

Protective features of *Myrtus communis* leaves against the genotoxic effects of arsenic in Wistar rats

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Abstract

Our study was aimed to investigate the effect of arsenic chloride (AsCl₃) on the production of the tumor suppressor protein (P53) in the rat and study the ameliorate effect of *Myrtus communis* leaves on decreasing the toxicity of AsCl₃. Here, 12 Wistar rats were used and randomly divided into 4 groups (3 rats/group). The 1st-group, T1, rats were administrated 10mg/kg BW of AsCl₃ only. The 2nd-group, T2, rats were given 10mg/kg BW of AsCl₃ and 3mg/ml of myrtle extract. The 3rd-group, T3, rats were supplied with 3mg/ml of myrtle extract only. The 4th-group rats were provided with 0.2ml of distilled water as a control group, C. A P53-based gene expression (PGE) analysis was introduced using a real time PCR (RT-PCR) technique. The results recorded higher levels of the PGE in the T3 group and followed by lower values in the T1 group when both compared with other groups. This study shows that AsCl₃ induces negative effects on the PGE levels and probably the protein activity of P53 gene resulting in low gene expression; however, *Myrtus communis* improves the status by increasing the levels of PGE if used alone or mixed with AsCl₃.

Keywords: Arsenic chloride, genotoxic effect, *Myrtus communis*, P53, rats.

INTRODUCTION

Exposure to heavy metals is a common environmental and health problem induced by waste product contaminants such as arsenic (As) via the misuse of pesticides, herbicides, and insecticides. Some risky health situations caused by this exposure such as neurotoxicity, genotoxicity, or carcinogenicity. As an example for such dangerous contaminants is As that causes physiological, pathological, and genetic toxicological effects (Flora, Mittal and Mehta, 2008). There are some toxic compounds formed out of this element that expose the environment via soil and ground seeping water contamination. However, toxicity in animals and humans are generated when exposing for long time to this contamination via multiple methods such as drinking water. This problem affects the health of humans and animals in some countries that could cause serious problems such as cancers in skin, kidneys, bladders, or lungs. The immediate symptoms of this intoxication include abdominal pain, vomiting, and esophageal pain (Ratnaik, 2003; Hughes *et al.*, 2011). In addition to the physiological and chemical changes induced by arsenic, some genetic intoxication represented by changes in the levels of gene expression of some important genes such as the tumor suppressor protein (P53) is revealed (Hsu *et al.*, 1999; Sandoval *et al.*, 2007). Revealed studies about treating As toxicity are generously many via the use of certain substances such as Biochanin (Jalaludeen *et al.*, 2016), Plantain Root (Israel Oyewole *et al.*, 2015), antioxidants (Kanou K. and Belyagoubi-Benhammou, N., Ghembaza, N. and Atik Bekkara, 2014; Varghese *et al.*, 2016), *Triticum aestivum* Linn (Lakshmi *et al.*, 2015), edible freshwater (Sajed Ali and Maiti, 2017), and Metallothionein (Agrawal *et al.*, 2014). *Myrtus communis* was studied for its effects on improving the health status after exposing to arsenic toxicity as *M. communis* has pharmacological and anti-toxicological effects (Sumbull *et al.*, 2011; Chokri Messoud, Messoud and Boussaid, 2013; Yildirim *et al.*, 2015; Miraj and Kiani, 2016). Our study was aimed to investigate the effect of arsenic chloride (AsCl₃) on the production of the tumor suppressor protein (P53) in the rat and study the ameliorate effect of *Myrtus communis* leaves on decreasing the toxicity of AsCl₃.

MATERIALS AND METHODS

Animals and experimental design

Here, 12 Wistar rats (200-220gm), obtained from the College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq, were acclimatized to the experiment environment for 7 days prior to the beginning of the experiment. The experimental environment includes 12hrs light/dark cycle,

housing in plastic cages in a well-ventilated room at 25±2 °C, and food and water were provided ad libitum.

The rats were randomly divided into 4 groups (3 rats/group). The 1st-group, T1, rats were administrated 10mg/kg BW of AsCl₃ only. The 2nd-group, T2, rats were given 10mg/kg BW of AsCl₃ and 3mg/ml of myrtle extract. The 3rd-group, T3, rats were supplied with 3mg/ml of myrtle extract only. The 4th-group rats were provided with 0.2ml of distilled water as a control group, C. The experiment period was 30 days, supplying of the variables. The AsCl₃ was obtained from the Central Laboratory of the mentioned university above. The AsCl₃ was administered intraperitoneally (IP), and the myrtle extract was added to the drinking water.

Extraction of *Myrtus communis* Linn leaves

Ethanol-based extraction of the *Myrtus communis*, myrtle, was performed using 1kg of the *Myrtus communis* Linn leaves, purchased from local markets, that were ground until getting powder form. Utilizing 1L of hydroethanol (80/20, v/v) for an 8hr-period that was repeated for 4 times, the extraction process was done. The resulted products were pooled and concentrated using vacuuming at no more 60°C. The final extract was kept frozen at -20°C for later use. The concentrated product was poured into sterile Petri dishes and oven-dried at 40 °C. Then, the dried powder was collected and mixed with distilled water at 3 mg/ml.

Tissue sampling

After the period of the experiment was done, the rats were sacrificed, and liver tissues were dislocated using a sterile scissor and snapped-frozen in liquid N₂ to be directly place in 1.5ml tubes preloaded with diethyl pyrocarbonate (DEPC). Immediately after that, these samples were sent to the RT-PCR unit in the college mentioned above to perform the PGE.

Total RNA extraction and reverse transcription (cDNA production)

The process of this extraction was performed employing 200mg of the liver tissue that was extracted via the use of Accuzol kit (Bioneer Company, Korea) and following the kit instructions. Nanodrop (THERMO, USA) was used for evaluating the resulted total RNA quantitatively and qualitatively. To get rid of the most DNA, DNase I enzyme kit was used according to (Promega Com., USA). The reverse transcription (RT) of the mRNA, 100ng/μl, into cDNA was performed using AccuPower-RocktScript RT (Bioneer Inc., Korea) and following the kit instructions. The conditions used are 50 °C for 1hr to perform cDNA process and 95 °C for 5mins to do heat inactivation process.

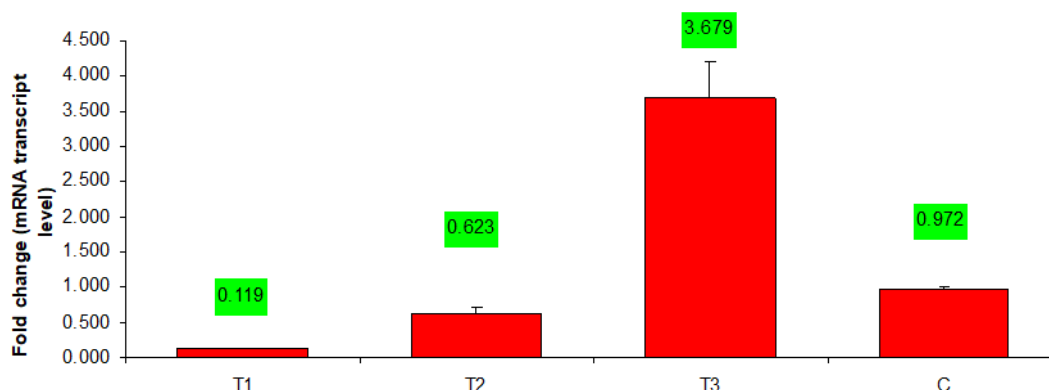


Figure 1: The means of the P53-related gene expression. T1: The AsCl₃ group, T2: the AsCl₃+myrtle group, T3: the myrtle group, and C: the control group.

RT-PCR technique

The relative gene expression used is $2^{-\Delta\Delta CT}$ Livak method (Livak and Schmittgen, 2001). The PGE was done using BioRad-RT-PCR System, USA, employing SYBER Green master mix. The housekeeping gene was β -actin. Primer3 plus was utilized to design the primers, and they are NM_030989.3 (F: ATCCTATCCGGTCAGTTGTTGG and R: AATGCAGACAGGCTTTGCAG) to amplify a 143bp region in the P53 gene and NM_031144.3 (F: CTAGGCACCAGGGTGTGATG and R: GTCAGGATGCCTCTCTTGCTC) to amplify an 85bp piece of the β -actin gene. The mixes for the reactions of the RT-PCR for both genes were generated using AccuPower-2XGreen kit (Bioneer Inc., Korea) and following the protocol provided with the kit. The conditions introduced to the reactions in the thermocycler were 1cycle-50°C-1hr for initial denaturing, 30cycles-(95°C-20s for main denaturing and 60°C-30s for annealing/extension/detection), and 1cycle at 60-95°C-0.5s for the melting process.

RESULTS

The relative PGE in the T1, T2, T3, and C were 0.119, 0.623, 3.679, and 0.972 respectively. The results recorded higher levels of the PGE in the T3 group and followed by lower values in the T1 group when both compared with other groups, figure 1

DISCUSSION

Heavy metals generate very dangerous impacts on lives of huge ranges of animal species plus humans. These compounds, such as cadmium, mercury, and arsenic, induce accumulative properties in different living tissues leaving improper physiological actions (Mussali-Galante *et al.*, 2013; Shaheen *et al.*, 2016; Umme *et al.*, 2016).

Arsenic chloride alone showed lower PGE in T1 group than that in the other groups which indicates the high detrimental effects of AsCl₃ on this gene. This could be an evidence for high probability of tumor incidence in these tissues. When oxidation processes in cells are induced by toxic compounds, DNA-damaging processes are initiated leading to decreasing the expression of P53 gene if the damage is extensive (Hsu *et al.*, 1999; Ozaki and Nakagawara, 2011; Noman *et al.*, 2015). The induced intoxication by AsCl₃ is linked to glutathione leading to increasing the levels of free radicles such as the hydroxyl, superoxide, H₂O₂, singlet oxygen, and isoprostanes and peroxides. When AsCl₃ intoxication is initiated, increasing the levels of highly toxic substances such as dimethylarsenic peroxy and dimethylarsenic radicles that cause complete DNA destruction are released (Jomova *et al.*, 2011).

Interestingly, T3 group that was administrated myrtle extract revealed higher PGE, 3.679, comparing that to other groups. This indicates that *Myrtus communis* places an important effect in increasing the P53 gene expression and probably its protein activities. *Myrtus communis* has some therapeutic and pharmacological effects such as anti-microbial, anti-anxiety, antioxidant, herbicidal, and anti-cytotoxic, and anti-tumor features (Miraj and Kiani, 2016). Our results agree with many reports and studies that showed the anti-toxicological effects of bioactive substances present in different substances such as essential oil, tannins, and flavonoids (Sumbull *et al.*, 2011; Ganapathy *et al.*, 2014). The oil of *Myrtus communis* contains monoterpenes hydrocarbons and related compounds and α -pinene that play important roles in challenging the reactive oxygen species (ROS) via combing to those ROS leading to deactivating process and fighting diseases such as cancers and cardiovascular conditions (Snoussi *et al.*, 2015). *Myrtus communis* Linn has high amounts of linoleic acid, octane 3,5- dimethyl, oleic acid and other compounds that act against the effects of toxicants via activating different cell pathways such as programmed cell death to provide the cells with protection against the toxicological properties of heavy metals (Qader, Al-Saadi and Al-Saadi, 2017; Shahnazi *et al.*, 2017).

This study shows that AsCl₃ induces negative effects on the PGE levels and probably the protein activity of P53 gene resulting in low gene expression; however, *Myrtus communis* improves the status by increasing the levels of PGE if used alone or mixed with AsCl₃.

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