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Banat's Journal of Biotechnology
2019, X(20)

In vitro mutagenic effect of cedar (*Cedrus libani* A. Rich) tar in the salmonella/microsome assay system

DOI: 10.7904/2068–4738–X(20)–13

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Abstract. Cedar tar is obtained by pyrolytic breakdown of *Cedrus libani* A. Rich (*Pinaceae)* wood. *Cedrus libani* A. Rich (Pinaceae) grows on the Taurus Mountains in Southern Turkey, Eastern Mediterranean, Western Syria and Lebanon. *Objective*: The main objective of this study is to investigate the mutagenic and antioxidant effect of Cedar tar produced from cedarwoods collected from Gume village of Mut, Mersin, Turkey. *Methods*: The mutagenic activity of Cedar tar was screened by using *Salmonella typhimurium* TA98 and TA100 strains, with and without S9 metabolic activation in this study. Three concentrations of (10, 15 and 25 mg/plate) Cedar tar were examined in AMES assays. *Results*: Cedar tar was found to be non–mutagenic against strains TA98 and TA100 ($p>0.05$) in the presence and absence of S9 metabolic activation. Cedar tar did not show any antioxidant activity based on DDPH radical scavenging. The total phenolic and flavonoid contents of tar were 0.85±0.06 mg GAE/g and 0.068±0.02 mg RE/g, respectively. *Conclusions*: Antimutagenic, cancerogenic and cytotoxic effects of Cedar tar should also be investigated by using different test systems.
Keyword: Ames test, Cedar tar, Folk medicine, Mutagenicity.

Introduction

Cedrus libani A. Rich is a species of *Cedrus* genus belongs to *Pineaceae* family. It naturally grows in the eastern Mediterranean, western Syria and Lebanon [BOYDAK, 2003].

Cedrus libani, occurred the wide parts of forests in the Taurus Mountains of Turkey, is referred to as Lebanon cedar and Taurus cedar [KURT et al., 2008].

Taurus cedar has historical, cultural, ecological aesthetic, scientific, economic and pharmacological significance [BILGEN et al., 2012; GHANEM and OLAMA, 2014].

Structures such as temples, churches, houses, dams, palaces and ships have been built by using the cedarwood because of being resistance to rottenness since ancient times [BOYDAK, 2003; KURT et al, 2008].

Cedarwood has been traditionally used for treatment of animal and human diseases in folk medicine from past to present [GHANEM and OLAMA, 2014, RASHED and BUTNARIU, 2014, BUTNARIU, 2016].

Especially, tar obtained from cedarwood is extensively used as protective against intestinal parasites and repellent against insects and snakes in veterinary [PEKGOZLU et al., 2017].

It is produced by pyrolysis process that is a thermal conversion of biomass into gas, liquid and char in the absence of oxygen [KURT and ISIK, 2012; PEKGOZLU et al., 2017].

Tar including various chemical compounds has characteristic smoky smell and dark brown/black color [PEKGOZLU et al., 2017]. Tar is called as 'Katran' in Turkey [ARI et al., 2014, SAMFIRA, *et al.,* 2014, BUTNARIU, 2016].

The objective of this study is to investigate the mutagenic and antioxidant activity of Katran that is traditionally obtained from *Cedrus libani* A. Rich wood by local people.

Material and methods

Chemicals and Strains

β–nicotinamide adenine dinucleotide phosphate (NADP), D–Glucose–6–phosphate, 2–Aminofluorene,

Sodium aside, L–histidine, D–biotin and rat liver S9 fraction were purchased from Sigma Aldrich (St. Louis, MO, USA). *Salmonella typhimurium* TA98 (*HisD3052, pKm101, rfa, uvrB*) and TA100 (*HisG46, pKm101, rfa, uvrB*) strains were granted by Prof. Dr. Nuran Diril, Hacettepe University, Ankara, Turkey.

Preparation of Cedar tar

The traditional production of Katran is based on pyrolytic breakdown of woods. In order to obtain Cedar tar, cedarwoods were collected from Gume village of Mersin, Mut district in East Mediterranean Region of Turkey.

Traditional tar production from these woods is thermal conversion of biomass in the absence of oxygen. For this, the hole (ignition compartment) was dug in the ground depending upon the amount of wood. Two metal cans, one of them is lidless, were obtained. Dry cedarwood was cut into small pieces and the covered metal can was filled with these pieces.

They were provided to connect each other by placing the collecting can (lidless metal can) in the hole and the other one onto collecting can. The burning process was started by igniting pieces and continued at high temperature (average 400 ºC) for 1–2 h for a better tar yield. After then, Cedar tar was collected with distinctive smoky smell and dark brown–black color from collecting can. This extract having characteristic smoky smell is black and a complex mixture.

***In vitro* Phytochemical Analysis**

Antioxidant activity of Cedar tar was performed according to DPPH Radical Scavenging Method defined by [UCAN TURKMEN and MERCIMEK TAKCI, 2018].

Quantification of the total phenolic compounds was examined by colorimetric reaction of the Folin–Ciocalteu reagent [STANKOVIC, 2011]. The flavonoid contents of Katran were determined by the aluminum chloride method using catechol as standard [SHARM and VIG, 2013].

Mutagenic Assay

The Salmonella/microsome assay developed by [MARON and AMES, 1983] was performed to detect the mutagenicity of Cedar tar. Prior to initiation analysis, it was tested mutagen properties of *Salmonella typhimurium* TA98 (*HisD3052, pKm101, rfa, uvrB*) and TA100 (*HisG46, pKm101, rfa, uvrB*) strains. For cytotoxicity analysis, it was used tar concentrations of 25–50–75–100–150–200 mg/plate.

To determine the cytotoxic dose of Cedar tar, 100 µL Cedar tar and 100 µL of bacteria strains grown overnight (approximately 1–2x10^9 cfu/mL) were mixed with test tubes including 2 mL top agar. These tubes were poured into Nutrient agar plate and incubated at 37 ºC for 24–72 h. After incubation, non–toxic tar dose was detected by comparing the number of colonies in the test and control plate. The mutagenicity assay was performed with the selected doses.

It was used the standard plate incorporation assay of the AMES, with or without the liver S9 mix. The liver S9 mix was prepared according to the procedure declared by Ames.

In all assays, negative controls (DMSO and distilled water) and positive controls (1 mg/plate sodium for TA100 and 200 µg/plate 2–aminoflourene for TA98) were used according to strains [EKMEKCI, 2010].

100 µL of overnight culture (approximately 1–2x10^9 cfu/mL), Cedar tar at different concentrations (10–15–25 mg/plate) and 500 µL of S9 mix or 500 µL of phosphate buffer were added to 2 mL top agar. The entire mixture spread on minimal glucose agar plate.

All experiments were performed in triplicate. After incubation for 24–72 h at 37 °C, the revertant colonies per plate were counted. Mutagenicity of Cedar tar was evaluated by comparing the number of revertants in the test plate with the number of colonies in the control plate. When the number of revertant colonies per plate doubled that counted in control plates, the test substance is mutagen.

Statistical Analysis

The significant difference *($p<0.05$)* between the numbers of revertants per plate in association with the mutagenicity was determined by using variance analysis (ANOVA) and Tukey multiple comparison tests.

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Results and discussion

Cytotoxicity of Cedar tar on Strains

When the number of His^+ revertants in strains TA98 and TA100 at all doses tested is not lower than half of the colonies in control plate, the tested dose is indicated to be non–cytotoxic.

The viable cells for strains TA98 and TA100 were encountered in test plate including Cedar tar at the 50–75–100–150–200 mg/plate concentrations as shown Table 1. However, the His^+ colonies in control plate were observed in tester strains TA98 and TA100 as a result of spontaneous reversion mutations at 24 and 72 h. So, these doses of Cedar tar tested showed a toxic effect against strains TA98 and TA100. The number of bacterial colonies calculated at the dose of 25 mg/plate for both strains was not lower than half of the negative control (Table 1).

Table 1.

Cytotoxic activity of Cedar tar on strains TA98 and TA100

	TA 98	Control (distilled water)	TA 100	Control (distilled water)
200 mg/plate	–*	57.5±0.50	–*	184 ±3.0
150 mg/plate	–*		–*	
100 mg/plate	–*		–*	
75 mg/plate	–*		–*	
50 mg/plate	–*		–*	
25 mg/plate	33.5±1.50		123 ±8.0	

* Bacteria colonies were not observed on nutrient agar plate

The low numbers of colonies in *S. typhimurium* TA98 may indicate to be more sensitive than the strain TA100 against Cedar tar. According to this result, 10–15 and 25 mg/plate doses were performed for mutagenicity analysis.

Mutagenic and Antioxidant Activity of Cedar tar

Regarding mutagenicity, the number of His^+ revertants colonies induced by positive control for strains TA98 and TA100 decreased in all doses tested (Table 2 and 3).

The mutagenicity test showed that the number of His^+ revertants in strains TA98 and TA100 was not a positive dose–related increase. According to the number of His^+ colonies in negative control plate (28.5±1.5), the number of revertants increased (32.5±1.5) at the 10 mg/plate dose without S9 mix for TA98.

The number of His^+ revertants at the 10–15 mg/plate dose tested in mutagenicity analysis with S9 mix for strain TA98 increased by comparison negative control. Frameshift mutation activity of katran (10–15 mg/plate dose) induced with S9 mix for TA98. On the other hand, the number of His^+ revertants in all doses tested with or without metabolic activation was lower than negative control plate for strain TA100.

Table 2.

Mutagenic activity of treatments with different doses of the Cedar tar on strain TA98

Treatments	Concentrations	Number of His+ revertants colonies/plate (mean ± SD)	
		S. typhimurium TA98 without S9 mix	*S. typhimurium* TA98 with S9 mix
Negative control (distilled water)	100 µL/plate	28.5±1.50	41.5±4.50
Positive control (2–AF)	200 µg/plate	–*	277.5±22.50
Cedar tar (Katran)	25 mg/plate	20.5±0.50	37±5.0
	15 mg/plate	23.5±2.50	43±0.0
	10 mg/plate	32.5±1.50	55.5±0.50

*: Revertant colonies belong to TA98 were not observed on minimal glucose agar plate

The significant difference between the number of His^+ revertants in the test and the positive control groups was not found ($p>0.05$). % inhibition of mutagenicity ranged between 0–25 % for strains TA98 and TA100. In brief, it was appeared that all doses of Cedar tar were not any mutagenic effect for both TA98

and TA100. 2–AF and sodium aside were performed as the mutagenic agents in this study. Sodium aside produces reactive metabolites and induces reactive oxygen species (ROS) associated with the activity of caspases, causing base–pairs mutations. 2–AF induces intracellular oxidative stress and can binds to guanine residues of DNA, causing frameshift mutations [Da SILVA LIMA et al., 2017; CARNEIROA et al., 2018, CAUNII, *et al.,* 2015, SAMFIRA, *et al.,* 2015].

Reactive oxygen species (ROS) is caused the oxidative modification of biomolecules such as DNA, protein, lipids and other cellular molecules.

Recently, the secondary metabolites such as flavonoids, phenolic and polyphenolic compounds, terpenoids and nitrogen–containing alkaloids and sulphur–containing compounds in plants are accepted to defend the cellular molecules against this oxidative modification [IANCULOV, *et al.,* 2004, PETRACHE, *et al.,* 2014, SAMFIRA, *et al.,* 2014]. This defense is described as antioxidant activity. This natural antioxidant activity of plant extracts is decreased the mutagenic and carcinogenic effects of some chemicals [ZAHIN et al., 2014].

Table 3.

Mutagenic activity of treatments with different doses of the Cedar tar on strain TA100

Treatments	Concentrations	Number of His+ revertants colonies/plate (mean ± SD)	
		S. typhimurium TA100 without S9 mix	*S. typhimurium* TA100 with S9 mix
Negative control (distilled water)	100 µL/plate	173±30.0	199.7±23.7
Positive control (Sodium azide)	1 mg/plate	1538±208.0	1583±9.0
Cedar tar (Katran)	25 mg/plate	156.5±3.5	186±29.0
	15 mg/plate	162±6.0	185±4.0
	10 mg/plate	158.5±1.50	186±29.0

Cedar tar that was traditionally produced was determined to have the low phenolic content (0.85±0.06 mg GAE/) in this study. The amount of flavonoids, are a class of low–molecular–weight phenolic compounds was 0.068±0.02 mg RE/g.

These low contents of secondary metabolites may be expressed to affect adversely the antioxidant and mutagenic activity of Katran. During traditional production of Katran, the ignition process at average 300 ºC temperature may be caused the loss of most secondary metabolites in çira.

AMES is the most important *in vitro* test system that is investigated the relationship between carcinogenic and mutagenic potentials of test substances.

But, the studies over the last two decades are indicated to determine carcinogens that are not mutagenic.

Therefore, genotoxic effects of a test substance should be researched by *in vitro* and *in vivo* test systems such as micronucleus, comet, sister chromatid exchange, chromosomal abnormalities and transgenic rat mutation following bacterial gene mutation analysis.

Based on this literature data, further studies of Cedar tar will carry out in these test systems.

Conclusions

To our knowledge, this is the first study of the antioxidant and mutagenic activities of Cedar tar (Katran) produced from *Cedrus libani* A. Rich. The results of this study indicated that Cedar tar is not source of natural antioxidant and has not any mutagenic activity.

Acknowledgments: This present study was presented at III. International Vocational and Technical Sciences Congress (UMTEB'18), Gaziantep, Turkey and published as abstract. The authors would like to thank Kilis 7 Aralık University for laboratory opportunity and Prof. Dr. Nuran Diril for *S. typhimurium* TA98 and TA100 strains.

Compliance with Ethical Standards

Disclosure of potential Conflict of Interest: The authors have no conflicts of interest to declare. (N)

Funding: This study was not funded by any national/international establishment. (N)

Research involving Human Participants and/or Animals: This article does not contain any studies with human participants or animals performed by any of the authors. (N)

Informed consent: This study does not include any individual participants. (N)

References

1. Arı, S.; Kargıoglu, M.; Temel, M.; Konuk, M. Traditional Tar Production from the AnatolianBlack Pine [*Pinus nigra* Arn. subsp. *Pallasiana* (Lamb.) Holmboe var. pallasiana] and its usages in Afyonkarahisar, Central Western Turkey. *Journal of ethnobiology and ethnomedicine,* **2014**, 10(29), 1–9.
2. Bilgen, B.B.; Kurt, Y.; Kaya, N. Mating system in natural populations of Taurus cedar (*Cedrus libani* A.Rich.). *Turkish journal of agriculture and forestry,* **201**2, 36, 379–387.
3. Boydak, M. Regeneration of Lebanon cedar (*Cedrus libani* A. Rich.) on karstic lands in Turkey. *Forest ecology and management*, **2003**, 178, 231–243.
4. Butnariu M. Methods of analysis (extraction, separation, identification and quantification) of carotenoids fromnatural products. *Journal of Ecosystem & Ecography*, **2016**;6:193.
5. Butnariu, M.; Samfira, I.; Sarac, I.; Negrea, A.; Negrea, P. Allelopathic effects of *Pteridium aquilinum* alcoholic extract on seed germination and seedling growth of *Poa pratensis*, *Allelopathy journal*, **2015**, 35(2), 227–236a.
6. Butu, M.; Butnariu, M.; Rodino, S.; Butu, A. Study of zingiberene from *Lycopersicon esculentum* fruit by mass spectometry, *Digest journal of nanomaterials and biostructures*, **2014**, 9(3), 935–941a.
7. Carneiroa, C.C.; Véras, J.H.; Góesa, B.R.L.; Pérez, C.N.; Chen–Chen, L. Mutagenicity and antimutagenicity of *Salacia crassifolia* (mart. Ex. Schult.) G. Don. evaluated by Ames test. *Brazilian journal of biology,* **201**8, 78(2), 345–350.
8. Caunii, A.; Butu, M.; Rodino, S.; Motoc, M.; Negrea, A.; Samfira, I.; Butnariu, M. Isolation and Separation of Inulin from *Phalaris arundinacea* Roots, *Revista de chimie*, **2015**, 66(4), 472–476.
9. Da Silva Lima, D.C.; do Vale, C.R.; Veras, J.H.; Bernardes, A.; Noda Perez, C.; Chen–Chen, L. Absence of genotoxic effects of the chalcone (*E*)–1–(2–hydroxyphenyl)–3–(4–methylphenyl)–prop–2–en–1–one) and its potentialchemoprevention against DNA damage using *in vitro* and *in vivo* assays. *Plas one,* **2017**, 12(2), 1–15.
10. Ekmekci, N. Investigation of Antimutagenic Effects of Some Local Honey Kinds in Turkey by Salmonella/ Microsome (Ames) Test System. Dissertation. University of Selcuk, Konya, **2010**.
11. Ghanem, S.; Olama, Z. Antimicrobial Potential of Lebanese Cedar Extract against Human Pathogens and Food Spoilage Microorganisms. *European journal of botany plant science and phytology*, **2014**, 1(1), 13–26.
12. Ianculov, I.; Gergen, I.; Palicica, R.; Butnariu, M.; Dumbrava, D.; Gabor, L. The determination of total alkaloids from *Atropa belladona* and *Lupinus sp* using various spectrophotometrical and gravimetrical methods, *Revista de chimie*, **2004**, 55(11), 835–838.
13. Kurt, Y.; Isık, K. Comparison of Tar Produced by Traditional and Laboratory Methods. *Ethno–Medicine,* **2012,** 6(2), 77–83.
14. Kurt, Y.; Kacar, M.S.; Isık, K. Traditional Tar Production from *Cedrus libani* A. Rich on the Taurus Mountains in Southern Turkey. *Economic Botany,* **2008**, 62(4), 615–620.
15. Maron, D.M.; Ames, B.N. Revised methods for the *Salmonella* mutagenicity test. *Mutation Research,* **1983,** 113(3–4), 173–215.
16. Pekgozlu, A.K.; Kustas, S.; Mercan, B.; Bicer, A. Chemical Characterization of Lebonan Cedar tar. *Drvna Industrija,* **2017**, 68(2), 107–112.
17. Petrache, P.; Rodino, S.; Butu, M.; Pribac, G.; Pentea, M.; Butnariu, M. Polyacetylene and carotenes from *Petroselinum sativum* root, *Digest journal of nanomaterials and biostructures*, **2014**, 9(4), 1523–1527.
18. Rashed, K.; Butnariu, M. Isolation and antimicrobial and antioxidant evaluation of bio-active compounds from *Eriobotrya japonica* stems. Advanced pharmaceutical bulletin, **2014**, 4(1), 75–81.
19. Samfira, I.; Butnariu, M.; Rodino, S.; Butu, M. Structural investigation of mistletoe plants from various hosts exhibiting

diverse lignin phenotypes, Digest Journal of Nanomaterials and Biostructures, **2014**, 8(4), 1679–1686.
20. Samfira, I.; Rodino, S.; Petrache, P.; Cristina, R.T.; Butu, M.; Butnariu, M. Characterization and identity confirmation of essential oils by mid infrared absorption spectrophotometry. *Digest journal of nanomaterials and biostructures*, **2015**, 10(2), 557–566.
21. Sharm, S.; Vig, P.A. (Evaluation of *in vitro* antioxidant properties of methanol and aqueous extracts of *Parkinsonia aculeata* L. leaves. *The scientific world journal* **2013,** 2013, 1–7.
22. Stankovic, M.S. Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac Journal of Science*, **2011,** 33, 63–72.
23. Ucan Turkmen, F.; Mercimek Takci, H.A. Ultraviolet–C and ultraviolet–B lights effect on black carrot (*Daucus carota* ssp. sativus) juice. *J Food Meas Charact* **2018,** 12, 1038–1046.
24. Zahin, M.; Ahmad, I.; Gupta, R.C.; Aqil, F. Punicalagin and Ellagic Acid Demonstrate Antimutagenic Activity and Inhibition of Benzo[a]pyrene Induced DNA Adducts. *BioMed Research International***, 2014**, 2014, 1–10.

Received: January 31, 2019
Article in Press: November 04, 2019
Accepted: Last modified on: November 20, 2019