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Research Article

CHRONIC EFFECTS OF HYDRO-ALCOHOLIC ARTEMISIA ABSINTHIUM EXTRACT ON THE LIVER ENZYMES AND TISSUE CHANGES OF ADULT MAL RAT

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ABSTRACT

Artemisia absinthium has many pharmacological effects, but toxic effects of it, were seen on nervous system and liver. Therefore, the aim of this study was to evaluate the chronic effects of different doses of *Artemisia absinthium* extract on the enzymes and histopathological changes of the liver tissue of adult normal male rat. Method and materials: In this experimental study, forty eight male Wistar rats were randomly divided into 6 groups of 8 as follows: Control, sham (recipient of distilled water) and 4 experimental groups that received *Artemisia absinthium* hydro- alcoholic extract at doses of 125, 250, 500 and 1000 mg/kg intraperitoneally.

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The data were analyzed using one-way ANOVA and Duncan post hoc tests. P≤0.05 was considered statistically significant. Results: After 8 weeks, doses of 125, 250 and 500 mg/kg could significantly reduce amount of ALT, AST and ALP. Dose of 1000 mg/kg increased ALT, AST and ALP. From the standpoint of histopathological study, doses of 125, 250 and 500 *Artemisia absinthium* had no significant side effect on liver tissue, but 1000 mg/kg caused destruction of liver cell membranes, enlargement of sinusoidal space, sporadic leukocyte infiltration, Kupffer cells hypertrophy, and vascular congestion.

Conclusion: Maximum dose of Artemisia absinthium extract (1000 mg/kg) increased liver enzymes and destroy liver tissues of normal male rats.

Keywords: Artemisia Absinthium, Enzymes, Histopathology, Liver, Rat

1. INTRODUCTION

Wormwood plant with the scientific name Artemisia absinthium is from the Asteraceae family which is native to Europe [1], as well as different regions of Iran [2]. Some studies have demonstrated the protective effect of wormwood [3-5]. In traditional medicine it can be used to treat anorexia, atonic bowel, gastritis, stomach pain, intermittent fever and intestinal parasites [1, 6].The most important pharmaceutical active ingredients of wormwood include glycoside absinthium, tannins, resins, potassium nitrate, organic acids, komazoline and tujone [7]. Previous researches suggested that alpha tujone as a main part of wormwood has many pharmacological effects such as analgesic, anti-parasite, and anti-pyretic [8-10]. However, this combination is highly toxic and can cause neurological disorders but beta tujone, its stereoisomer, is less toxic because of its different spatial structure [11]. Although, it was found that the toxic effects of alpha and beta tujone was done via cannabinoid receptors[11] a different study proved that worm wood extract has low affinity for them [12]. Another study stated that tujone reduced threshold of neurons by blocking gaba A receptors, and so, caused convulsion and muscle spasms [13]. Hepatocyte cells contain large amounts of complex metabolic enzymes which leak into the plasma due to liver damage[14]. The enzymes such as aspartate aminotransferase (ASP),

alanine aminotransferase (ALT) and alkaline phosphatase (ALP) can be useful to identify the level of liver damage[15]. Some studies have reported that wormwood has protective effects on the liver damage induced by lead,[16],facetaminophen and carbon tetrachloride [4]. In another study, the protective effect of aqueous extract of wormwood to hepatotoxicity dose dependently (50, 100 and 200 mg/kg) has been related to its antioxidant and immune modulatory properties in mice [3]. There are controversies about absithium effects on health. Although some studies have shown protective effect ,others found its toxicity on nervous system and liver like porphyrogenicity, convulsion, hallucination and psychosis[11, 13, 17].

It was shown that toxic effects of absinthium were related to alpha and beta thujone accumulation in the tissues [11]. On the other hand, alcoholic beverages containing absinthe have chronic and high daily consumption. In addition, nowadays, societies try to use traditional medicine and medicinal herbs in accordance with World Health Organization recommendations .So it can be toxic during long term use. Considering our knowledge, the aim of this study was to investigate chronic effects of hydro-alcoholic Artemisia absinthium extract on the liver enzymes and tissue changes of adult male rat.

2. RESULTS

Effect of hydroalcoholic extract of Artemisia absinthium absinthe on the serum AST levels, although AST levels in control group had no significant difference with sham group but Hydroalcholic extract of absinthe with doses of 125, 250 and 500 mg /kg significantly decreased compared with the control group. While 1000 mg /kg of it, showed the significant elevation of AST relative to the control group. (Table1).

2.1. Effect of hydroalcoholic extract of Artemisia absinthium on ALT serum levels

There was no significant difference between the levels of serum ALT of animals from control group and sham. Serum ALT significantly reduced in the groups receiving hydroalcholic extract at the doses125, 250 and 500 mg/ _kg compared with the control group. The most effective dose

was 500 mg/ -kg, but, the animals which received hydroalcholic extract at the dose1000 mg/kg showed ALT levels higher than controls and sham groups (Table 1).

2.2. Effect of hydroalcoholic extract of Artemisia absinthium on ALP serum levels

No significant changes were observed between levels of ALP of the control group in relation to the sham. ALP serum levels in the rats pretreated with hydroalcoholic extract of Artemisia absinthium at doses of 125, 250 and 500 mg/ kg were significantly lower than control group and sham. The most effective dose was 500 mg /kg which was comparable to the others in reducing the rate of ALP. Meanwhile, ALP levels of rats pretreated with different dose of hydroalcoholic extract (1000 mg/ kg) were higher than control or sham groups (Table 1).

Table 1. The effect of hydroalcoholic extracts of Artemisia absinthium on rat serum enzymes ALT, AST, and ALP

Variables / Group	AST	ALT	ALP
Control	158.88±6.89 b	104.75±7.28 b	377.5±9.58 c
Sham	168.38±5.34 b	110.38±6.53 b	401.38±9.53 c
Absinthium 125	114.5±11.35 a	82.88±2.12 a	346.38±14.24 bc
Absinthium 250	109.13±9.92 a	75.88±3.71 a	260.75±1047 a
Absinthium 500	101.75±8.84 a	69.88±2.48 a	290.25±6.54 ab
Absinthium 1000	202.75±8.33 c	163.38±3.91 c	638.25±45.8 d

2.3. Sample staining with hematoxylin and eosin

Pretreatment with hydroalcoholic extract at doses of 125, 250 and 500 mg/kg) has not produced histological changes in the liver tissue. However we observed destruction of liver cells, large membrane space sporadically, leukocyte infiltration, hypertrophy of the Kupffer cells and the hyperemia in the latter group (1000 mg /kg) rather than control or sham groups (Fig 1-2) .

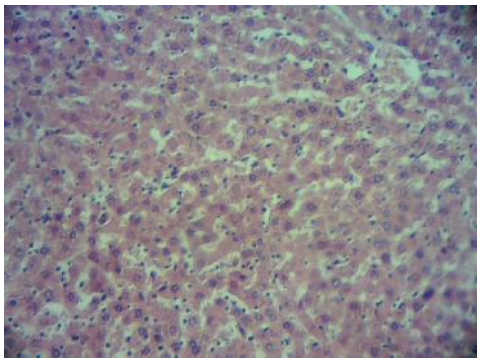

Fig.1. Microscopic view of the liver in control group, with a natural structure (Hematoxylin-eosin, 40 X magnifications)

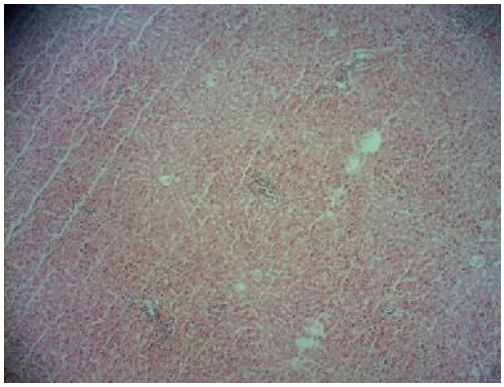

Fig.2. Microscopic view of the liver in the *Artemisia absinthium* 1000 mg/kg group: Infiltration of inflammatory cells around the portal space and the loss of cellular order toward the center and cell destruction (Hematoxylin-eosin, 40X magnification)

3. DISCUSSION

The results of this study have shown that although pretreatment of animals with low doses of hydroalcoholic extract of absinthe (125, 250 and 500 mg/kg), reduced the average amount of liver enzymes, the highest dose (1000 mg/kg) backfired. In accordance with biochemical analysis, we found some histological changes in the liver tissue such as edema, degeneration of cells and infiltration of leucocytes with high dose of it (Fig 6).

Previous studies showed that this plant has protective effect on brain injury induced by ischemia/reperfusion(I/R) injury [21], wound, eczema and gastritis irritation [22]. In agreement

with our study, 500 mg/kg wormwood aqueous extract has been effective in reducing chemical and immunological damage to the liver in mice [3], and, 200 mg/kg inhibited lipid peroxidation and cellular leakage due to cell rupture as well [3]. According to a study, by Craciunescu et althe Wormwood prevented oxidative damage in fibroblast-like cells via its antioxidant activity and cytoprotective effects [23]. According to absinthium,s effects on liver, it is necessaryto, pay attention to a few points:

Some controversies exist about the poisoning effects of absinthium[24]. These paradoxes may be due to dose, duration of consumption, type of animals or plant which was used during previous studies [25].

The conversion of alpha tujone to 7_ hydroxyl metabolite is less toxic [26]. These metabolites in the liver microsomes are produced by the system p450 , conjugated and excreted quickly [13].

However, toxicity at high doses and prolonged consumption is reported on the nervous system [13] .The cause of neurotoxicity can be related to accumulation of 7_ hydroxyl metabolites in this area[13]. Meanwhile Bonkovsky et al (1992) showed that thujone caused porphyrinogenic effect on cultured liver cells of chick embryo. This opposite effect can be related to race or type of animals [17]. In our results, low doses of wormwood extract (<1000 mg/kg) were not toxic and only enzyme levels that are indicative of damage to liver tissue increased with the highest dose. It seems that the system of conjugation in the liver was saturated and cannot dispose of all of it by high dose (1000 mg/kg).

It is possible that protective effects of absinthium at doses lower than 1000 mg/kg can be related to anti-inflammatory properties [3,27]. Recent studies have shown that wormwood has a weak antioxidant activity [28] , anti-inflammatory effect via cytokines, TNFalpha inhibition and immune system modulation [29]. But another study has proved that thujone content in wormwood is quiet variable [25]. It is possible that absinthium plays a prooxidant and inflammatory role such as gallic acid [30] in high dose so that hepatotoxicity was induced by inflammation and oxidation dependent on its thujone content, drying and harvesting conditions or climate that the plant was growing in [22, 25].

Contrary to our result, Muto, et al, (2003), observed no toxic changes related to the administration of the worm wood extract in Wistar rats which had been treated with 2% wormwood extract in drinking water for 13 weeks [31]. This administration route is different from intraperitoneal injection in our study. Histopathological study confirmed destruction of liver cell membranes, enlargement of sinusoidal space; sporadica leukocyte infiltration, Kupffer cells hypertrophy, and vascular congestion in the tissue of liver were seen using the highest dose of absintium extract (1000 mg/kg).

4. METHODS

4.1. Statements on Ethical Approval

The protocol of the study was approved by the Iran- Jahrom University Ethics Committee (JUMS.REC.1393.008), and the study was conducted according to the university's guidelines for the care and use of laboratory animals.

This study was an experimental research and was done in Jahrom university of Medical Sciences.

4.2. Preparation of Artemisia absinthium Extract

Aerial parts of absinthium were collected from dasht naz , Mazandaran, Iran and authenticated by Dr B. Eslami (voucher specimen is 345)[18]. Then they were ground to powder, and hydro alcoholic extract was prepared through soxhlet method. After that, 10 gr of this powder was soaked in 200 ml distilled water plus ethanol.Finally, solvent was separated using Rota vapor device[19].

4.3. Experimental design

Froty eight male Wistar rats (180-200gr) were obtained from Animal Breeding Center of Jahrom University of Medical Sciences. Animals were housed for 8 weeks in a light (12 hrs light/ 12hrs dark), temperature (24±1°C) condition with standard rat chow and tap water ad libitum.

Rats were randomly assigned to 6 groups (n =8 each). The first group was control rats that received no treatment. The second group was rats that received distilled water. The 3rd, 4th, 5th

and 6th groups received hydro alcoholic extract of Absinthe intra-peritoneal (IP) at 125, 250, 500 and 1000 mg/kg/day, respectively [3]. Duration of protocol was eight weeks.

5. EXPERIMENTAL PROTOCOL

At the end of 8 weeks, animals were anesthetized with diethyl ether and blood samples (5 ml) were withdrawn by cardiac puncture. The blood samples were centrifuged (3000 rpm, 15 min) and serum was obtained to determine ALT, ALP and AST using commercial kit (Quimica clinica applicada, S.A. spain). Total protein(TP) and serum albumin(Alb) were measured according to the protocols available in the commercial kits (Lab Care Diagnostics (India) Pvt. Ltd) and spectrophotometry method (auto analyzer, Selectera XL, Holand) [3].

5.1. Histopathological studies

For histopathological analysis, liver tissue was fixed in 10% buffered formalin. Fixed tissues were dehydrated through ascending grades of ethanol .They were cleaned in xylene, embedded in paraffin wax . Sections were cut at (5µm) on a rotatory microtome. They were flattened on warm water mounted onto slides and dried overnight. The sections were dewaxed in xylene and hydrated through descending grades of ethanol.The resulting slides are then viewed under the light [20] microscope (Olympus, CX-21) to record the liver pathological changes like hepatocyte necrosis or edema [3]. The photomicrographs were printed at a total magnification of x40, x100 and x400.

5.2. Statistics Analysis

The data, presented as mean±SEM, was analyzed through one way analysis of variance. In cases of significant difference with these tests, comparisons were performed by Duncan post hoc test. Calculations were performed using SPSS software (version: 16). $P \leq 0.05$ was considered statistically significant.

6. CONCLUSION

Maximum dose of Artemisia absinthium extract (1000 mg/kg) increased liver enzymes and destroy liver tissues of normal male rats. Absinthium may have acted like a double-edged sword, so in high dose it has worked as an oxidative and inflammatory substance.

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Conflict of Interests

The authors declare that there is no conflict of interests

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