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Hepatotoxicity effect of some Iranian medicinal herbs formulation in rats

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Abstract: The public conviction that "herbal remedies are safe" has led to increased consumption of these products. This study was performed in view of the wide distribution of herbal remedies, risks posed by self-treatment with these products, and existing reports about the toxic effects of some medicinal herbs. The effect of some most used herbal drops of A, B, C, and D on liver function of rat was examined at different doses, namely minimum dose, maximum dose, and 2.5 times the maximum dose indicated in the brochures. The animals were administered the said doses via feeding tube for 50 days. Liver function parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total serum protein, albumin and urea were measured using the spectrophotometric method. The animals' liver tissues were examined pathologically. A drop did not change liver function parameters significantly. B drop increased LDH by 34% compared to controls at maximum administered dose. C and D drops increased ALT, AST and LDH significantly compared to controls. Histological findings suggest the possible effect of C and D drops on the function of hepatocytes. We recommend that herbal formulations available in pharmaceutical markets be more closely controlled in terms of quality, as well as toxicity, especially as regards possible effects on the hepatic function.
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Introduction

Liver injury induced by drugs or chemicals, also known as toxic hepatopathy, is a major clinical challenge. It has been demonstrated by epidemiological studies that the prevalence of drug/chemicals-induced hepatopathy has increased over the past decades, especially in developing countries (1-3).

Based on World Health Organization estimates, 80% of world inhabitants use medicinal herbs to benefit from their primary therapeutic properties. Widespread dissatisfaction with high prices of chemical drugs towards the end of the 20th century has been associated with recourse to natural remedies, especially medicinal herbs. German citizens have access to nearly 600 of 700 herbal medicines, which are prescribed by 70% of German physicians (4-7).

Herbal products have been generally portrayed as "natural" and "safe" remedies; however, consumers do not seem fully aware of the potential side effects. The side effects reported following the use of herbal treatments has increased on a par with the rising demand for herbal medicines. Hepatitis and hepatic veno-occlusive disease are two perilous side effects of some herbal medicines (8, 9).

The use of Chaparral, an antioxidant used to slow aging, treat common cold and some skin lesions, and Germander, an herb used to treat obesity, can lead to acute hepatitis (8). In this study four hydro alcoholic based herbal medicines (A, B, C, D) which are prescribed as over- the counter drugs in Iran are studied for there hepatotxicoty damage.

Cuminum cyminum and *Tribulus terrestris* are among medicinal herbs used as ingredients of some herbal products such as A, B and C,D; there have been reports of hepatic damage resulting from administration of these products in animals (10-13). This study was conducted to investigate the possible

hepatotoxic effect of a number of herbal products available in Iran's pharmaceutical market.

Materials and methods:

Animals and treatments:

This is a controlled empirical study using male Wistar rats. The rats were supplied from Tehran Pasteur Institute. All animals were kept under the same laboratory conditions of temperature (25±2 ◦C) and lighting (12:12 h light:dark cycle) and were given free access to standard laboratory chow and tap water. All rats were allowed to acclimatize for 1 week prior to experimentation. All experimental procedures involving animals were approved by the Animal Research Ethics Committee of Isfahan Cardiovascular Research Center, Isfahan, Iran.

The animals were randomly divided into 13 groups, 5 rats in each. Each herbal drop was tested on three groups of five rats. Based on information printed in the products' brochures, minimum and maximum doses, as well as 2.5 times the maximum dose of each product were administered to rats orally, using feeding tubes. The administered doses were as follows (all doses in ml/kg.b.w):

- A which is made from Cucurbita pepo, Urtica dioica, Matricaria chamomilla, Tribulus terrostris, Pimpinella anisum: 4.5, 6 and 15
- B which is made from Foeniculum Vulgare, Cuminum cyminum, Trigonella foenum-gracum, Anethum graveolens: 3, 4.5 and 11.5
- C which is made from Foeuiculum vulgare, Cuminum cyminum, Laurus nobilis, Cerasus avium, Zea mays, Tribulus terrestris, Cucumis melo: 7.5, 9 and 22.5
- D which is made from Hypericum perforatum: 2, 4.5 and 11.5

The herbal drops were hydroalcoholic-based; hence the control group was administered only with the alcohol (70%).

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Sample collection and hepatic function tests:

At the end of the experiment, all animals were anesthetized and blood samples were obtained from their hearts. After coagulation, serum was separated by centrifuge at 3000 × g for 15 minutes to determination of the biochemical parameters. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total protein, albumin and urea as markers of hepatic function were determined with an automated Hitachi Analyzer Model 902(Hitachi).

Histological examination:

The animals were sacrificed and their liver tissues were removed immediately and fixed overnight in 10% neutral formalin solution, then embedded in paraffin wax and sectioned for histological evaluation. Paraffin sections (6µm thickness) were stained with haematoxylin and eosin (H&E) using a standard protocol, and then analyzed by light microscopy.

Statistical analysis:

Data were expressed as mean ± SD and were analyzed with SPSS, Version 12.0 software. Differences between means values corresponding to the groups under study were calculated by one-way variance analysis (ANOVA). Results were considered statistically significant when *P* value was below 0.05.

Results

Drug A did not cause any significant change in any of the measured parameters in the case group, compared to controls for all three used doses (Table 1). Drug B drops increased significantly LDH by 34% at a dose of 11.5 ml/kg.b.w (Table 2). But it was not any significant changes for other doses and liver tests. Pathological examination of liver in this group revealed dilated portal spaces, arteries and veins. The biliary ducts were also dilated, with some monocytic infiltration in the spaces between venous, arterial and biliary ductal spaces (Figure 1).

Administration of C drops at a dose of 22.5 ml/kg.b.w significantly increased AST, ALT and LDH (19%, 35% and 59%, respectively) (Table 3). and there was not any significant changes for other dosese and liver tests. Examination of liver tissue in this group revealed dilation of some central lobular veins and edematous fluid accumulation (Figure 2).

Administration of D at a dose of 11.5 ml/kg.b.w significantly increased AST, ALT, and LDH by 25%, 33%, and 286%, respectively (Table 4). Histological examination of livers of rats in this group showed dilation and congestion of sinusoid and central lobular veins, with considerable monocytic infiltration (Figure 3). D drop at doses of 4.5 and 2 ml/kg.b.w did not have any significant effect on the measured biochemical parameters.

TABLE 1. Biochemical parameters showing liver function of rats following administration of A drop with different doses (Mean ±SD)

parameter	Control	4.5 ml/kg.b.w	6ml/kg.b.w	15 ml/kg.b.w
Alanine amino transferase (IU/L)	70.0±9.0	73.0±6.0	68.0±7.0	68±7.0
Aspartate amino transferase (IU/L)	140.0±21.0	145.0±12.0	133.0±18.0	139.0±11.0
Alkaline phosphatase (IU/L)	136.0±17.0	143.0±17.0	147.0±17.0	134.0±10.0
Lactate dehydrogenase (IU/L)	360.0±32.0	342.0±44.0	337.0±44.0	361.0±48.0
Total protein (g/dl)	8.0±0.6	7.6±0.6	7.8±0.4	6.6±0.4
Albumin (g/dl)	4.5±0.3	4.1±0.3	4.3±0.4	4.3±0.2
Urea (mg/dl)	38.0±6.0	50.0±8.0	48.0±6.0	37.0±5.0

Results are expressed as mean ± standard deviation. rats in each group(N=5)
*P values less than 0.05 were considered significant
IU: International units

TABLE 2. Biochemical parameters showing liver function of rats following administration of B drop with different doses (Mean ±SD)

parameter	Control	3 ml/kg.b.w	4.5 ml/kg.b.w	11.5 ml/kg.b.w
Alanine amino transferase (IU/L)	70.0±9.0	66.0±8.0	70.0±8.0	72.0±10.0
Aspartate amino transferase (IU/L)	140.0±21.0	148.0±18.0	144.0±20.0	151.0±15.0
Alkaline phosphatase (IU/L)	136.0±17.0	137.0±13.0	141.0±21.0	143.0±19.0
Lactate dehydrogenase (IU/L)	360.0±32.0	356.0±26.0	340.0±45.0	*419.0±58.0
Total protein (g/dl)	8.0±0.6	7.1±0.8	7.1±0.6	6.8±0.5
Albumin (g/dl)	4.5±0.3	4.1±0.4	4.5±0.5	4.5±0.6
Urea (mg/dl)	38.0±6.0	36.0±5.0	49.0±8.0	38.0±8.0

Results are expressed as mean ± standard deviation.
rats in each group(N=5)
*P values less than 0.05 were considered significant
IU: International units

TABLE 3. Biochemical parameters showing liver function of rats following administration of C drop with different doses(Mean±SD)

parameter	Control	7.5 ml/kg.b.w	9 ml/kg.b.w	22.5 ml/kg.b.w
Alanine amino transferase (IU/L)	70.0±9.0	71.0±0.0	66.0±8.0.	*84.0±7.0
Aspartate amino transferase (IU/L)	140.0±21.0	136.0±0.0	145.0±21.0	*184.0±15.0
Alkaline phosphatase (IU/L)	136.0±17.0	145.0±16.0	133.0±21.0	142.0±19.0
Lactate dehydrogenase (IU/L)	360.0±32.0	337.0±61.0	340.0±41.0	*498.0±43.0
Total protein (g/dl)	8.0±0.6	7.9±0.8	7.9±0.7	7.6±0.9
Albumin (g/dl)	4.5±0.3	4.7±0.7	4.2±0.6	4.7±0.4
Urea (mg/dl)	38.0±6.0	48.0±14.0	47±7.0	43.0±6.0

Results are expressed as mean ± standard deviation.
rats in each group(N=5)
*P values less than 0.05 were considered significant
IU: International units

TABLE 4. Biochemical parameters showing liver function of rats following administration of D drop with different doses(Mean±SD)

parameter	Control(0)	2 ml/kg.b.w	4.5 ml/kg.b.w	11.5 ml/kg.b.w
Alanine amino transferase (IU/L)	70.0±9.0	74.0±10.0	69.0±8.0	*88.0±9.0
Aspartate amino transferase (IU/L)	140.0±21.0	148.0±19.0	139.0±18.0	*180.0±25.0
Alkaline phosphatase (IU/L)	136.0±17.0	140.0±16.0	144.0±15.0	147.0±11.0
Lactate dehydrogenase (IU/L)	360.0±32.0	387.0±31.0	352.0±57.0	*1205.0±121.0
Total protein (g/dl)	8.0±0.6	7.2±0.8	6.8±0.6	7.5±0.9
Albumin (g/dl)	4.5±0.3	4.1±0.3	4.5±0.3	4.1±0.4
Urea (mg/dl)	38.0±6.0	42.0±6.0	37.0±8.0	50.0±9.0

Results are expressed as mean ± standard deviation.
rats in each group(N=5)
*P values less than 0.05 were considered significant
IU: International units

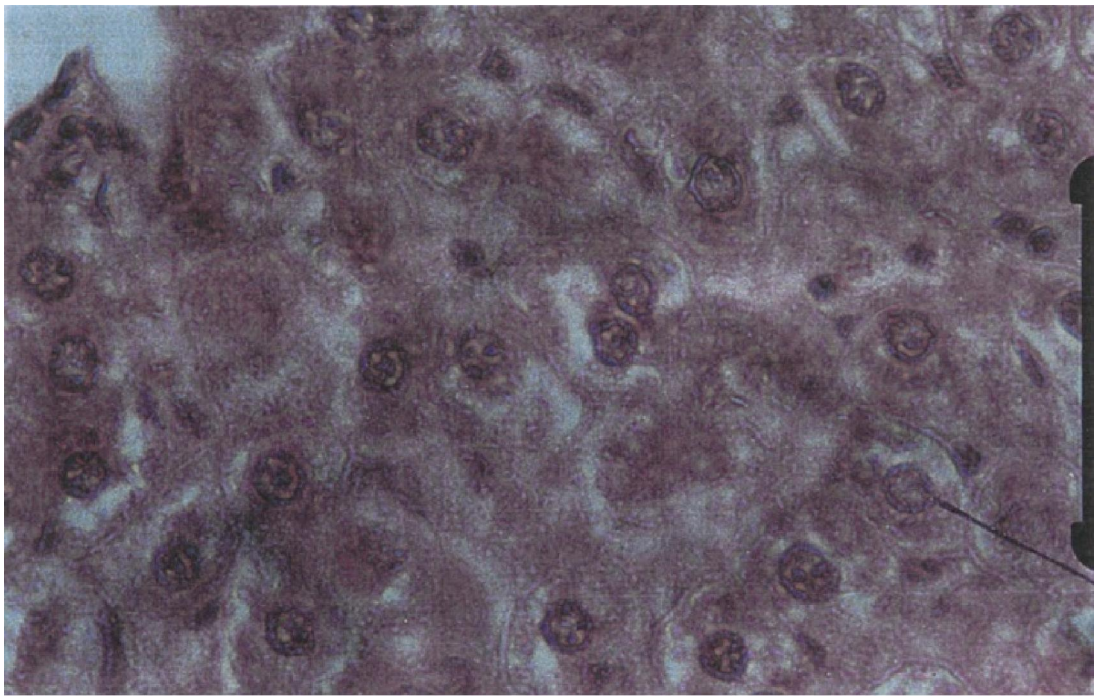

FIGURE 1. Liver tissue in the group receiving B drop with a dose of 11.5 ml/kg: dilation and congestion of portal spaces of arteries and veins, dilation of biliary ducts, monocytic infiltration in spaces between arteries, veins, and biliary ducts

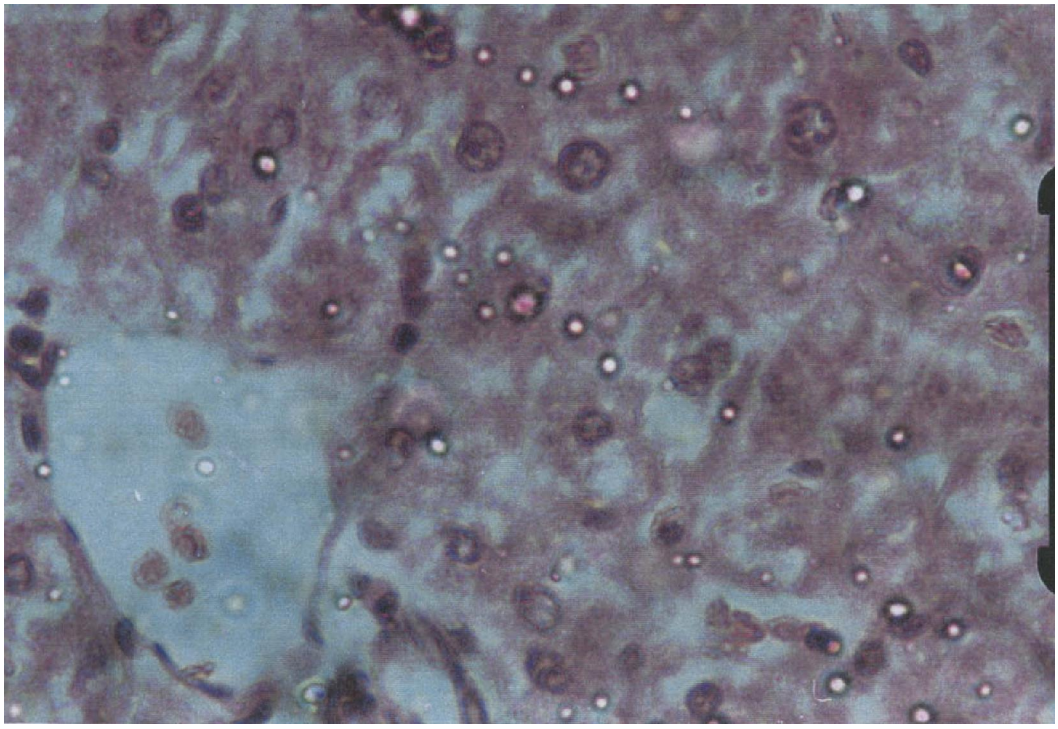

FIGURE 2. Liver tissue in the group receiving C drops with a dose of 22.5 ml/kg: dilation of some central lobular veins and edematous fluid accumulation

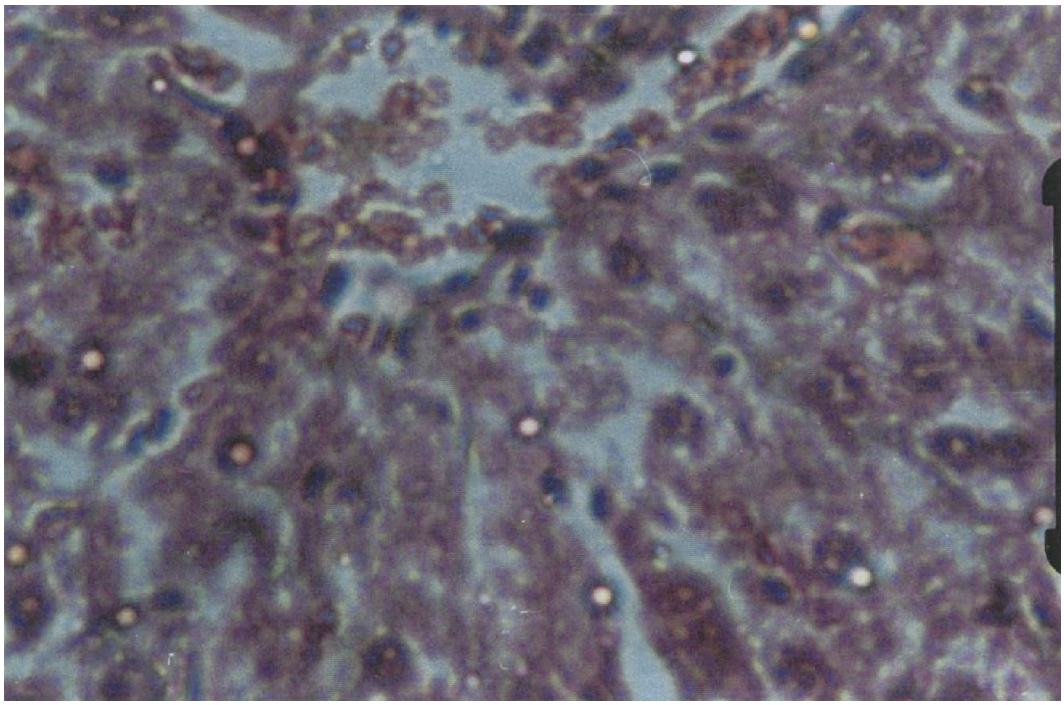

FIGURE 3. Liver tissue in the group receiving D drops with a dose of 11.5 ml/kg: dilation and congestion of sinusoid and central lobular veins, with considerable inflammatory monocytic infiltration

Discussion

A drop is used to treat acute and chronic inflammation of the prostate, as well as urinary urgency and frequency. This herbal product contains 20% Tribulus terrestris. Reports suggest liver damage in animals feeding on this herb (10-12). In this study, however, administration of A drop did not affect liver function of any of the treated rats (Table 1).

B drop mainly used to increase milk in breastfeeding mothers, contain 20% Cuminum cyminum. This herb has been reported to increase AST and serum urea in rats (13). In this study, oral administration of B at low doses did not affect liver function, however, it increased serum LDH by 34% compared to controls at a dose of 11.5 ml/kg.b.w (Table 2). Nonetheless, LDH has different isoenzymes and measurement of total LDH does not suffice for assessment of liver function. Histological examination of rat livers in this group showed dilation and congestion of portal spaces of arteries and veins, as well as dilation of biliary ducts and some monocytic infiltration in spaces between arteries, veins, and biliary ducts. B drop also contain 50% Foeniculum vulgare; reports are suggestive of this herb's protective effects against hepatotoxicity induced by carbon tetrachloride in rats (14). Thus, the presence of Foeniculum vulgare in this herbal product may have offset the hepatotoxic effects of Cuminum cyminum in rats.

C drop contains 12.5% Cuminum cyminum and 12.5% Foeniculum vulgare. At low dose, C did not affect liver function in rats; however, at 2.5 times the maximum dose printed in brochures (22.5 ml/kg.b.w), the herb significantly increased ALT, AST and LDH compared to controls (Table 3). Histological examination of rat livers also showed dilation of some central lobular veins and accumulation of edematous fluid, as well as limited monocytic infiltration in biliary ducts.

D contains the hydroalcoholic extract of Hypericum perforatum and is used in the treatment of depression, insomnia, anxiety, neurological headaches and migraine. This herb causes sensitivity to ultraviolet radiation (UV) and may promote allergic reactions, as well as gastric discomfort. Some studies have shown Hypericum perforatum to interfere with some drugs, including warfarin and contraceptives (15). Thus far, there have been no reports of the hepatotoxicity of this herb, however, administration of D at a dose of 11.5 ml/kg.b.w significantly increased ALT, AST, and LDH compared to controls. Histological examination of liver tissue in this group confirmed its effect on liver function of treated rats.

It is in certain situations and use higher doses, herbal products may be just as harmful as conventional drugs. Although findings on the hepatotoxicity of drugs in rats cannot be reliably generalized to humans, they are of high predictive value. In the present study, administration of medicinal herbs at doses indicated in brochures was not associated with hepatotoxicity. At higher doses, however, B, C and D affected liver function; this was evidenced by changes in the concentration of hepatic enzymes in rats. Further studies are warranted to assess the hepatotoxic properties of these herbs. We recommend that herbal formulations available in pharmaceutical markets be more closely controlled in terms of quality, as well as toxicity, especially as regards possible effects on the hepatic function.

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