

Original Article

Hepatotoxicity of *Dorema Aucheri* (Bilhar) in Albino Mice

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Abstract

Background: The geographic map of cancer prevalence differs due to environmental and dietary factors in various populations. High prevalence of a number of cancers in some regions is thought to be attributed to local dietary habits. *Dorema aucheri* (Bilhar) is used commonly as an herbal medicine in some regions including Iran. The aim of this study was to evaluate whether *Dorema aucheri* has carcinogenic effects in albino mice or not.

Methods: The *Dorema aucheri* leaves were extracted by Soxhlet method and were injected intraperitoneally and randomly into 28 healthy albino mice which were divided into seven groups. One was put aside as the non-injected control group. The second control group was chosen to be injected by a known carcinogen. Another group was injected by carcinogen and then, Bilhar extract. The left four groups were injected the extracts in a dose-dependent manner, increasingly in the range of 0.4 – 3.2mL/kg. Extract injections were repeated every 48-hour intervals for three times. Then, liver and serum samples were analyzed biochemically and pathologically.

Results: The pathologic and biochemical studies showed that the injection of plant extracts caused necrosis, inflammation of the liver tissue, cell proliferation, cholestasis, and there were significant increases in release of liver enzymes [ALP, ALT (SGPT) and AST (SGOT)] and bilirubin compared to the non-injected control group. The level of liver damage was dose dependent.

Conclusions: *Dorema aucheri* has potential hepatotoxic capacities and possibly this may be related to the high prevalence of cancer in some regions of Iran.

Keywords: Albino mice, *Dorema aucheri*, hepatotoxic, neoplasms

Cite this article as: Mostafavi SH, Fazilati M, Mostafavi SA, Vahhabi MR, Mostafavi F, Omidvarinia Sh, et al. Hepatotoxicity of *Dorema aucheri* (Bilhar) in albino mice. *Arch Iran Med*. 2013; **16(9)**: 530 – 532.

Introduction

Cancer is a major cause of mortality worldwide,¹ but the geographic map of its prevalence and the body organs which more frequently affected by neoplasm is widely different in various countries and ethnic groups.^{1,2} Some of these diversities may be attributed to different environmental factors and various food and dietary habits among different populations.³ According to Armstrong and Doll, dietary factors may be highly correlated with several types of cancers. They declared that incidence rates for 27 cancers in 23 countries, and mortality rates for 14 cancers in 32 countries correlated with a variety of dietary variables.⁴ Food components either could have a positive (carcinogenic) or negative (preventive) effects.³ The macro- components of foods play an indirect role but the micro- components have a clearly defined action.³ For example, consumption of high-caloric and fat-rich foods appears to have a strong positive influence on

cancer incidence associated with increases in breast, colon, and prostate cancers.⁵ Conversely vegetables rich in antioxidants and fibers tend to reduce cancer incidence.⁶ Carcinogenic plant agents like alkaloids, mycotoxins, genotoxins, aromatic hydrocarbons, and heterocyclic amines (HCAs) generated by heat-cooking via combustion and through reactions involving certain, sugar, and amino acids in meat and other dietary contaminants frequently enter our body by eating different foods. Some epidemiologic investigations positively correlate with HCA intake and cancer incidence.³

The incidence of a number of cancers (like esophageal, stomach, colon, liver, and prostate) which are related to the individual and community lifestyle is highly associated with dietary factors and varies by the local dietary habits in different parts of society.⁷

There are great concerns about increasing cancer incidence worldwide. In Iran, it is estimated that cancer is the third main cause of mortality and its incidence increasing as time goes by.⁸ Recently, Iran's Health Minister warned people and government that according to the available data there will be a 10-fold increase in the rate of cancer in Iran in about 10 years. Accordingly, there are lots of investigations to determine different aspects of domestic causes of cancer in Iran.

In some parts of Iran, various records show that the percentage of some kinds of cancer is much higher than the average and many believe that this should be caused by local dietary habits.^{7,9}

Dorema aucheri (Bilhar) is consumed regularly in a great amount by the people of central regions of Iran and although it is used as an herbal medicine to decrease the blood triglycerides and to control diabetes, it is also used as a pain killer. However, there is no reliable research about its advantages or its possible side effects.

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Accepted for publication: 10 July 2013

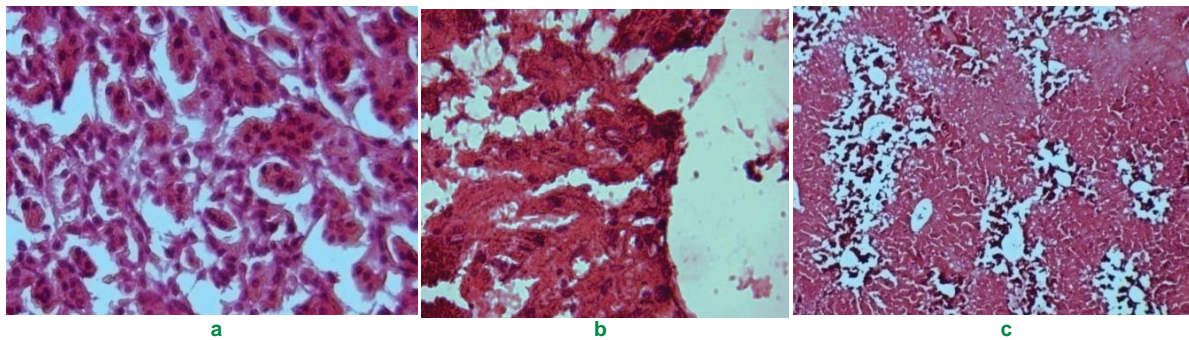


Figure 1. The pathology results of liver species, stained by using hematoxylin-eosin dye, and observations under microscope; **a)** Normal control liver (non-injected), hepatocytes are seen with normochrome round nucleus and eosinophilic cytoplasm which are normal; **b)** Control, induced liver cancer (tio-injected), proliferation of neoplastic cells is seen; **c)** Liver tissue treated with *Dorema auseri* (Bilhar) extract (3.2 mL/kg), necrosis and degeneration of some hepatocytes are seen with little centers of inflammation.

Table 1. The Biochemistry test results for liver homogenate samples

Parameters	SGPT (IU/L)	SGOT (IU/L)	Indirect bil (mg/dL)	Direct bil (mg/dL)	Totalbil (mg/dL)	ALP (IU/L)
Control1 (non-injected)	86.43 ± 0.23	37.81 ± 0.34	0.89 ± 0.32	0.29 ± 0.22	1.18 ± 0.27	181.43 ± 0.61
Control2 (tio-injected)	181.22 ± 0.42*	95.53 ± 0.21*	0.96 ± 0.10	0.35 ± 0.36	1.31 ± 0.32	282.4 ± 0.43*
Tio+extract (3.2 mL/kg)	355.45 ± 0.73*	155.55 ± 0.34*	1.41 ± 0.29	0.60 ± 0.34	2.01 ± 0.21	309 ± 0.54*
Extract (0.4 mL/kg)	179.89 ± 0.14*	93.60 ± 0.85*	0.94 ± 0.18	0.31 ± 0.55	1.25 ± 0.34	189.09 ± 0.52
Extract (0.8 mL/kg)	204.54 ± 0.84*	102.67 ± 0.86*	0.99 ± 0.46	0.38 ± 0.37	1.37 ± 0.39	195 ± 0.71
Extract (1.6 mL/kg)	230.43 ± 1.3*	115.89 ± 0.39*	1.2 ± 0.15	0.42 ± 0.49	1.62 ± 0.92	212 ± 0.81
Extract (3.2 mL/kg)	263.5 ± 1.8*	131.51 ± 0.2*	1.34 ± 0.24	0.52 ± 0.88	1.86 ± 0.85	295.44 ± 0.99*

Each value represents mean of four data obtained from mice in each trial group ± SEM; *: significant increments compared to non-injected control at P ≤ 0.05 level.

Table 2. The Biochemistry test results for serum samples

Parameters	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Control 1 (non-injected)	88.12 ± 0.04	43.55 ± 0.07	115.44 ± 0.24
Control 2 (tio-injected)	177.23 ± 0.43*	86.40 ± 0.19*	206.55 ± 0.11*
Tio+ extract (3.2 mL/kg)	197.24 ± 0.14*	121.33 ± 0.15*	213.44 ± 0.23*
Extract (0.4 mL/kg)	95.27 ± 0.11	62.56 ± 0.21	140.51 ± 0.63
Extract (0.8 mL/kg)	143.65 ± 0.2	74.89 ± 0.44	154.67 ± 0.21
Extract (1.6 mL/kg)	152.91 ± 0.18	82.12 ± 0.51	182.68 ± 0.31
Extract (3.2 mL/kg)	165.56 ± 0.38*	107.33 ± 0.33*	194.56 ± 0.46

Each value represents mean of four data obtained from mice in each trial group ± SEM; *: significant increments compared to non-injected control at P ≤ 0.05 level.

In this study, an attempt was made to determine whether *Dorema auseri* is hepatotoxic and/or carcinogenic in albino mice.

Materials and Methods

Startup procedure

The green leaves of *Dorema auseri* were collected from the mountains 50 miles off the Semirrom town in the first of May. The species were verified by the experts of Natural Resources College in Isfahan University of Technology. The leaves were washed, dried in shadow, and then powdered to fine particles of 1mm mesh size.

Extraction procedure

The extraction of plant carried out by Soxhlet method. The powder of plant materials (50 g) was extracted by 200 mL of 95 % ethanol for 24 hours by Soxhlet method. In all of the extraction time, the temperature did not reach the boiling point of the solvent. Following the filtration of the extract by Whatman No.1 filter paper, the extract was concentrated by a rotary evaporator at 40° C.¹⁰ Extraction percentage was estimated to be about 100 % and each ml of extract was equal to 1 mg of the plant.

Injections

A total of 28 healthy albino mice (mean weight ± SD: 25 ± 1 g) was selected and each four of them was randomly placed in separate cages, and then were allowed to live collectively about one week before the study. It helped them to be accustomed with each other

and surroundings. The light, temperature, and humidity were well controlled. All study conditions were even for each and every group.

After a week and before any injections, all mice were completely normal. One group of mice was put aside as the control group and left without any injections. The second control group was chosen to be injected intraperitoneally (IP) by a proven carcinogenic substance (50 µL of 0.013 molar tioacetamide, once at the beginning).¹¹ Another group was randomly selected to be injected IP by 50 µL tioacetamide (0.013 molar) once at the beginning and after 36 hours. Bilhar extract (3.2 mL/kg) was injected three times every 48 hours to test whether this extract is protective against carcinogenic effects of tioacetamide or not. The left four groups were injected IP in a dose- dependent manner, increasingly in the order of 0.4, 0.8, 1.6, and 3.2 mL/kg. All injection procedures of Bilhar extracts were repeated in every 48- hour intervals for three times.

End process

After all injections were done, a 2mL blood sample was collected from each mouse and then centrifuged at 5000 rpm for 15 minutes.¹² The serum was separated and then kept under -50 °C for further analysis. Then animals were killed by spine cutting on their neck, dissected, and half of their livers were homogenized in TRIS buffer (20 % w/v) on pH equal to 7.2. The homogenates were centrifuged at 5000 rpm for 15 minutes, and then were sent to the biochemistry laboratory for estimation of serum glutamate pyruvate transaminase (SGPT/ALT), serum glutamate oxaloacetate transaminase (SGOT/AST), alkaline phosphatase (ALP),

and direct and indirect bilirubin tests. The other part of livers was labeled and sent to the pathology laboratory, stained by using hematoxylin-eosin dye, and examined under microscope.

Statistics

The statistical analysis was done by using IBM® SPSS Statistics v. 17. The intergroup variations were measured by one way analysis of variance (ANOVA) with Bonfironi post-hoc analysis. The results were articulated as mean of measurements obtained from four mice in each trial group \pm SEM.

Ethics

The study was approved by the Ethics Committee of Isfahan University of Technology. Adverse effects on the animals were minimized as far as possible. The highest standards of animal welfare were upheld.

Results

The serum and liver homogenate biochemical results showed that ascendant-dose injections of Dorema aucheri extracts caused a highly increased bilirubin levels and there were significant increments in ALP, SGPT, and SGOT compared to the non-injected control group (Tables 1 and 2).

The pathologic study of liver homogenates showed that in the non-injected control group the livers were completely normal and no inflammation, cholestasis, or fibrosis was found in this category. While, the injection of plant extract caused necrosis, inflammation of the liver tissue, cell proliferation, cholestasis, and a great release of liver enzymes (Figure 1). The level of liver damage was dose dependent.

Discussion

The liver plays a central role in whole body metabolism of carbohydrates, proteins, and fats. Some key end products and enzymes of the metabolic pathways which are so sensitive to abnormalities, such as bilirubin, SGOT, SGPT, and ALP can clinically be used as biomarkers of liver impaired functioning.¹³⁻¹⁵

We injected the carcinogenic tioacetamide and then Dorema aucheri extract to look whether it is protective against hepatic toxicity or not. Surprisingly, the biochemical results showed a dramatic increment in SGPT, SGOT, ALP, and bilirubin levels compared to the non-injected control and tioacetamide injected control groups. Furthermore, progressive elevation of ALP, SGOT, and SGPT in liver and then in serum^{15,16} by a gradually increasing dose of Dorema aucheri extract shows its potential toxicity especially in relation to liver cells.

Considering that tioacetamide is among potential carcinogenic substances,¹¹ it can be inferred from the results that the lowest dose of extract injection (0.4mL/kg) has the toxicity similar to tioacetamide.

Pathologic studies showed that herbal injection of Dorema aucheri leads to necrosis, hepatic inflammation, proliferation, and cholestasis. In other words, IP injection of extract changes cellular membrane permeability and leads to release of liver enzymes. As a result of hepatocellular membrane dysfunction, some hepatic enzymes leak into the blood stream and are reflected in serum biochemical tests.^{17,18}

Drug substance or any other solution, quickly absorbs into the blood flow and especially the liver and efficiently affects liver

metabolism when they are injected IP.¹⁹ While IP injection is used as an investigatory method to study the biologic effects, but in normal physiologic situations digestion and absorption processes of food passing through gastrointestinal tract will decrease the bioavailability and hence modifies the effects. For this reason, it is not so far-fetched that in the real feeding conditions the effects of Dorema aucheri plant maybe less than trial circumstances but still the risk of habitual consumption of this plant in relation to liver damage and possibly liver malignancy is strongly warning.

Acknowledgments

The authors are thankful for the financial aids of Isfahan University of Technology and Payam Noor University.

Conflict of interest: *The authors declare that they have no conflict of interest.*

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