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RESEARCH ARTICLE

Arum conophalloides Aqueous Extract Induced Hepatotoxicity in Rat; Histopathological, Biochemical, and mir-122 Assessments

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ARTICLE HISTORY

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Abstract: ***Background*:** *Arum conophalloides (A. conophalloides)* is a wild edible delicate plant, widely used in traditional medicine.

***Objective*:** This study aimed to examine the effects of *A. conophalloides* extracts on biochemical, molecular, and histopathological changes in the rat.

***Methods*:** Fifty adult male Sprague-Dawley rats were divided into 5 groups (10 each) as follows: G1 or control, received distilled water; G2 and G3, treated with the aqueous extract at doses of 200 and 400 mg/kg; G4 and G5, treated with the hydroalcoholic extract at doses of 200 and 400 mg/kg. Prior to and at the end of the experiments, the serum levels of biochemistry parameters and the relative expression of miR-122 were assessed. Moreover, the liver and kidney tissues were examined microscopically.

***Results*:** Liver and kidney tissues showed normal structure in all groups. There were no significant changes in biochemical indices or the expression of miR-122 in the extract-treated groups at the dose of 200 mg/kg. However, the group that received the aqueous extract at the dose of 400 mg/kg exhibited a significantly lower level of HDL, LDL, ALT, and ALP in comparison to the control. Additionally, miR-122 expression in this group exhibited a 10-fold increase (P=0.009).

***Conclusion*:** The serum level of hepatocyte-specific miR-122 will be more helpful in detecting hepatic changes in early stages than ALT and AST activity or histopathological evaluations of liver sections. Our findings highlight the potential hepatotoxicity of *A. conophalloides* aqueous extract in a rat model.

Keywords: *Arum conophalloides*, hepatotoxicity, miR-122, rat, histopathological, HDL, ALT, AST.

1. INTRODUCTION

The genus *Arum* is made up of about 26 species of flowering plants, native to Asia, Europe, and northern Africa, and belonging to *Araceae* family. *Arum conophalloides Kotschy Ex Schott* (*A. conophalloides*), locally known as Kardeh, is a seasonal (spring) and edible delicate plant, widely used in some regions of Iran, Iraq, Syria, and Turkey as cooked in a stew and rice-vegetable dish [1-3]. It is a well-known aromatic and medicinal plant and its leaves were used in traditional medicine as a painkiller, blood purifying, stomach and intestine tonic, and to treat animal mastitis [3-5]. Nowadays, the demands for plant-based medicines are rising in both developing and industrialized countries due to its medical and economic benefits [6-8]. Medicinal plants and other plant-derived remedies are widely available and self-prescribed; therefore, the quality, safety, standardization, and efficacy of these products have become a major concern by policy-makers, health professionals as well as the general public. Hepatotoxicity, acute liver failure, and renal impairment are major complications that these plants and herbs can cause [8-10].

Despite other tasks, the liver is responsible for processing, concentrating, metabolizing, and detoxifying the majority of the drugs and toxins; hence, it is a prime target for medication-induced damages. Although some medicinal plants have hepatoprotective activity, others contain substances that can lead to hepatotoxicity [11-13]. Among the liver injury markers, evaluation of the serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most common tools, but they are not specific to the liver and could be released from non-hepatic tissues.

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In addition, the alterations of these enzymes in asymptomatic cases might be attributed to the normal physiological phenomenon [14, 15]. Liver biopsy is considered as an alternative tool to assess cases with an unexplained elevation of transaminases. However, since liver injury evolution is gradual, merely minimal histological changes occur in a short duration. Moreover, liver biopsy has several limitations and disadvantages; therefore, this method is neither ideal nor sufficient for diagnostic purposes [16]. Hence, the research interests have focused on new non-invasive techniques to assess liver injuries in the shortest time possible [17, 18].

MicroRNAs (miRs) are a group of short ~22 nucleotides that play a vital role in the regulation of gene expression at the post-transcriptional level by accelerating degradation or by inhibiting the translation of messenger RNA (mRNA) into the protein [19-21]. The hepatocyte-specific miR-122 is highly conserved among vertebrate species, and involved in various physiological processes, hepatic function as well as liver pathology [22, 23]. MiR-122 almost exclusively is expressed in the liver (about 70% of total miRNA pool) and has a minor expression in other tissues. Moreover, circulating levels of miR-122 are highly stable; therefore, its deregulation in the blood might represent hepatic cell death, which is clinically important. Up-regulation of miR-122 can be observed prior to/in the absence of elevations in transaminases or observation of histopathological changes, making it a highly sensitive and specific biomarker for hepatocyte damages [19, 21, 24]. Therefore, the current study aimed to examine the effects of aqueous and hydroalcoholic extract of *A. conophalloides* on biochemical, molecular, and histopathological changes in male Sprague-Dawley rats.

2. MATERIALS AND METHODS

2.1. Ethics

Animal care was performed according to the guidelines of the local Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (Code: IR.SUMS.REC.1395.S1240).

2.2. Collection of the Plant and Preparation of the Extracts

Fresh *A. conophalloides* was collected from the local vegetable market in Shiraz, Fars Province, Iran. The voucher specimen of the plant (no. 790) was assigned and deposited at the herbarium in the Department of Traditional Pharmacy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. Air-dried leaves and stems were used to prepare the herbal extracts. Briefly, 500 g of the powder were separately macerated in 2 liters of either distilled water or 70% ethanol (ethanol/water 70/30v/v) for the preparation of aqueous or hydroalcoholic extract, respectively. The mixtures were kept at room temperature for 3 days. After filtration using Whatman filter paper, the filtrate was concentrated in the water bath (45-50°C) and evaporation of the solvent was performed under vacuum condition.

2.3. Animals and Study Design

Fifty adult male Sprague-Dawley rats (weighing 200-220 g) were acclimatized for two weeks at the Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences, Shiraz, Iran (temperature 22 ± 2°C; humidity 50-60%; light/dark cycle 12:12 h). Prior to any experiment, the blood samples were collected separately from each rat under the general anesthesia using an intramuscular injection of 10% ketamine (90 mg/kg) and 2% xylazine (10 mg/kg) (Alfasan, Woerden, Holland). Next, the rats were randomly assigned into five groups (each containing 10 cases) as follows: G1: the control group; treated with distilled water; G2 and G3 were treated with the aqueous extract at doses of 200 and 400 mg/kg body weight, respectively; G4 and G5 were treated with the hydroalcoholic extract at doses of 200 and 400 mg/kg body weight, respectively. All the extracts were dissolved in distilled water to make the required concentrations and administered orally by gavage (20 gauge) for 10 consecutive days. During the experiment, animals were allowed to have free access to water and a standard pellet diet. At the end of the experiment and under anesthesia, the blood samples were collected again, then the animals were euthanized and liver and kidney tissue specimens were harvested.

2.4. Biochemical Analysis

The blood samples were transferred into a non-heparinized tube (Mediplus, China), allowed to clot, and centrifuged for 5 min at 3000 RPM. The blood biochemistry parameters including Fasting Blood Sugar (FBS), Triglycerides (TG), Cholesterol (Chol), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Blood Urea Nitrogen (BUN), Creatinine (Cr), ALT, AST, and alkaline phosphatase (ALP) were measured by an automated biochemical analyzer (Alpha-Classic AT Plus, Tajhizat Sanjesh, Iran) with a commercially available reagents (Pars Azmoon, Tehran, Iran).

2.5. Molecular Assessment

For the purpose of total RNA extraction, 1mL of cold QIAzol lysis reagent (Qiagen, USA) was added to 300 µL of the serum sample and vortexed vigorously for 1 min. After incubating the homogenate at room temperature for 5 min, 0.2 mL of chloroform (Merck, Germany) was added to the tube, and the solution was mixed by inverting for 1 min. The mixture was centrifuged for 15 min at 14000 RPM at 4°C, and the aqueous phase was carefully removed and transferred into a new RNase, DNase, and Pyrogen free tube. Total RNA was directly precipitated, using 1 mL of cold absolute ethanol (Merck, Germany) and 2 µL of Glycogen (20 mg/mL) (ABM Inc., Canada). The solution was then sedimented and the supernatant was discarded. The RNA pellets were washed with 75% ethanol and after one more centrifugation step, it was allowed to air-dry, and finally eluted in 100 µL of DEPC-treated water. All RNA samples were stored at -70°C until further processing.

Reverse transcription was performed using the gene-specific primers (Table **1**) and the RevertAid first-strand cDNA synthesis kit (Thermo Scientific, USA) according to the manufacturer's instructions. Upon completion of the cDNA synthesis, products were diluted 1:5 in nuclease-free water and used as a template in a subsequent SYBR green

Table 1. The list of gene-specific primers used in real-time PCR assay.

Gene	Primer Name	Sequence, 5' --- 3'	Accession No.
miR-122	RT primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAAACA	MI0000891
	Forward primer	GTATACTGGAGTGTGACAATG	
	Reverse primer	GTGCAGGGTCCGAGGT	
miR-16	RT primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGCCAA	MI0000844
	Forward primer	GTATACTAGCAGCACGTAAAT	
	Reverse primer	GTGCAGGGTCCGAGGT	
miR-221	RT primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGAAATC	MI0000961
	Forward primer	GTATACACCTGGCATACAATG	
	Reverse primer	GTGCAGGGTCCGAGGT	
Let-7a	RT primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACTAT	MI0000827
	Forward primer	GTATACTGAGGTAGTAGGTTG	
	Reverse primer	GTGCAGGGTCCGAGGT	
snRNA U6	RT primer	AACGCTTCACGAATTTGCGT	K00784
	Forward primer	CTCGCTTCGGCAGCACA	
	Reverse primer	AACGCTTCACGAATTTGCGT	

real-time PCR assay. Real-time PCR reaction mixture containing the equal amounts of diluted cDNAs (2 µL), 10 pmol of each primer, and 10 µL of the 2X SYBR green PCR master mix (Applied Biosystems, USA) was prepared and the total volume reached 20 µL in nuclease-free water. No-Template Controls (NTC) also ran at the same time and the reactions were processed with a Rotor-Gene Q PCR cycler (Qiagen, Germany) using the following conditions: 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Following the final cycle, the specificity of the amplicons was verified by conventional melting curve analysis (60-95°C with a heating rate of 0.5°C/s). The PCR was analyzed, using either relative expression or absolute quantification. Relative expression was performed using gene-specific primers for miR-122 as a target gene and mir-16, miR-221, let-7a, and U6 RNA as reference genes and the result was calculated by the 2^{-ddCt} formula. Additionally, absolute quantification of miR-122 was performed in parallel. In brief, the synthetic miR-122 oligoribonucleotides were serially diluted to span a range between 1,000,000 and 10 copies/µL, then reverse-transcribed and amplified in each round of real-time PCR. Finally, a six-point standard curve was used to calculate the quantity of miR-122 in the serum samples.

2.6. Histopathological Evaluations

After fixation of tissues in 10% neutral-buffered formalin, the routine histological process was performed using an automated tissue processing machine (DID SABZ Co., Iran) and the tissues were embedded in paraffin. The specimen slices were cut at a thickness of 5µm using a microtome (DID SABZ Co., Iran), and then stained with conventional hematoxylin and eosin method (Merck, Germany). Finally, the tissue slides were examined under the light microscope (Nikon Eclipse Ni, Tokyo, Japan) by a pathologist, who was blinded to the study.

2.7. Statistical Analysis

Statistical analyses were carried out using the statistical package for the social sciences (IBM SPSS, version 21). Variables were checked for normality by the Kolmogorov-Smirnov test. All values are expressed as means and Standard Deviations (SD), and comparisons were made by one-way Analysis of Variance (ANOVA) with a post-hoc Tukey test. Pearson's $\chi 2$ test was used to assess the differences between categorical variables. A P value < 0.05 was considered to be statistically significant.

3. RESULT

The aqueous and hydroalcoholic extracts had no significant effect on the subjects' body weight (Table **2**). Based on the histopathological findings, the liver and kidney tissues showed normal structure without any functional impairment in all experimental groups that had received extracts at doses of 200 and 400 mg/kg as well as the control (Figs. **1** and **2**).

Various biochemical parameters were assessed at the baseline and at the end of the experimental period (Table **2**). There were no significant differences in the serum biochemical indices as well as a miR-122 expression between the control and the pre-treatment values; hence, these parameters in each extract-treated group were compared with the control. Both extract-treated groups at the dose of 200 mg/kg (G2

Table 2. Body-weight and the serum level of biochemical indices in each experimental group.

Variables	Experimental Groups (Mean ± SD)					
	Pre-Treatment	Control	Aqueous Extract		Hydroalcoholic Extract	
			200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg
Body-weight (g)	212.3 ± 5.1	219.6 ± 3.7	220.1 ± 5.2	218.8 ± 5.4	220.2 ± 3.3	222.1 ± 2.3
FBS (mg/dL)	148.6 ± 8.3	180.1 ± 43.3	250.6 ± 97.1	271.4 ± 59.9	237.8 ± 130.3	213.1 ± 2.3
TG (mg/dL)	79.1 ± 5.2	100.8 ± 28.5	80.8 ± 18.7	78.6 ± 44.2	83.1 ± 33.6	83.1 ± 13.8
Chol (mg/dL)	72.2 ± 8.1	67.4 ± 10.7	57.6 ± 14.9	48.4 ± 5.2	49.8 ± 16.2	59.1 ± 3.4
HDL (mg/dL)	30.1 ± 2.4	30.4 ± 1.1	28.2 ± 6.1	**19.8 ± 4.4***	25.4 ± 7.2	26.5 ± 7.5
LDL (mg/dL)	19.1 ± 4.1	24.8 ± 3.7	19.1 ± 3.6	**16.8 ± 5.2***	20.6 ± 3.4	19.5 ± 0.7
BUN (mg/dL)	23.1 ± 2.3	24.4 ± 3.7	21.6 ± 1.3	26.8 ± 3.4	22.1 ± 3.3	21.1 ± 1.1
Cr (mg/dL)	0.71 ± 0.09	0.58 ± 0.08	0.56 ± 0.05	0.56 ± 0.05	0.62 ± 0.19	0.55 ± 0.05
AST (IU/L)	118.8 ± 11.8	138.4 ± 20.4	139.2 ± 15.5	147.2 ± 12.6	148.4 ± 3.5	129.5 ± 29.4
ALT (IU/L)	62.6 ± 4.7	65.8 ± 6.8	59.8 ± 5.7	**48.1 ± 4.3***	58.2 ± 13.1	**41.5 ± 10.9***
ALP (U/L)	820.6 ± 145.1	733.2 ± 120.1	703.1 ± 223.4	**396.1 ± 148.3***	737.1 ± 113.2	**402.5 ± 15.5***

Asterisks show significant differences (P<0.05) compared to control group.

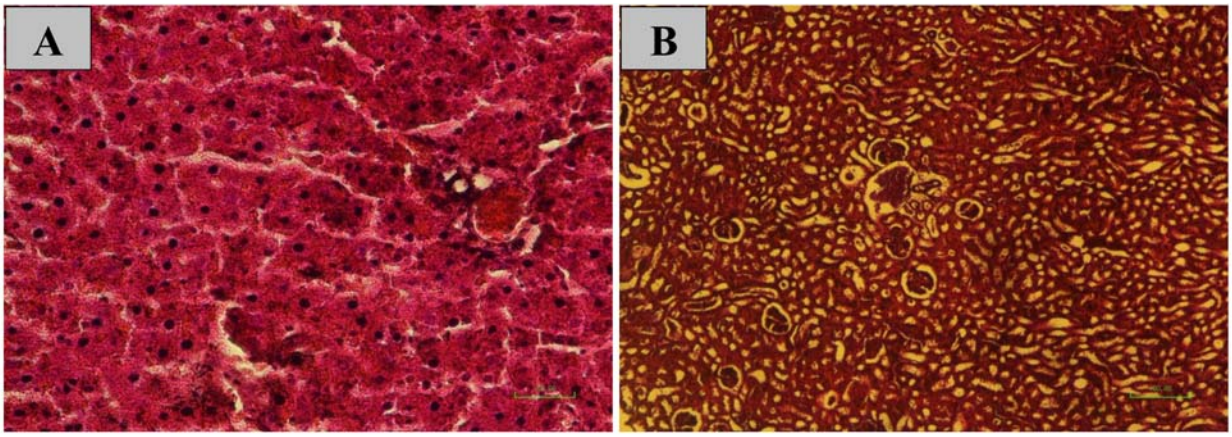

Fig. (1). (**A**) A normal structure of the liver and (**B**) kidney tissues in the control group (G1) which was treated with distilled water (×100, H & E).

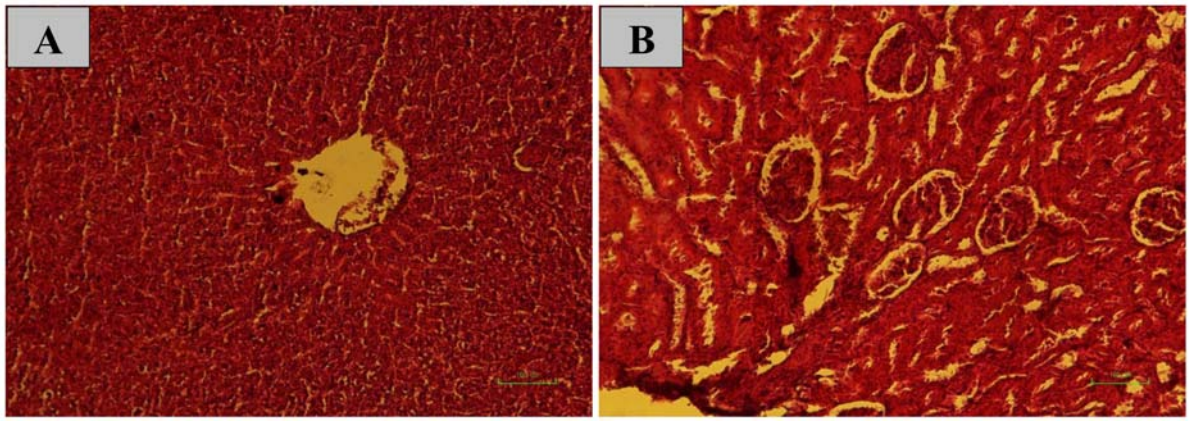

Fig. (2). (**A**) Histopathological evaluation of the liver and (**B**) kidney tissues in group 2 which was received the aqueous extract of *Arum conophalloides* at the dose of 200 mg/kg body weight (×100, H & E). Both tissues showed a normal structure.

Table 3. Results of circulatory miR-122 expression in the *Arum conophalloides* extract-treated groups in comparison to control (Figs. 3 and 4).

Groups	Type of Extract	Dose	Fold Change	Standard Error	*P* Value
G2	Aqueous	200 mg/kg	1.872	0.782-8.424	0.230
G3		400 mg/kg	10.416	2.028-27.309	0.009
G4	Hydroalcoholic	200 mg/kg	0.955	0.760-1.228	0.792
G5		400 mg/kg	1.113	0.927-1.464	0.794

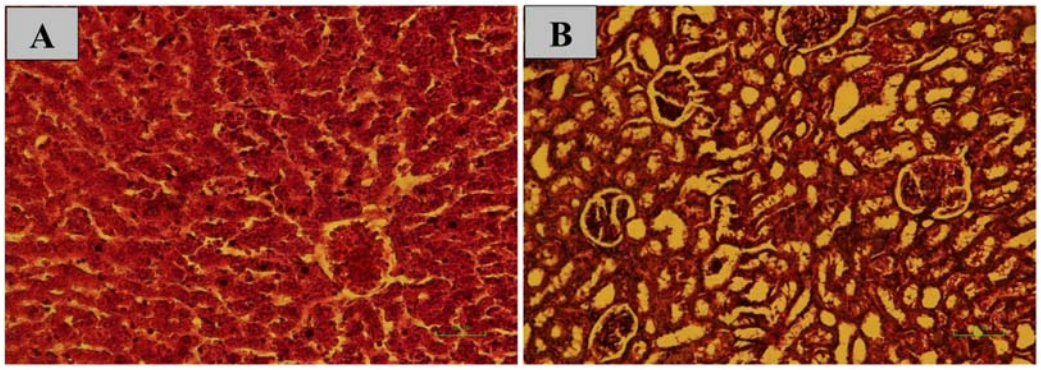

Fig. (3). (**A**) Histopathological evaluation of the liver and (**B**) kidney tissues in group 3 which was received the aqueous extract of *Arum conophalloides* at the dose of 400 mg/kg body weight (×100, H & E). Both tissues showed a normal structure.

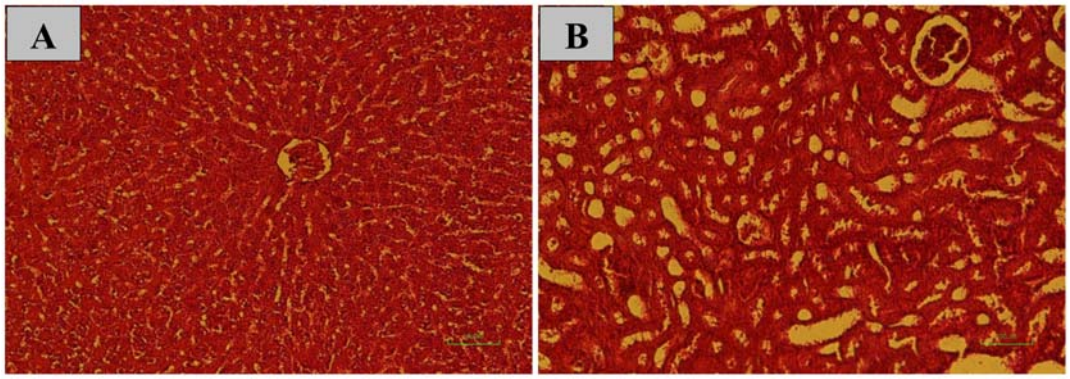

Fig. (4). (**A**) Histopathological evaluation of the liver and (**B**) kidney tissues in group 4 which was received the hydroalcoholic extract of *Arum conophalloides* at the dose of 200 mg/kg body weight (×100, H & E). Both tissues showed a normal structure.

and G4) had no significant differences regarding the mean values of any biochemical indices in comparison to the control group. However, the experimental group which was treated with the aqueous extract at the dose of 400 mg/kg, exhibited lower levels of HDL (P=0.037), LDL (P=0.030), ALT (P=0.020), and ALP (P=0.013) in comparison to the control group. In addition, hydroalcoholic extract at a dose of 400 mg/kg caused a significant reduction in ALT (P=0.002) and ALP (P=0.025) levels in comparison to the control group.

Blood samples were obtained pre- and post-treatment in order to assess whether miR-122 expression had changed in the circulation. Overall, our findings indicate that the expression and quantitation of hepatocyte-specific miR-122 were not significantly changed in any of the experimental groups, except for those treated with the aqueous extract at the dose of 400 mg/kg (Table **3**) (Figs. **3** and **4**). The mean quantity of miR-122 in this group was significantly higher than that observed in the control group (3910 copies/mL *vs*. 289 copies/mL, P=0.025). Moreover, the mean values of miR-122 in the abovementioned group exhibited a 10-fold increase (P=0.009). However, the relative miR-122 expression in this group did not correlate with other variables (P>0.05).

4. DISCUSSION

Despite potential pharmaceutical values, all medicines, including herbal or commercialized, might have a wide range of adverse effects, especially at higher doses. Early detection

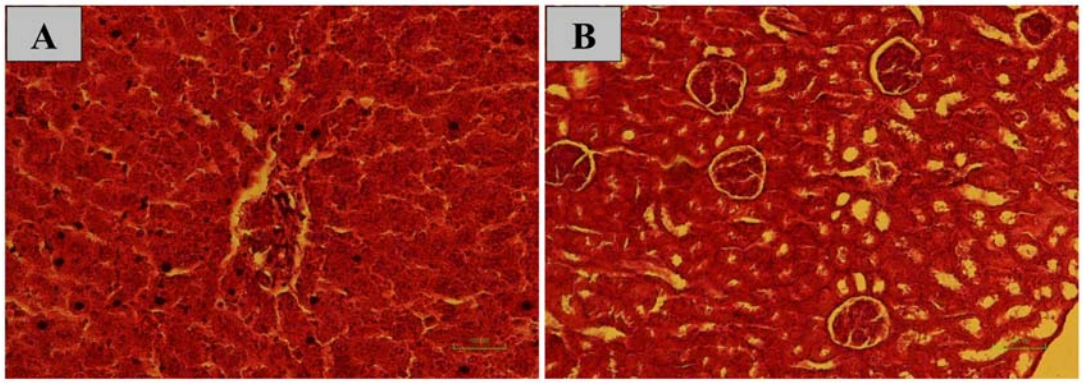

Fig. (5). (**A**) Histopathological evaluation of the liver and (**B**) kidney tissues in group 3 which was received the hydroalcoholic extract of *Arum conophalloides* at the dose of 400 mg/kg body weight (×100, H & E). Both tissues showed a normal structure.

of such side effects and their pathological impacts on the tissues is always difficult (Fig. **5**) [10]. Extracellular miRs, derived from various tissues/organs, are extremely stable in the circulation, and their expression could change during cellular damages or tissue injuries. Evidence shows that changes in miRNA expression occur earlier than conventional biomarkers, potentially offering more sensitivity and specificity of miRs compared to other available tests. Circulatory miR-122 is promising miRs that account for about 70% of all miRs in the liver, while its increase might be an implication for the development of liver diseases [19, 21, 23, 24]. Hence, the current research was designed to evaluate the potential of *A. conophalloides* extracts to cause liver injury.

Our findings showed that miR-122 serum levels were only increased in the experimental group treated with aqueous extract at the dose of 400 mg/kg once daily for 10 consecutive days. However, an examination of the liver sections showed the normal histological structure of the central vein and the surrounding hepatocytes. Accumulating evidences point to the role of circulating miR-122 as a replacement or supplement of the current serum biomarkers for liver injuries. It is highly abundant in the liver and almost absent in other organs, which represents it as a highly specific non-invasive circulating biomarker. Under normal conditions and the absence of overt cellular injuries, liver-specific miR-122 is released into the circulation in a constant manner *via* hepatic exosomes; however, its level in circulation is highly correlated to the hepatic expression [25, 26]. The predictive value of miR-122 for diagnosis of liver injury was later confirmed in rats; therefore, a marked increase in serum level of miR-122 corresponds well with a range of liver diseases [27, 28].

On the other hand, our results revealed that the elevation of miR-122 in the group treated with the aqueous extract at the dose of 400 mg/kg had occurred in the presence of a significant reduction in serum ALT and ALP levels. Overall, the elevation of circulatory miR-122 is preceded by a rise in biochemical liver markers, which makes it a promising biomarker for detecting hepatotoxic compounds at an early stage [29-31]. The inverse significant correlations of ALT and ALP with miR-122 expression were also reported in patients with hepatocellular carcinoma [32]. Although the AST, ALT, and ALP are the most commonly-requested tests for liver function screening, they are not necessarily specific to liver and changes can be associated with several extra-hepatic conditions. Moreover, emerging evidence has highlighted the role of genetic variations in the alteration of these enzymes [33, 34].

To the best of our knowledge, the major/active chemical compounds of *A. conophalloides* extracts have not been described, yet. However, T-muurolol (25.4%) is the main essential oil composition of the *A. conophalloides* leaves followed by nonanal, β-Ionone, T-cadinol, Fitone, and Methyl palmitate [1]. Several reports are also available in the literature regarding the chemical composition of other *Arum* species [35-38]. Although no histopathological lesions were observed in the current study, the hepatotoxicity and elevation of ALT, AST, and ALP, and histopathological changes such as aggregation of lymphocytes, steatosis, congestion, and necrosis were observed in female rats that had received the *Arum palaestinum* ethanolic extract at the doses of 500 mg/kg/day, for 30 days [39]. The main differences between our study and the aforementioned one might be the dose and treatment period. Moreover, a significant increase in the serum level of FBS was also noted following the *Arum palaestinum* ethanolic extract consumption [39], which is in contrast with our results.

CONCLUSION

Our data suggest that the serum level of miR-122 will be more helpful in detecting hepatic changes in the early stages rather than ALT and AST activity or histopathological evaluation of liver sections. Our findings highlight the potential hepatotoxicity effect of *A. conophalloides* aqueous extract; hence, the preparation manner can have a strong and direct impact on the results. Due to the popularity and consumption of this herb amongst the Iranian population, further comprehensive studies are warranted to discover its biochemical composition and its hepatotoxicity. It is important to point out that, despite our effort, there are some limitations to this investigation. For example, only one treatment duration was examined; therefore, the toxicological determination is limited. The quality of *A. conophalloides* extracts and the overall quality control need to be better determined. Besides, as

one miR was investigated merely, the overall impact is not known. Therefore, our report should be considered as a preliminary one which will be expanded in the future.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animal care was performed according to the guidelines of the local Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (Code: IR.SUMS.REC.1395.S1240).

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. Animal care was performed according to the guidelines of the local Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Haghighi H. Essential oil of the leaves of *Arum conophalloides* (Araceae) from Iran. Indian J Pharm Sci 2016; 12(3): 11-6. http://dx.doi.org/10.4236/ijcm.2015.69084

[2] Kaval İ, Behçet L, Çakilcioğlu U. Survey of wild food plants for human consumption in Geçitli (Hakkari, Turkey). Indian J Tradit Knowl 2015; 14(2): 183-90.

[3] Mosaddegh M, Naghibi F, Moazzeni H, Pirani A, Esmaeili S. Ethnobotanical survey of herbal remedies traditionally used in Kohghiluyeh va Boyer Ahmad province of Iran. J Ethnopharmacol 2012; 141(1): 80-95. http://dx.doi.org/10.1016/j.jep.2012.02.004 PMID: 22366675

[4] Erbay MŞ, Anıl S, Melikoğlu G. Plants used as painkiller in traditional treatment in Turkey-II Headache. Marmara Pharm J 2018; 22(1): 29-36. http://dx.doi.org/10.12991/mpj.2018.37

[5] Seifi Zangeneh M, Rafieirad M, Sazgar H. The effect of Kardeh (Biarum Bovei) hydro-alcoholic extract on pain threshold in STZ induced diabetic rats. J Herbal Drugs 2015; 6(3): 137-42.

[6] Hosseinzadeh S, Jafarikukhdan A, Hosseini A, Armand R. The application of medicinal plants in traditional and modern medicine: a review of *Thymus vulgaris*. Int J Clin Med 2015; 6(9): 635-42. http://dx.doi.org/10.4236/ijcm.2015.69084

[7] Atanasov AG, Waltenberger B, Pferschy-Wenzig E-M, *et al.* Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv 2015; 33(8): 1582-614. http://dx.doi.org/10.1016/j.biotechadv.2015.08.001 PMID: 26281720

[8] Chikezie PC, Ojiako OA. Herbal medicine: yesterday, today and tomorrow. Altern Integr Med 2015; 4(3): 195-9. http://dx.doi.org/10.4172/2327-5162.1000195

[9] Zhang J, Onakpoya IJ, Posadzki P, Eddouks M. The safety of herbal medicine: from prejudice to evidence. Evid Based Complement Alternat Med 2015; 2015: 316706. http://dx.doi.org/10.1155/2015/316706 PMID: 25838831

[10] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 2014; 4: 177. http://dx.doi.org/10.3389/fphar.2013.00177 PMID: 24454289

[11] Teschke R, Frenzel C, Glass X, Schulze J, Eickhoff A. Herbal hepatotoxicity: a critical review. Br J Clin Pharmacol 2013; 75(3): 630-6. http://dx.doi.org/10.1111/j.1365-2125.2012.04395.x PMID: 22831551

[12] Guan Y-S, He Q. Plants consumption and liver health. Evid Based Complement Alternat Med 2015; 2015(9): 824185. PMID: 26221179

[13] Licata A, Macaluso FS, Craxì A. Herbal hepatotoxicity: a hidden epidemic. Intern Emerg Med 2013; 8(1): 13-22. http://dx.doi.org/10.1007/s11739-012-0777-x PMID: 22477279

[14] Newsome PN, Cramb R, Davison SM, *et al.* Guidelines on the management of abnormal liver blood tests. Gut 2018; 67(1): 6-19. http://dx.doi.org/10.1136/gutjnl-2017-314924 PMID: 29122851

[15] Malakouti M, Kataria A, Ali SK, Schenker S. Elevated liver enzymes in asymptomatic patients - what should I do? J Clin Transl Hepatol 2017; 5(4): 394-403. http://dx.doi.org/10.14218/JCTH.2017.00027 PMID: 29226106

[16] Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol 2014; 20(2): 475-85. PMID: 24574716

[17] Fallatah HI. Noninvasive biomarkers of liver fibrosis: an overview. Adv Hepatol 2014; 2014(1): 357287. http://dx.doi.org/10.1155/2014/357287

[18] Halász T, Horváth G, Pár G, *et al.* miR-122 negatively correlates with liver fibrosis as detected by histology and FibroScan. World J Gastroenterol 2015; 21(25): 7814-23. http://dx.doi.org/10.3748/wjg.v21.i25.7814 PMID: 26167081

[19] Laterza OF, Scott MG, Garrett-Engele PW, Korenblat KM, Lockwood CM. Circulating miR-122 as a potential biomarker of liver disease. Biomarkers Med 2013; 7(2): 205-10. http://dx.doi.org/10.2217/bmm.12.107 PMID: 23547815

[20] Wang K, Yuan Y, Cho J-H, McClarty S, Baxter D, Galas DJ. Comparing the microRNA spectrum between serum and plasma. PLoS One 2012; 7(7): e41561. http://dx.doi.org/10.1371/journal.pone.0041561 PMID: 22859996

[21] Roderburg C, Benz F, Vargas Cardenas D, *et al.* Elevated miR-122 serum levels are an independent marker of liver injury in inflammatory diseases. Liver Int 2015; 35(4): 1172-84. http://dx.doi.org/10.1111/liv.12627 PMID: 25039534

[22] Hu J, Xu Y, Hao J, Wang S, Li C, Meng S. MiR-122 in hepatic function and liver diseases. Protein Cell 2012; 3(5): 364-71. http://dx.doi.org/10.1007/s13238-012-2036-3 PMID: 22610888

[23] Zhang Y, Jia Y, Zheng R, *et al.* Plasma microRNA-122 as a biomarker for viral-, alcohol-, and chemical-related hepatic diseases. Clin Chem 2010; 56(12): 1830-8. http://dx.doi.org/10.1373/clinchem.2010.147850 PMID: 20930130

[24] Starkey Lewis PJ, Dear J, Platt V, *et al.* Circulating microRNAs as potential markers of human drug-induced liver injury. Hepatology 2011; 54(5): 1767-76. http://dx.doi.org/10.1002/hep.24538 PMID: 22045675

[25] Wang Y-L, Yu W. Association of circulating microRNA-122 with presence and severity of atherosclerotic lesions. PeerJ 2018; 6: e5218. http://dx.doi.org/10.7717/peerj.5218 PMID: 30013851

[26] Willeit P, Skroblin P, Kiechl S, Fernández-Hernando C, Mayr M. Liver microRNAs: potential mediators and biomarkers for metabolic and cardiovascular disease? Eur Heart J 2016; 37(43): 3260-6. http://dx.doi.org/10.1093/eurheartj/ehw146 PMID: 27099265

[27] Sharapova T, Devanarayan V, LeRoy B, *et al.* Evaluation of miR-122 as a serum biomarker for hepatotoxicity in investigative rat toxicology studies. Vet Pathol 2016; 53(1): 211-21. http://dx.doi.org/10.1177/0300985815591076 PMID: 26123229

[28] Starckx S, Batheja A, Verheyen GR, *et al.* Evaluation of miR-122 and other biomarkers in distinct acute liver injury in rats. Toxicol Pathol 2013; 41(5): 795-804. http://dx.doi.org/10.1177/0192623312464436 PMID: 23143187

[29] Antoine DJ, Dear JW, Lewis PS, *et al.* Mechanistic biomarkers provide early and sensitive detection of acetaminophen-induced acute liver injury at first presentation to hospital. Hepatology 2013; 58(2): 777-87. http://dx.doi.org/10.1002/hep.26294 PMID: 23390034

[30] Park H-K, Jo W, Choi H-J, *et al.* Time-course changes in the expression levels of miR-122, -155, and -21 as markers of liver cell damage, inflammation, and regeneration in acetaminophen-induced liver injury in rats. J Vet Sci 2016; 17(1): 45-51. http://dx.doi.org/10.4142/jvs.2016.17.1.45 PMID: 27051339

[31] Peng X, Li Q, Lu S, He X, Yu S, Zhang Z, *et al.* The plasma miR-122 basal levels respond to circulating catecholamine in rats. bioRxiv 2018; 2018(1): 257402.

[32] Amr KS, Elmawgoud AHA, Elazeem ERA, Ezzat WM. Early diagnostic evaluation of miR-122 and miR-224 as biomarkers for hepatocellular carcinoma. Genes Dis 2017; 4(4): 215-21. http://dx.doi.org/10.1016/j.gendis.2017.10.003 PMID: 30258925

[33] Yuan X, Waterworth D, Perry JR, *et al.* Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. Am J Hum Genet 2008; 83(4): 520-8. http://dx.doi.org/10.1016/j.ajhg.2008.09.012 PMID: 18940312

[34] Robles-Díaz M, Medina-Caliz I, Stephens C, Andrade RJ, Lucena MI. Biomarkers in DILI: one more step forward. Front Pharmacol 2016; 7: 267. http://dx.doi.org/10.3389/fphar.2016.00267 PMID: 27597831

[35] Christie WW. 13-Phenyltridec-9-enoic and 15-phenylpentadec-9-enoic acids in *Arum maculatum* seed oil. Eur J Lipid Sci Technol 2003; 105(12): 779-80. http://dx.doi.org/10.1002/ejlt.200300865

[36] Kite GC. The floral odour of *Arum* maculatum. Biochem Syst Ecol 1995; 23(4): 343-54. http://dx.doi.org/10.1016/0305-1978(95)00026-Q

[37] Salik S, Alpinar K, Imre S. Fatty acid composition of the seed oil of *Arum* italicum miller. J Food Lipids 2002; 9(2): 95-103. http://dx.doi.org/10.1111/j.1745-4522.2002.tb00212.x

[38] El-Desouky SK, Kim KH, Ryu SY, Eweas AF, Gamal-Eldeen AM, Kim Y.K. A new pyrrole alkaloid isolated from *Arum* palaestinum Boiss and its biological activities. Arch Pharm Res 2007; 30(8): 927-31. http://dx.doi.org/10.1007/BF02993958 PMID: 17879743

[39] Al-Qudah M. Histological and biochemical studies on liver of female rats treated with different concentrations of ethanolic extract of *Arum* palaestinum. J Appli Environ Biol Sci 2016; 6(7): 7-16.