Advances in Bioresearch
Adv. Biores., Vol 6 (5) September 2015: 78-82
©2015 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV 7.20 [Poland]

Advances in Bioresearch

ORIGINAL ARTICLE

Effect of Essential Oil of Dill (*Anethum graveolens* L.) on Aflatoxin-Induced Liver Damage in Male Rats

Omid Hajikandi [1], Maryam Eidi [2,*], Fatemeh Noorbakhsh [1]
[1]Department of Microbiology, College of Biological Sciences, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
[2]Department of Biology, College of Biological Sciences, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
***Corresponding author's** e-mail: eidi@iauvaramin.ac.ir, maryameidi@gmail.com

ABSTRACT
Different parts of the Anethum graveolens L. (dill) (Umbeliferae) are used to treat liver disorders, traditionally. It is one among the constituents in various folk medicines used for the treatment of liver disorders and other diseases. The aim of the present study is to evaluate the hepatoprotective effect of dill against experimentally induced liver injury. The essential oil of dill was evaluated for the hepatoprotective activity against Aspergillus parasiticus aflatoxin induced hepatotoxicity in rats. Various biochemical parameters like serum alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (AP) and total protein (TP) levels were determined. The treatment of aflatoxin at dose 450 microg/kg interperitoneally increased serum ALT, AST and AP levels, while decreased total protein levels in poisoned rats in comparison to control normal rats. Treatment of essential oil of dill at doses 25, 50, 100 and 200 mg/kg mg/kg body weight decreased the raise of serum AST, ALT and AP levels and increased serum total protein level in treated rats in comparison to control rats. Also, histophatological study showed that treatment of essential oil of dill attenuate liver damage in poisoned rats in compared control group. This study demonstrates the hepatoprotective activity of Anethum graveolens and thus scientifically supports the usage of this plant in traditional medicine for treatment of liver disorders.
Keywords: *aflatoxin, Anethum graveolens, Aspergillus parasiticus, dill, hepatotoxicity, hepatic enzymes, histology, rat.*

Received 22.05.2015 Accepted 03.09.2015

How to cite this article:
Omid H, Maryam E, Fatemeh N. Effect of Essential Oil of Dill (Anethum graveolens L.) on Aflatoxin-Induced Liver Damage in Male Rats. Adv. Biores., Vol 6 [5] September 2015:78-82**.** DOI: 10.15515/abr.0976-4585.6.5.7882

INTRODUCTION

Aflatoxins are biologically active secondary metabolites produced by certain strains of *Aspergillus parasiticus, Aspergillus nominus* and *Aspergillus flavus* [1]. The aflatoxin producing fungi are widely distributed in nature and can grow over a wide range of environmental conditions [2]. Aflatoxins have been detected in cereal grains, oil seeds, fermented beverages made from grains, milk, cheese, meat, nut products, fruit juice and numerous other agricultural commodities [3]. Aflatoxins have been shown to be hepatotoxic, carcinogenic, mutagenic and teratogenic to different species of animals [4-6]. Aflatoxin B1 (AFB1) is the most prevalent and carcinogenic of the aflatoxins and the International Agency for Research on Cancer (IARC) classify AFB1 as a group I carcinogen (that is, an agent that is carcinogenic to humans). Epidemiological studies also indicate that areas in the world with high levels of aflatoxin are correlated with high incidence of liver cancer [7].

AFB1 caused damage by two different ways in the cells. Firstly, AFB1 ($C_{17}H_{12}O_6$) is activated to AFB1-8,9-oxide and forms adduct primarily at N7 position of guanine and is responsible for its mutagenic and carcinogenic effects [8,9]. Secondly, aflatoxins especially AFB1, produce reactive oxygen species (ROS) such as superoxide radical anion, hydrogen peroxide and lipid hydroperoxides; though these do not appear to interact with DNA, but they are precursors to the hydroxyl radical. The hydroxyl radicals interact with DNA and produces mutations [10]. Numerous diverse compounds and extracts containing activity inhibitory to aflatoxin biosynthesis have been reported. The most of these inhibitors are plant-

derived such as phenylpropanoids, terpenoids and alkaloids [11]. A group of plant-derived inhibitors is essential oils that possess antifungal activities against *A. parasiticus* and/or *A. flavus* [12,13]. Up to date, no review directly has been carried out to evaluate the protective effects of essential oils against the aflatoxins.

There is no report about hepatoprotective effect of dill essential oil against *Aspergillus parasiticus* aflatoxin-induced liver damage in male rats. So, in the present study, we evaluated the protective effect of essential oil of *Anethum graveolens* against aflatoxin induced hepatotoxicity in rats.

MATERIALS AND METHODS

Dill leaves (*Anethum graveolens* L.) were purchased from Varamin in June 2014, identified by department of botany of Islamic Azad University (Voucher number: 037420, Director: Dr. Ali Mazooji). The plant was cleaned, shed dried at 25°C, and the dried leaves of the plant were ground with a blender, and the powder was kept in nylon bags in a deep freezer until the time of experiments. Dried and ground leaves (about 500 g) were submitted to steam distillation in a clevenger-type apparatus with 1000 ml of water for 2 h to obtain the essential oil.

In this study, male Wistar rats weighing 200–250 g were housed in clean cages with temperature (22–24 °C), 12-h light/12-h dark cycle and relative air humidity 40–60%. Rats had continuous access to food and to tap water. Permission for the study was obtained from the Pastour institute, Tehran, IRAN.

In the present experiment, 48 rats (40 poisoned, 8 intact rats) were used. The rats were divided into six groups. Group 1: Normal control rats (intact) were administrated 0.5 ml of saline as aflatoxin vehicle every week, interperitoneally. Group 2: Control rats were co-administrated 0.5 ml of aflatoxin at dose 450 microg/kg every week, interperitoneally and 0.5 ml sunflower oil as essential oil vehicle, orally for 4 weeks, daily. Groups 3–6: rats were co-administrated 0.5 ml of aflatoxin every week, interperitoneally and essential oil of dill at doses 0.0125, 0.025, 0.05 and 0.1 ml/kg body wt. daily for 4 weeks, orally.

After 4 weeks of treatment, the animals were anesthetized by ether and blood samples were drawn from heart. Serum total protein, aspartate aminotransferase (AST), alanine amino transferase (ALT) alkaline phosphatse (AP) and total protein (TP) levels were determined by kit (Parsazmoon, Iran).

For qualitative analysis of liver histology, the tissue samples were fixed for 48 h in 10% formalin-saline and dehydrated by passing successfully in different mixtures of ethyl alcohol–water, cleaned in xylene and embedded in paraffin. Sections of the tissue were prepared by using a rotary microtome and stained with haematoxylin and eosin dye, which was mounted in a neutral deparaffinated xylene medium for microscopic observations. Histological damage including fatty change in hepatocyte, dilation of sinusoid and congestion in high lipid diet fed. Each damage is given 1 score.

All the data were expressed as mean ± S.E.M. of eight rats (n = 8). Statistical analysis was carried out using one-way ANOVA followed by Tukey post hoc test. The criterion for statistical significance was $p<0.05$.

RESULTS AND DISCUSSION

The results showed significant elevations in serum ALT ($p<0.001$), AST ($p<0.001$) and AP ($p<0.001$) levels in poisoned rats, while significant attenuation in serum TP level in the poisoned control rats in comparison with control normal rats ($p<0.001$). The present results showed that treatment of essential oil of dill decreased serum ALT ($p<0.001$), AST ($p<0.001$) and AP ($p<0.001$), while increased serum TP level ($p<0.001$) in treated poisoned rats in compared to control rats ($p<0.001$) (Table 1).

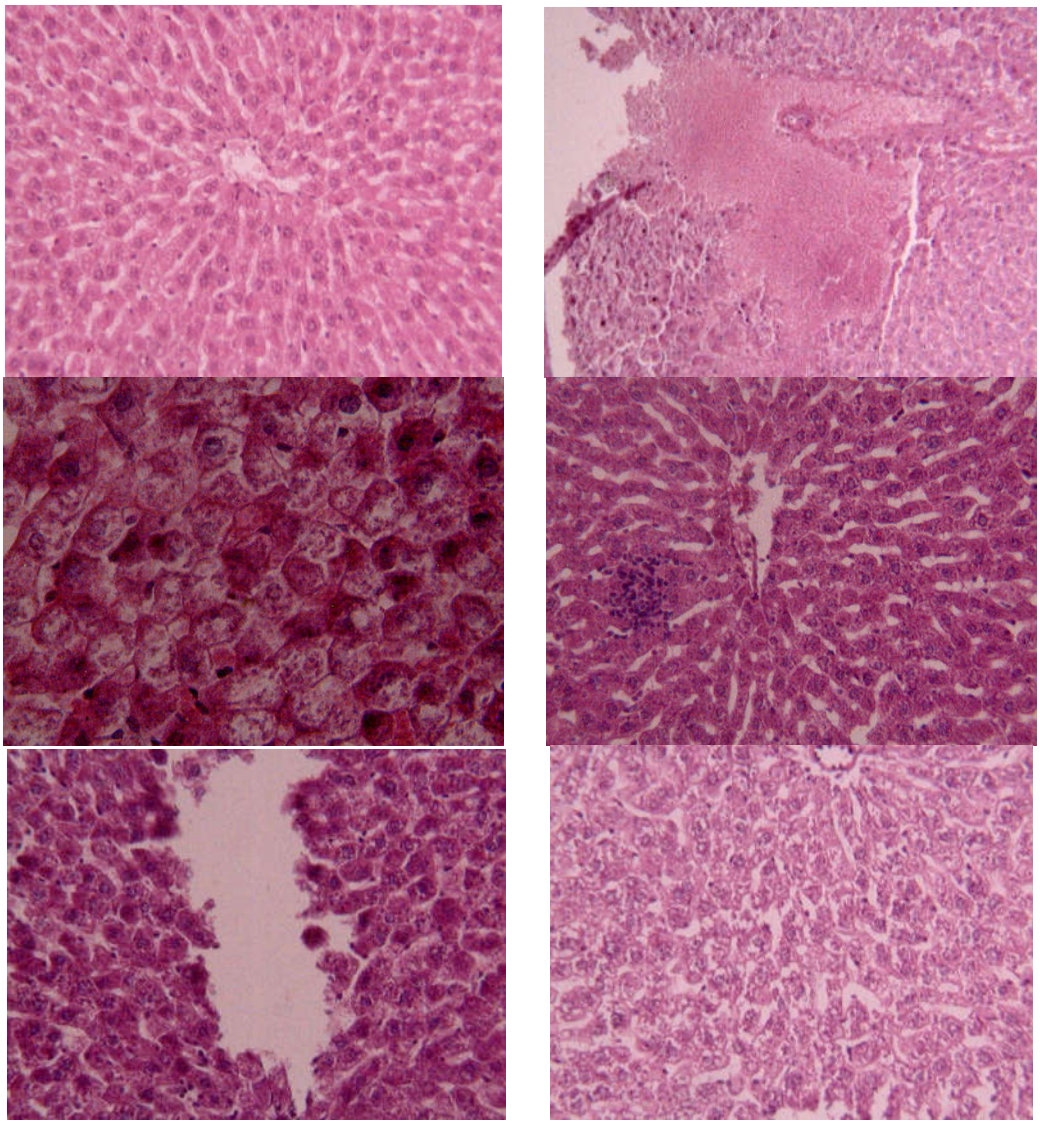
Histopathological study showed that the administration of the essential oil of dill (0.0125, 0.025, 0.05 and 0.1 ml/kg body wt.) significantly decreased histopathological damages of liver including fatty change in hepatocyte, dilation of sinusoid, picnotic nucleus of hepatocytes, necrosis of tissue, cell detachment from tissue and congestion in liver tissue (Picture 1) in treated poisoned rats with aflatoxin in comparison to control poisoned rats (Table 1). Each damage was given 1 score and whole scores of each sample is shown damage level in liver.

Table 1. Effect of dill essential oil on hepatic enzyme levels in serum and liver tissue damages in poisoned rats with aflatoxin.

Serum parameters	Intact	Control	Essential oil (ml/kg)			
			0.0125	0.025	0.05	0.1
ALT	79.33 ±6.1	110.43±4.9 ***	102.7±4 *** +	99±4 *** +++	89.57±4 ** +++	86.67±2.3 ns +++

AST	160.33±6.2	198±4 ***	193.43±4.6 ***	187.29±5.3 *** ++	172.14±3.4 *** +++	169±2.8 * +++
AP	190.67±7.2	240.57±4.1 ***	233.14±3.3 *** ns	229.29±4.68 *** ++	218.71±6.6 *** +++	209.67±7.1 *** +++
TP	7.805±0.19	6.28±0.1 ***	6.617±0.09 *** +	6.897±0.36 *** +++	7.21±0.18 *** +++	7.43±0.7 * +++
Score of damage	0	3.83±0.3 ***	3.17±0.4 *** ns	2.33±0.4 *** +	1.33±0.3 *** +++	0.83±0.3 *** +++

Ns=not significant
*p<0.05, **p<0.01, ***p<0.001 different from intact rats.
+p<0.05, ++p<0.01, +++p<0.001 different from control poisoned rats.



Picture 1- Histopathology of liver tissue in intact and poisoned rats (hematoxylin-eosin). (top-left) Normal liver tissue (×100), (top-right) Congestion in liver tissue (×100), (middle-left) Fatty change in hepatocyte (×400), (middle-right) Dilation of sinusoid (×100), (down-left) Cell detachment from tissue, (down-right) Picnotic nucleus of hepatocytes.

Mechanism underlying the hepatotoxicity of aflatoxins is not fully understood. Several reports suggest that toxicity may ensue through the generation of intracellular ROS during the metabolic processing of

AFB1 by cytochrome P450 in the liver [14]. Free radicals provoked by various environmental chemicals as well as endogenous metabolism are involved in a number of diseases like tumors, inflammation, shock, atherosclerosis, diabetes, infertility, gastric mucosal injury and ischemia due to the oxidative damage to DNA, lipids and proteins and which can result in failure of cellular functions [15]. Free radicals also contribute to G. C + T. A transversions by the production of 8OHGua in liver DNA, therapies designed to reduce damage by oxygen free radicals [16] during chronic hepatitis would be predicted to delay the onset of primary liver cancer [17].

To control the level of ROS and to protect cells under stress conditions, living tissues contain several enzymes and many antioxidant substances. The effect of ROS is balanced by the antioxidant action of non-enzymatic antioxidants, as well as by antioxidant enzymes. Such antioxidant defences are extremely important as they represent the direct removal of free radicals (pro-oxidants), thus, providing maximal protection for biological sites [18]. A lot of antioxidant compounds such as essential oil, phenolics compounds and secondary metabolites, which are synthesized by plants, serve in defence against ROS. The antioxidant properties of essential oil of plant origin have been studied in recent years. A strong correlation has been found between the essential oil level and the antioxidant activity potential. Some essential oils and other extracts (vitamins, riboflavin, carotenoids, beta-carotene, alfa-carotene, lycopene, ascorbic acid, curcumin, several flavonoids, phenolic compouds and synthetic phenolic compounds) of plants could potentially provide protection against aflatoxins especially AFB1 [12,13,19]. In addition, many essential oils could reduce toxic and mutagenic effect of aflatoxins. Most studies indicated that anti-aflatoxigenic properties may be due to inhibition of penetration of *A. flavus* and *A. parasiticus* [12,13].

The essential oils can decrease the damaged effect of aflatoxins by two different ways. Firstly, DNA binding formation of aflatoxins is reduced by essential oils. Secondly, aflatoxins cause increase of reactive oxygen species and essential oils react with ROS. Therefore, essential oils protect the cells from harmful impact of aflatoxins. Recently, the natural products such as plant extracts have been identified as potential candidates against AFB1. A few study show that essential oils reduce DNA binding of aflatoxin. Essential oils from common spices such as nutmeg, ginger, cardamom, celery, xanthoxylum, black pepper, cumin and coriander were tested for their ability to suppress the formation of DNA adducts by AFB1 *in vitro* in a microsomal enzyme-mediated reaction. All oils were found to inhibit adduct formation very significantly and in a dose-dependent manner. The adduct formation appeared to be modulated through the action on microsomal enzymes, because an effective inhibition on the formation of activated metabolite was observed with each oil. The enzymatic modulation is perhaps due to the chemical constituents of the oils and this could form a basis for their potential anticarcinogenic roles [20]. In another research, it is shown garlic oil significantly decreased AFB1-induced DNA damage in cultured primary rat hepatocytes [21].

Therefore, the study scientifically supports the usage of this plant in various traditional medicines for treatment of liver disorders. The present study suggests that the essential oil of dill is a potential source of natural detoxificant agents. After this screening experiment, further work should be performed to isolate the active constituents and evaluate their activities against aflatoxins.

ACKNOWLEDGEMENTS

This work was MSc thesis and supported by the Deputy Research, Varamin-Pishva Branch, Islamic Azad University.

REFERENCES

1. Cotty, P.J., Bayman, P., Egel, D.S., Elias, D. (1994). Agriculture, aflatoxins, and *Aspergillus*. In The Genus *Aspergillus* Edited by Powell K A Fenwick A and Peberdy J F New York: Plenum Press, pp. 1-27.
2. Holmquist, G.U., Walker, H.H., Stahr, H.M. (1983). Influence of temperature, pH, water activity and antifungal agents on growth of *Aspergillus flavus* and *A. Parasiticus*. J Food Sci, 48: 778-782.
3. Bullerman, L.B. (1986). Mycotoxins and food safety. Food Technol, 40: 59-66.
4. Abdel-Wahhab, M.A., Nada, S.A., Amra, H.A. (1999). Effect of aluminosilicate and bentonite on aflatoxin-induced developmental toxicity in rats. J Appl Toxicol, 19: 199-204.
5. Eaton, D.L., Gallagher, E.P. (1994). Mechanisms of aflatoxin carcinogenesis. Annu Rev Pharmacol Toxicol, 34: 135-172.
6. Wogan, G.N., Paglialunga, S., Newberne, P.N. (1974). Carcinogenic effects of low dietary levels of aflatoxin B1 in rats. Food Cosmet Toxicol, 12: 681-685.
7. IARC. (1985). Aflatoxin, IARC Monographs (Suppl. 7), pp. 83-87.
8. Denissenko, M.F., Cahill, J., Kondriakova, T.B., Gerber, N., Pfeifer, G.P. (1999). Quantitation and mapping of aflatoxin B1-induced DNA damage in genomic DNA using aflatoxin B1-8, 9-epoxide and microsomal activation systems. Mutat Res, 425: 205-211.

9. Wang, J.S., Groopman, J.D. (1999). DNA damage by mycotoxins. Mutat Res, 424: 167-181.
10. Halliwell, B., Gutteridge, J.M.C. (1999). Oxidative stress: adaptation damage, repair and death in: Halliwell B Gutteridge J M C (Eds.) Free radicals in biology and medicine Oxford University Press, New York, pp. 246-350.
11. Holmes, R.A., Boston, R.S., Payne, G.A. (). Diverse inhibitors of aflatoxin biosynthesis. Appl Microbiol Biotechnol, 78: 559-572.
12. Rasoli, I., Fakoor, M.H., Yadegarinia, D., Gachkar, L., Allameh, A., Rezaei, M.B. (2008). Antimycotoxigenic characteristics of *Rosmarinus officinalis* and *Trachyspermum copticum* L. essential oils. Int J Food Microbiol, 122: 135-139.
13. Bluma, R.V., Etcheverry, M.G. (2008). Application of essential oils in maize grain: Impact on *Aspergillus* section Flavi growth parameters and aflatoxin accumulation. Food Microbiol, 25: 324-334.
14. Towner, R.A., Qian, S.Y., Kadiiska, M.B., Mason, R.P. (2003). *In vivo* identification of aflatoxin-induced free radicals in rat bile. Free Radic Biol Med, 35: 1330-1340.
15. Kasai, H. (2002). Chemistry-based studies on oxidative DNA damage: formation, repair, and mutagenesis Free Radical. Biol Med, 33: 450-456.
16. Ames, B.N. (1983). Dietary carcinogens and anticarcinogens - Oxygen radicals and degenerative diseases. Science, 221: 1256-1264.
17. Cheng, K.C., Cahill, D.S., Kasai, H., Nishimura, S., Loeb, L.A. (1992). 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G/T and A/C substitutions. J Biol Chem, 267: 166-172.
18. Valko, M., Rhodes, C.J., Moncola, J., Izakovic, M., Mazura, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico Biol Interact, 160: 1-40.
19. Alpsoy, L., Yldırım, A., Agar, G. (2009). The antioxidant effects of vitamin A, C and E on aflatoxin B1-induced oxidative stress in human lymphocytes. Toxicol Ind Health, 25: 121-127.
20. Hashim, S., Aboobaker, V.S., Madhubala, R., Bhattacharya, R.K., Rao, A.R. (1994). Modulatory effects of essential oils from spices on the formation of DNA adducts by aflatoxin B1 *in vitro.* Nutr Cancer, 21: 169-175.
21. Shenn, L.Y., Wu, C.C., Lii, C.K., Tsai, S.J. (2001). Effect of diallyl sulfide and diallyl disulfide, the active principles of garlic, on the aflatoxin B1-induced DNA damage in primary rat hepatocytes. Toxicol Lett, 122: 45-52.