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Short Communication

Genotoxic Evaluation of an Endemic Plant *Thermopsis turcica* Extracts on Liver Cancer Cell Line

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ABSTRACT

Traditional medicines are used for therapeutic purposes all over the world. Many endemic plants all over the world have magical therapeutic potential. These could be explored further for medicinal purposes and hence can be preserved for their proper propagation. *Thermposis turcica* is endemic to Turkey. It's general anti-oxidant and anti-cancerous activities are explored, but no study has been observed on liver cancerous cell line in term of its genotoxicty. So, genotoxic evaluation was carried out for the alcoholic and hexane extracts of *T. turcica*. Methanol extracts showed the highest DNA damage (20±1) at 200 µg/ml concentration and 11.67±2.52 at 50 µg/ml. Ethanol extracts showed the 2nd highest DNA damage (19±2) at 200 µg/ml concentration and 11 ±1 at 50 µg/ml. While least was observed in the hexane extract (10.33±1.15; 6±2) at both concentrations, respectively. All groups were significantly different ($P<0.05$) from the control group at both concentrations. A current study concluded that *T. turcica* had the genotoxic effects on the liver cancerous cell line and alcoholic extracts showed the more DNA damage on HepG2 cells.

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Authors' Contributions
IHC conceived the idea and supervised the research. MMA performed the experiments and drafted the manuscript.

Key words
Endemic plant, Genotoxicity, DNA damage.

The incidence of hepatocellular carcinoma has increased in the last decade and is the fifth commonest neoplasm in the world, and third commonest cause of cancer-related death (Irfan and Dileep, 2006). Medicinal plants play an important role in amelioration of some diseases such as infectious and cancerous diseases in Turkey. Their parts used as a drog to combat the disease due to their effective compounds (Kültür, 2007).

Their usage has increased on account of heir low adverse effects and healthful features all over the world (Basgel and Erdemoglu, 2006). Plants of the genus *Thermopsis Fabaceae* includes poisonous and harmful species with low feeding value. *Thermopsis* species are believed to be the source of poisoning in children (McGrath-Hill and Vicas, 1997) and toxic to grazing herbivores such as cattle (Keeler and Baker, 1990). This specie contains many alkaloids and flavonoids (Bali *et al*., 2014), but It is not known whether any of these compounds cause cell cycle arrest or genotoxic effects. *T. turcica,* that is endemic to turkey, but poorly studied in terms of its genotoxicity on different cell lines. Literature states that extracts prepared from *Thermopsis* species are cytotoxic to cancer cells (Bali *et al*., 2014; Ali and Cigerci, 2017).

So, consistent with its cytotoxic and anti- cancer properties, genotoxicity of *T. turcica* plant parts was observed in a dose-dependent manner to the liver cancerous cell line. Liver cancerous cell line is commonly being studied as it generate the similar conditions for the toxicological studies near to the to vivo conditions (Blaauboer *et al*., 1998).

Materials and methods

Thermposis turcica was collected from Afyon, Sultandagi, near Aksehir Lake in Turkey during the flowering period in May. The collected plants was dried under shade, grounded into powder and extracted with 400 mL of ethanol, methanol, and n-hexane at ambient temperature for 24 h. Dimethyl sulfoxide (DMSO; 1%), was used as solvent to dissolve the all plant extracts (Ali and Cigerci, 2017).

Frozen liver cancer cells (HepG2) were obtained from Anadolu University, thawed quickly at 37°C water bath. This procedure was done not for more than a minute. Quickly pipetted out into a flask, added the appropriate amount of medium, centrifuged it at 1000 rpm for 5 min. Supernatant discarded and pellet was resuspended with 2

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ml medium. The cells were transfered from tube to the 25 cm^2 flasks. 4 ml medium added into a flask and placed in incubator (5% CO_2). The HepG2 was grown in RPMI 1640 medium, containing supplemented with 15% fetal bovine serum, 1% Penicillin-Streptomycin (10,000 U/mL). After 24 h cells were attached. The culture medium was changed to remove non-adherent cells and replenish nutrients. Again cells were incubated until 80% confluence. Medium was changed at every 3 day.

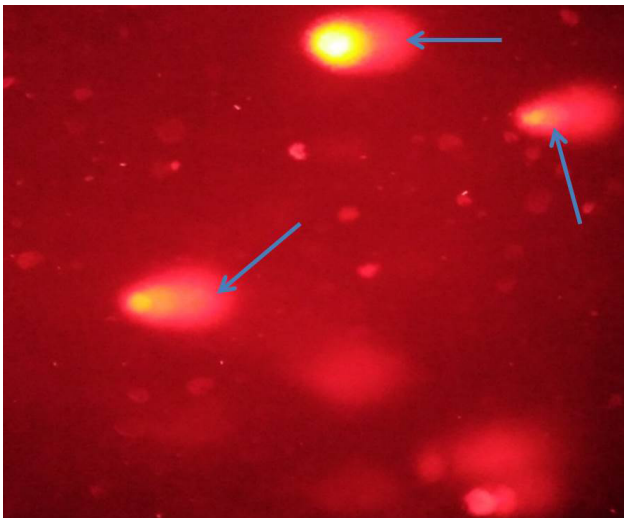

Fig. 1. DNA damage is shown in the form of tail. Arrows pointing to the tails of a damaged DNA.

When cells reached up to 80% confluence then Cells were passaged in to 75 cm^2 flask. Removed medium directly by aspiration. Trypsin-EDTA (0.02 to 0.03 ml per cm^2) for 4-6 min. Then detached cell with trypsin-EDTA added in to Fresh medium. Medium quantity was equal to the quantity of trypsin EDTA. This mixture was centrifuged at 1000 rpm for 5 min. Supernatant was discarded. Pellet was mixed with 2-3 ml fresh medium. Then it is converted into 75 cm^2 flask and kept in an incubator. Again medium was changed at every 3rd day and passaging was done until 80 % confluence.

Genotoxic evaluation was carried out by the alkaline comet assay. Approximately 1 x 10^5 cells were cultured in each flask 25 cm^2, and kept for 24 h for stabilization. *T. turcica* extracts were exposed to 50 µg/ml and 200 µg/ml. Negative solvent control (DMSO) was also taken. After 24 h exposure, cell were washed with PBS, scrapped with cell scraper and 10 µl cells were mixed with LMP agarose (100 µL). Mixture of cells and LMP agarose was then spread over the slide already coated with the NMP agar. Slides were kept at the cold slabs for 2-3 min after covering with long coverslips. Then, coverslips were removed and slides were kept in an alkaline lysis solution for one and half hour at 4°C. Next, the slides were incubated in electrophoresis buffer for 20 min and then electrophoresis was carried out for 20 min at 25V/300 mA. Slides were washed with cold distilled water and stained with ethidium bromide (200 µg/ml). The DNA damage score was calculated as described by the Cigerci *et al*. (2015, 2016). Briefly, score was given on the basis of tail length from 0 to 4 as shown in Figure 1 (0= no damage to 4= highest damage).

The data from the comet assay was subjected to variance analysis (ANOVA), ($p < 0.05$).

Results and discussion

Although it is categorized as critically endangered by the Red Data Book (Ekim *et al*., 2000), the investigation of its biological activities might increase importance of *T. turcica* conservation in nature.

Results of genotoxicity caused by the alcoholic and hexane extracts are shown in Table I. Methanol extracts showed the highest DNA damage (20±1) at 200 µg/ml concentration and 11.67±2.52 at 50 µg/ml. Ethanol extracts showed the 2nd highest DNA damage 19±2 at 200 µg/ml concentration and 11 ±1 at 50 µg/ml. While least was observed in the hexane extract (10.33±1.15; 6±2) at both concentrations, respectively. All groups were significantly differ from the control group at 200 µg/ml concentration and 50 µg/ml. While hexane extract was significantly different ($P<0.05$) from the control group at the highest dose. There was no difference between methanol and ethanol ($P<0.05$).

Table I.- DNA damage by the alcoholic and hexane extracts of the *Thermopsis turcica* on HepG2 cells.

Extracts	DNA damage (Mean±SD)*	
	200 µg/ml	50 µg/ml
Ethanol	19±2^{ab}	11±1^{a}
Methanol	20±1^{ab}	11.67±2.52^{a}
Hexane	10.33±1.15^{a}	6±2^{b}
Control	2±1.0^{b}	1±1^{b}

* Means with the same letter do not differ statistically at the level of $P<0.05$. SD, standard deviation.

Comet assay was used to assess the genotoxic effects of *T. turcica* extracts on hepG2. The comet assay, is a very cheap and sensitive test for estimation of DNA damage and provides direct determination of DNA strand breaks in individual cells. DNA strand breaks in individual cells (Sohail *et al*., 2017). This test has already been used to evaluate the *in vitro/in vivo* genotoxicity/antigenotoxicity of several agents and chemicals with various cell lines (Valentin-Severin *et al*., 2003).

Genotoxic effects of various *T. turcica* extracts were observed in the present study. More genotoxicity was observed at the highest concentrations. Alcoholic extracts showed the more DNA damage effects on HepG2 cells. Similarly, genotoxic effects of *T. turcica* extract had also been observed on the root nuclei of onion bulbs in a dose dependent manner (Cigerci *et al*., 2015). It is already shown that genotoxic components of plant origin can be used as a chemotherapeutic agent, Like, podophyllotoxin, catarantine and scopolamine have the toxic properties and these have been used into therapy (Bali *et al*., 2014).

Thermopsis species have been constantly investigated in several areas. Total flavonoid contents like formononetin, chrysoeriol, apigenin, luteoln, thermopsoside and cynaroside could be the reason of its medicinal properties (Kotenko *et al*., 2001). The various extcracts had already been investigated to find its cytotoxic, antimicrobial and anti-oxidant properties (Korcan *et al*., 2009; Aksoy *et al*., 2013) reported that acetone and methanol extracts of *T. turcica* had radical scavenging effects at 50, 100 and 200 µg/mL concentrations and methanol extract showed the antioxidant results (Aksoy *et al*., 2013). An ethanolic extract prepared from *T. rhombifolia* was cytotoxic to HT-29 (colon) and SH-SY5Y (brain) cancer cell lines (Kerneis *et al*., 2014).

Although some studies have been practiced to *T. turcica*, but no study has been found on the genotoxic effects of this endemic plant on liver cancerous line by the alcoholic and hexane extracts. This study suggests that alcoholic extracts had the genotoxic effects on the liver cancerous cell line. This study provides the preliminary data on the DNA damage effects on HepG2 cells. Further investigations could be made to find its anticancerous effects on various cell lines at the molecular level.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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