

## ARTICLE

## HEPATOTOXICITY EVALUATION OF METHANOL LEAVES EXTRACT OF *ARUM MACULATUM*

Zahraa Hussein M Kadri\*, Niran A Ibrahim, Hanady S Al-Shmgani

Department of Biology, Collage of Education for Pure Sciences (Ibn AL- Haithum), University of Baghdad, Baghdad, IRAQ

## ABSTRACT

**Background:** *Arum maculatum* is one of Araceae family, commonly known as Kardi. This plant is used medically to treat various diseases such as rheumatic pain, cardiovascular and anti-inflammatory diseases. However, prolong consumption may cause harmful side-effects. This study aimed to investigate the hepatic toxicological effects of leave extract in albino male mice. **Methods:** Hepatotoxicity effects were assessed in albino male mice after inducing hepatic damage with carbon tetrachloride (CCl<sub>4</sub>). The liver function enzymes in serum, and histopathological were evaluated. **Results:** Histopathological study showed loss of hepatic architecture normal appearance associated with hepatocytes vacuolated cytoplasm. Serum enzymes level showed significant increase in GPT, GOT and ALP in homogenized liver tissue in group treated with methanol extract comparable with CCL<sub>4</sub> treated group (positive control) induced hepatotoxicity and negative control group treated with distilled water. **Conclusion:** Methanol leaves extract of *Arum maculatum* possess significant histological and biochemical damage in mice liver tissue.

## INTRODUCTION

The vast majority of people on this planet still rely on their traditional *materia medica* (medicinal plants and other materials) for their everyday health care needs. It is also a fact that one quarter of all medical prescriptions are formulations based on substances derived from plants or plant-derived synthetic analogs, and according to the World Health Organization (WHO), 80% of the world's population, primarily those of developing countries, rely on plant- derived medicines for their healthcare [1]. This is reasoned by the fact that medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health, and accordingly, they have been subjected to an intensive investigation to reveal their pharmaceutical potentials [2].

*Arum maculatum* is one of Araceae family, widely distributed in many countries in European and Middle East [3]. It is also can found in the north of Iraq where commonly known as Kardi. Traditionally *A. maculatum* used as a folk medicine to treated rheumatic pain, kidney stone disease, colitis, liver disease and hyperacidities [4]. Furthermore it has been clinically demonstrated to have an anti-inflammatory activity in the intestinal and respiratory tract. In addition *A. maculatum* has antimicrobial and antifungal activity [5]. However, this plant can be a double adage sword herb. Researchers have been reported the harmful side effects, Nabeel *et al.* 2008, investigated the cytological damage of water leave extract in bone marrow cell of mice and suggested a range of damages in all mitosis stage.

In a bid to better understand the impact of *A. maculatum* consumption, this study aimed to investigate the enzymatic and histopathological damages of the methanol leave extract in mice liver. For our knowledge this is the first study described the detrimental effects of this plant in Iraq.

## MATERIALS AND METHODS

## Arum maculatum

The *A. maculatum* was grown and collection in spring. The (whole plant) obtained from local markets in Erbil, and classified at the Herbarium of Biology Department (College of Education for Pure sciences/Ibn Al-Haitham, University of Baghdad). It was left at room temperature to dry and supplied as powdered dried material.

## Plant extract

The leaves powder was extracted with methanol 80%; 10 grams of the powder were extracted with 100 ml of solvent at 60°C for six hours using the Soxhlet apparatus. Then, the resulted extract solution was evaporated by a rotary evaporation. The collected crude deposit extract was frozen at -20°C until use to prepare the required doses [6]. One dose was prepare (100mg/Kg) based on [7].

## Laboratory animals

The study was carried out on Albino male mice (*Mus musculus*) obtained from Pharmaceutical Control Department (Health Ministry). Twenty four mice weighing 23 – 27 g, of 8-9 week age were housed in conventional conditions at animal house laboratory in Ibn Al-Haitham College and fed on a standard pellet and sterilized distilled water.

## KEY WORDS

*Arum maculatum*,  
Methanol extract,  
Liver enzymes, CCL<sub>4</sub>.

Received: 10 Oct 2016  
Accepted: 12 Nov 2016  
Published: 1 Dec 2016

\*Corresponding Author  
Email: zahraaa\_ali@yahoo.com  
Tel.: +964 7708882106

### Experimental design

Hepatotoxicity effects were assessed in albino male mice after inducing hepatic damage with carbon tetrachloride (CCl<sub>4</sub>). The liver function enzymes in serum, and histopathological were evaluated. Four groups of mice (each of six mice and the total was 26 animals), but with a different experimental design.

Animals were divided into four groups of six mice each and IP injected as follows:

First group: The negative control group was mice administrated with distilled water for 7 days once daily.

Second group: Mice were administered with a single dose of 0.2% CCl<sub>4</sub> in olive oil (0.1ml) in day 1 and 2, and then received distilled water (0.1 ml) as a single daily dose for 7 days (positive control).

Third group: Mice were administered with a single dose of *A. maculatum* extract (100 mg/kg), once daily for 7 days.

Forth group: Mice were administered with a single dose of 0.2% CCl<sub>4</sub> in olive oil (0.1ml) in day 1 and 2, and then received 0.1 ml of the dose (100 mg/kg) of *A. maculatum* methanol extract once daily for 7 days.

### Blood collection

After 8 days of experiment and before sacrificing the animals, blood was collected by heart puncture, transferred to Eppendorf tube and allowed to clot at room temperature for 15 minutes. Then serum was separated by centrifugation at 3000 rpm for 10 minutes. The serum was used for the assessment of liver function enzymes Glutamic-pyruvic transaminase (GPT), Glutamate oxaloacetate transaminase (GOT), and alkaline phosphatase (ALP). After blood collection, the mouse was sacrificed and dissected to obtain the liver.

### Determination of liver function enzyme

The enzyme activity of GPT and GOT were determined in mouse serum following the enzymatic colorimetric method [8], where Randox Company kit was used. While Alkaline Phosphatase (ALP) was assessed in mouse serum using a commercial kit produced by Bio Merieux Company and the most commonly used method is that [9], in which di-sodium phenyl phosphate is hydrolyzed with liberation of phenol and formation of sodium phosphate. The amount of phenol formed is estimated colorimetrically. The following equation was employed to assess the activity of ALP.

$$\frac{\text{Sample Absorbance} - \text{Control Absorbance}}{\text{Standard Absorbance} - \text{Blank Absorbance}}$$

### Histo pathological evaluation of liver

The liver tissue prepared for histopathological study as described by [10, 11]. Samples were fixed in 10% formalin for 24 h, followed by dehydration with a gradual series of alcohol (30 – 100%) for 5 min each. Then the samples cleared in two changes of xylene before embedded in paraffin wax for sectioning. Cross sections of 5 µm thickness were prepared and stained with hematoxylin (Harison) and eosin according to standard method. Histopathological changes were performed under light microscopy as compared to control group.

### Statistical analyses

The values of the investigated parameters were given in terms of mean ± standard error, and differences between means were assessed by analysis of variance (ANOVA) and Duncan test, using the computer programme SPSS version13. The difference was considered significant when the probability value was equal or less than 0.05.

## RESULTS

The hepatotoxicity evaluation include assessment of liver functional enzymes (GPT, GOT and ALP), in serum, as well as histopathological evaluation of liver tissue, in albino male mice.

### Liver functional enzymes

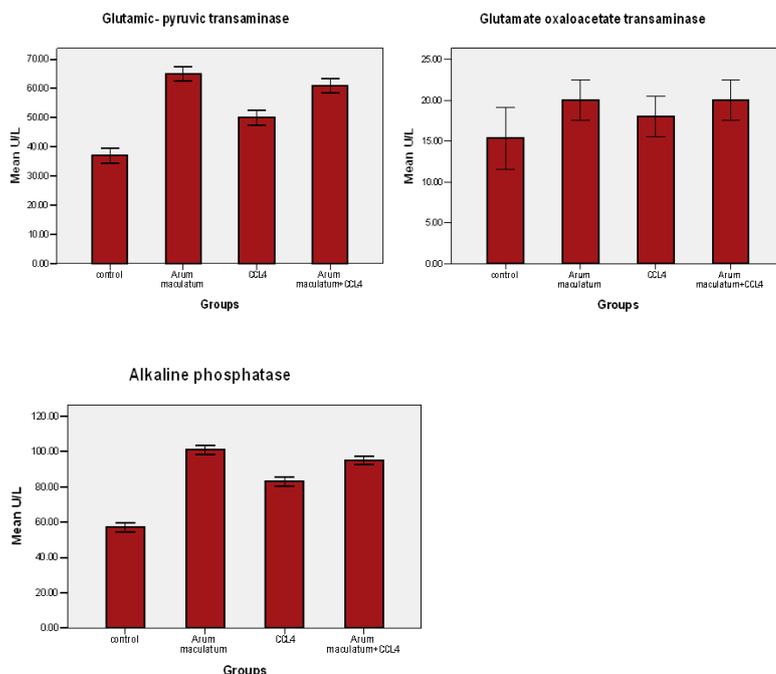
As shown in [Table 1], treatment with CCl<sub>4</sub> were significantly increased the activity of enzymes (18.00±0.58, 50.00±0.57 and 83.00±0.58) U/L, compared with negative control (15.33±0.88, 37.00±0.57 and 57.00±0.58) U/L, respectively. As well as, significantly increased the means of enzymes level (20.00±0.57, 65.00±0.58 and 101.00±0.57) U/L, in mice treated with methanol extract of *A. maculatum* compared with negative and positive controls, respectively, (P≤0.05) [Fig. 1].

**Table 1:** Effects of *A. maculatum* methanol extract on GPT, GOT and ALP in sera of carbon tetrachloride (CCl<sub>4</sub>)-treated albino male mice

Groups	GPT(M±SE)	GOT(M±SE)	ALP(M±SE)
--------	-----------	-----------	-----------

Negative control	37.00±0.57 <sup>A</sup>	15.33±0.88 <sup>A</sup>	57.00±0.58 <sup>A</sup>
<i>Arum maculatum</i>	65.00±0.58 <sup>D</sup>	20.00±0.57 <sup>B</sup>	101.00±0.57 <sup>D</sup>
CCL <sub>4</sub> (positive control)	50.00±0.57 <sup>B</sup>	18.00±0.58 <sup>B</sup>	83.00±0.58 <sup>B</sup>
Arum+CCL <sub>4</sub>	61.00±0.54 <sup>C</sup>	20.00±0.56 <sup>B</sup>	95.00±0.55 <sup>C</sup>

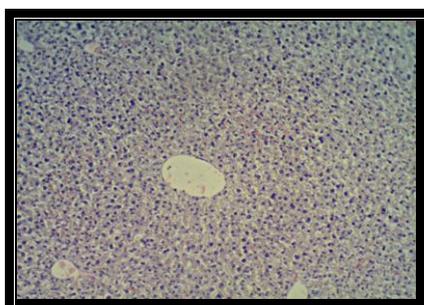
\*Different letters represent significant difference (P ≤ 0.05) between means of columns (Duncan test).



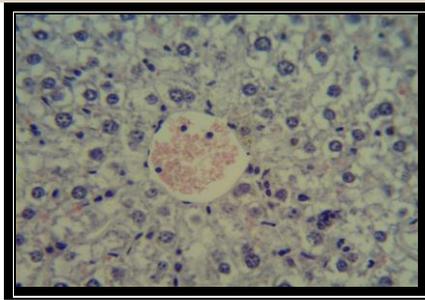
**Fig.1:** Graphics show effects of *A. maculatum* methanol extract on GPT, GOT and ALP in sera of carbon tetrachloride (CCL<sub>4</sub>)-treated albino male mice.

### Histopathological evaluation of liver

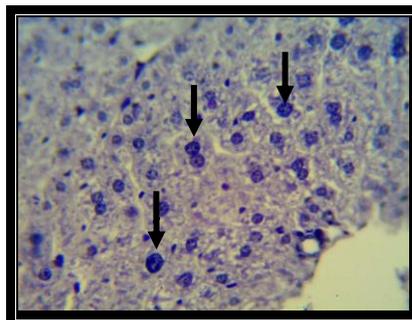
Liver sections from control group I revealed normal architecture with central vein with radiating cords of liver cells, the hepatocytes had vesicular nuclei and granular cytoplasm and blood sinusoids were evident between the cords of hepatocytes [Fig. 2 and 3]. Treated with CCL<sub>4</sub> showed Karyomegaly, enlargement of hepatocyte cytoplasm and nucleus, angular with fragmented and condensed nuclear material within the cytoplasm [Fig. 4 and 5]. However, treatment with methanol extraction showed dilated central vein (CV), loss of normal hepatic architecture and the cytoplasm of hepatocytes is vacuolated [Fig. 6].



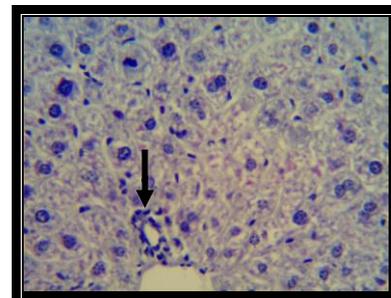
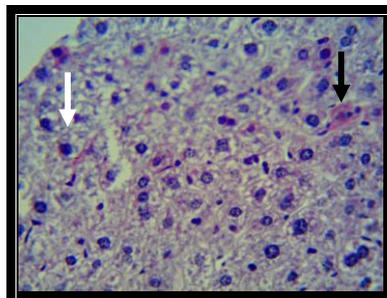
**Fig.2:** Cross section of the liver of albino mice (negative control), (H & E, 10X).



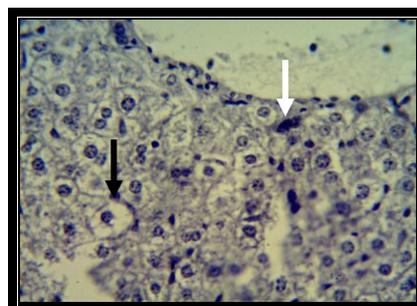
**Fig.3:** Cross section of the liver of albino mice (negative control) showed Glycogen accumulation (normal accumulation in hepatocytes), (H & E, 100X).



**Fig.4:** Cross section of the liver of albino mice treated with CCL4. Arrow indicates enlargement of hepatocyte cytoplasm and nucleus, (H & E, 400X).



**Fig.5:** Cross section of the liver of albino mice treated with CCL4. Affected hepatocytes are hyper eosinophilic and angular with fragmented and condensed nuclear material within the cytoplasm. (black arrow), pyknotic nuclei (white arrow), (Hx & E, 400X).



**Fig.6:** Cross section of the liver of albino mice treated with methanol extraction of *A. maculatum*. showing: dilated central vein (CV), loss of normal hepatic architecture. The cytoplasm of hepatocytes is vacuolated (black arrow) pyknotic nuclei (white arrow), (Hx & E, 400 X).

## DISCUSSION

Liver is the major site of detoxification and the primary target of drug exposure in the body. High levels of drugs cause various hepatic disorders by producing pro-oxidants/reactive oxygen species (ROS), which are able to induce cellular damage in a variety of ways by affecting the cellular biomolecules, such as lipids, DNA and proteins [12]. In this study, the results showed increase in functional liver enzymes (GPT, GOT and ALP), and damages in histopathological changes of liver section in mice treated with CCL<sub>4</sub>. The hepatotoxicity induced by CCL<sub>4</sub> is mainly due to its metabolite CCL<sub>3</sub><sup>-</sup>, which is a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids. In the presence of oxygen, lipid peroxides are produced, leading to liver damage, which is characterized by fatty liver, cirrhosis and necrosis [13][22]. In addition, in CCL<sub>4</sub>-induced hepatotoxicity, the extent of hepatic damage is assessed by the increased level of cytoplasmic enzymes (GPT, GOT and ALP), which leads to leakage of large quantities of the enzymes into the blood circulation and could be regarded as an index of the liver parenchymal cells damage [14]. Carbon tetrachloride-induced hepatotoxicity in mice caused a severe centrilobular necrosis, steatosis and damage to the structural integrity of liver and was reflected by increase in the liver hepato-specific enzymes in the serum, because they are cytoplasmic in location and are released into circulation after cellular damage causes the destruction of cell components, then cell death [15, 16].

*Arum maculatum* is medical plant used to treatment rheumatic pain [4][21], which contain many active compounds such as, alkaloid, saponin, cyanogenic, glycosides and lectin [17], but the research revealed that lectin isolated from *A. maculatum* effected on immune system, which found agglutinin presents in pro-inflammatory activity inducing neutrophil migration [18]. So many precaution from prolong consumption of this plant possible caused harmful side effects.

Moreover, the results revealed that methanol extract of *A. maculatum* at dose (100mg/Kg) caused hepatotoxicity by increased functional liver enzymes (GPT, GOT and ALP), as well as damage in liver tissue of mice treated with extract.

Nabeel and his colleagues [2008] illustrated that *A. maculatum* extract at lowest dose (125 mg/Kg), had cytotoxic effects on the bone marrow cells in albino male mice by induced abnormalities in restitution and multinuclei, abnormal prophase, C-metaphase, sticky chromosomes fragments, bridges, non-congression and laggards. However, disturbed metaphase, anaphase and telophase may be due to disturbance of spindle apparatus which allows that the chromosomes to spread irregularly over the cell; results c-mitosis, star-anaphase and star-telophase respectively [19], or may be attributed to the inhibitory effect of *A. maculatum* extract on DNA, RNA and protein synthesis on culture mammalian cells [20].

## CONCLUSION

As results, hepatotoxicity effects of the extract were overwhelmed by their in increasing the hepatic damage in mice group treated with methanol extract of *A. maculatum*.

### CONFLICT OF INTEREST

There is no conflict of interest.

### ACKNOWLEDGEMENTS

None

### FINANCIAL DISCLOSURE

None

## REFERENCES

- [1] Gurib-Fakim A. [2006] Medicinal plants: traditions of yesterday and drugs of tomorrow' Mol Aspects Med J. 27: 91-93.
- [2] Li Y, Hu Y, Shi S, Jiang L. [2014] Evaluation of antioxidant and immuno-enhancing activities of Purslane polysaccharides in gastric cancer rats' Int J Biol Macromol, 68:113-116.
- [3] Mohammed ZH, Ibraheem RM. [2015] Anti-oxidant activity of methanol extracts of *Arum maculatum* L. and *Physalis peruviana* L. Plants' Ibn AL-haitham J for pure and appl sci, 28(2).
- [4] Safari E, Amiri M, Bahador A, Amiri M, Esmaeili D. [2014] The study of antibacterial effects of alcoholic extracts of *Arum maculatum*, *Allium hirtifolium* and *Teucrium polium* against nosocomial resistance bacteria' Int J Curr Microbiol App Sci, 3(2): 301-605.
- [5] ÇolaK F, Savaro lu F, Ihan S. [2009] Antibacterial and Antifungal Activities of *Arum maculatum* L. Leaves Extracts' J App Biol Sci, 3(3): 13-16.
- [6] Sabahi M, Mansouri SH, Ramezani M, Gholam-Hoseinian A. [1987] Screening of plants from the southeast of Iran anti-microbial activity' Int J Crude Drug Res, 25: 72-76.
- [7] Nabeel M, Abderrahman S, Papini A. [2008] Cytogenetic effect of *Arum maculatum* Extract on the bone marrow cells of mice' International journal of cytology cytosystematics and cytogenetics, 61 (4):383-387.
- [8] Reitman S, Frankel S. [1957] A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases' Am J Clin Pathol. 28:56-63.
- [9] Gometi SA, Ogugua VN, Odo CE, Joshua PE. [2014] Effects of some anti-diabetic plants on the hepatic marker enzymes of diabetic rats' Afr J Biotechnol, 13: 905-909.
- [10] Camargo MMP, Martinez CBR. [2007] Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream' Neotrop Ichthyol, 5:327-336.
- [11] Rowe A, Zhang L, Hussain A, Braet F, Ramzan I. [2011] Assessment and histological analysis of the IPRL

- technique for sequential in situ liver biopsy' Comp Hepatol, 10: 7.
- [12] Ziech D, Franco R, Georgakilas AG, et al. [2010] The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development' Chem Biol Interact, 188:334-339.
- [13] Zeashan H, Amresh G, Singh S, Rao CV. [2008] Hepatoprotective activity of *Amaranthus spinosus* in experimental animals' Food Chem Toxicol. 46: 3417-3421.
- [14] Shankar NLG, Manavalan R, Venkappayya D, Raj CD. [2008] Hepatoprotective and antioxidant effects of *Commiphora berryi* (Arn) Engl bark extract against CCl<sub>4</sub>-induced oxidative damage in rats' Food Chem Toxicol, 46: 3182-3185.
- [15] Li Y, Hu Y, Shi S, Jiang L. [2014] Evaluation of antioxidant and immuno-enhancing activities of Purslane polysaccharides in gastric cancer rats' Int J Biol Macromol, 68:113-116.
- [16] Jin Xf, Qian J, Lu YH. [2011] The role of hepatoprotective effect of a flavonoid-rich extract of *Salvia plebeia* R.Br. on carbon tetrachloride induced acute hepatic injury in mice' J Med Plants Res. 5: 1558-1563.
- [17] Stuart M. [1979] The Encyclopedia of Herbs and Herbalism' Orbis Publishing, London
- [18] Alencar VB, Alencar NM, Assreuy AM, et al. [2005] Pro inflammatory effect of *Arum maculatum* lectin and role of resident cells' Int J Biochem Cell Biol. 37(9): 1805-1814.
- [19] Paul A, Nag S, Sinha K. [2013] Cytological Effects of Blitox on Root Mitosis of *Allium cepa* L' International Journal of Scientific and Research Publications. 3( 5): 2250-3153.
- [20] Al ahdal MN, Mcgarry TJ, Hannan MA. [1988] Cytotoxicity of Khat (*Catha edulis*) extract on cultured mammalian cells' Mutat Res. 204: 317-322.
- [21] Arunkumar N, Ram Kumar K, Venkataraman V. [2016] Automatic Detection of Epileptic Seizures Using Permutation Entropy, Tsallis Entropy and Kolmogorov Complexity. Journal of Medical Imaging and Health Informatics, 6(2);526-531.
- [22] Arun kumar N, Kumar KR, Venkataraman V. [2016] Automatic Detection of Epileptic Seizures Using New Entropy Measures. Journal of Medical Imaging and Health Informatics, 6(3): 724-730.