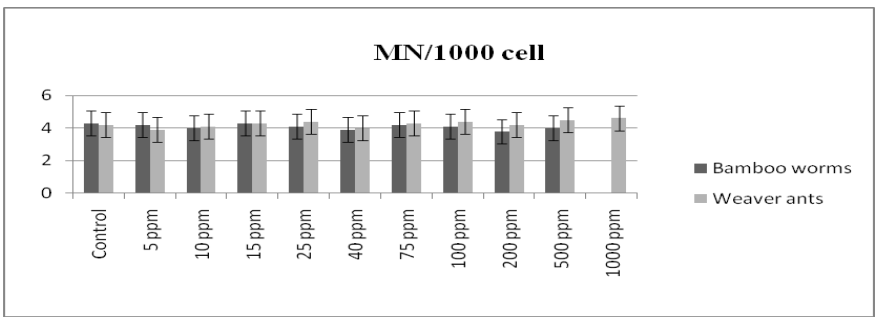

Figure 1. The effects of aqueous extracts from Weaver Ants and Bamboo Worms on MN / 1000 cell values in human blood cultures (Values are means ± standard deviation).

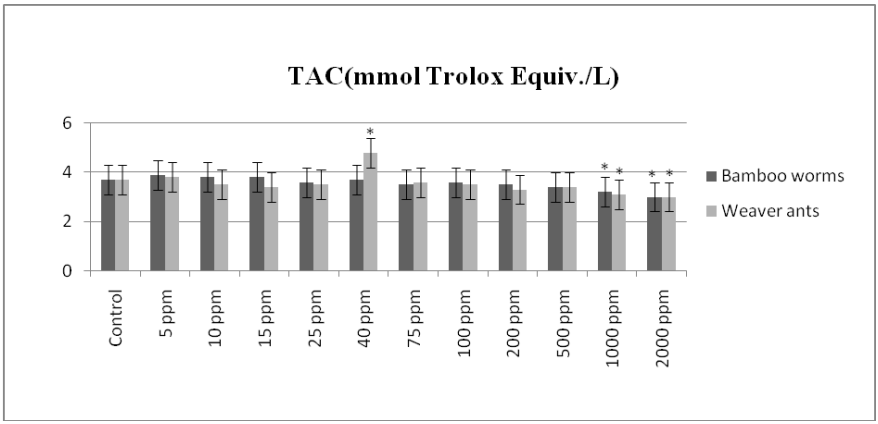

Figure 2. The effects of aqueous extracts from Bamboo Worms and Weaver Ants on TAC levels in human blood cultures (Values are means ± standard deviation, *symbol means statistically significant differences from control).

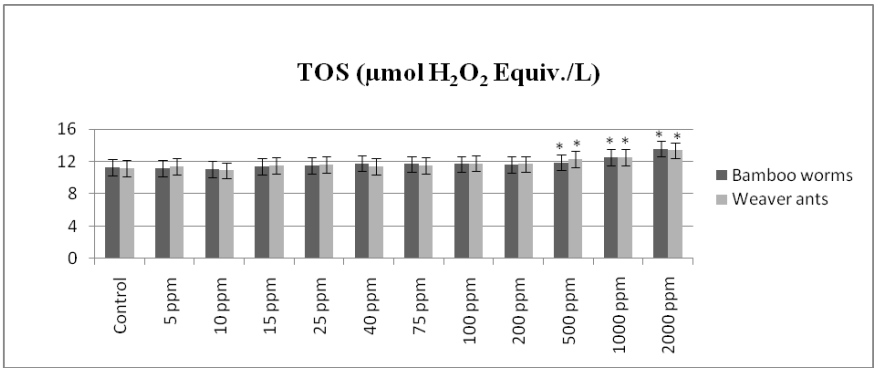

Figure 3. The effects of aqueous extracts from Bamboo Worms and Weaver Ants on TOS levels in human blood cultures (Values are means ± standard deviation, *symbol means statistically significant differences from control).