# Role of saffron (*Crocus sativus L.*) and honey syrup on aluminum-induced hepatotoxicity

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## ABSTRACT

**الأهداف**: يتناول هذه البحث دراسة تأثير مركب كلوريد الألومنيوم على زيادة مستوى سمية الكبد من الناحية البيوكيميائية والجزيئية والدور العلاجي للزعفران والعسل في التخلص من هذه السمية.

**الطريقة**: أُجريت هذه الدراسة في قسم الأحياء في كلية العلوم بجامعة الملك خالد، أبها، المملكة العربية السعودية وذلك خلال الفترة من يوليو 2009م إلى أغسطس 2009م. لقد تم استخدام سلالتين من الفئران وهما ( BALB/c وC57BL/6 )، وذلك باختيار 20 حيواناً من كل سلالة، ومن ثم قُسمت الحيوانات عشوائياً إلى أربع مجموعات وهي: مجموعة التحكم (المجموعة السليمة)، والمجموعة التي تعاني من سمية كلوريد الألومنيوم، والمجموعة التي تعاني من السمية وتم علاجها بالزعفران، والمجموعة التي تعاني من السمية وتم علاجها بالعسل. لقد تم قياس معدل أنزيمات الكبد وهي ناقلة ببتيد غاما غلوتاميل، وناقلة أمين الآلانين، ونازعة أمين الاسبارتات، وأنزيم فوسفاتازا القلوي، بالإضافة إلى مجموع البليروبين ومعدل تأكسد الدهون في جميع المجموعات. كما تم عمل مسح لمرسال الرنا ( mRNA )، وبعد ذلك عُزلت جينات التنظيم التناقصي والتزايدي للخلية وتم نسخها وتحديد تسلسلها الجيني.

**النتائج**: لقد كان هناك ارتفاعاً واضحاً في مستويات الكولسترول، والدهون الثلاثية، ومستويات ناقلة ببتيد غاما غلوتاميل، وناقلة أمين الآلانين، ونازعة أمين الاسبارتات، وأنزيم فوسفاتازا القلوي، ومعدل تأكسد الدهون بالإضافة إلى ارتفاع سكر الدم وذلك في المجموعات التي تعاني من سمية كلوريد الألومنيوم بالمقارنة مع مجموعة التحكم، إلا أن علاج هذه الحيوانات بالزعفران أوالعسل أدى إلى تحسن في وظائف الكبد وتقليل تأكسد الدهون. لقد تم الكشف عن 7 جينات ناتجة عن التنظيم التناقصي ( 3 في سلالة BALB/c و4 في سلالة C57BL/6 )، و5 جينات ناتجة عن التنظيم التزايدي للخلية ( 2 في سلالة BALB/c و3 في سلالة C57BL/6 )، كما لوحظ نشاط جين Aa2-245 في مجموعات الحيوانات التي تعاني من سمية كلوريد الألومونيوم والتي تم علاجها إما بالزعفران أو العسل وذلك في سلالة BALB/c.

**خاتمة**: تشير الدراسة بأن استخدام الزعفران أوالعسل يقلل من سمية كلوريد الألومنيوم على الكبد وذلك من خلال تخفيف تأثيره عليها من الناحية البيوكيميائية والجزيئية.

**Objectives:** To study the biochemical and molecular hepatotoxicity induced by aluminium chloride ($AlCl_3$) and the protective role of saffron and honey against such toxicity.

**Methods:** This study was performed in the Department of Biology, College of Science, King Khalid University, Abha, Kingdom of Saudi Arabia between July and August 2009. Two mice strains, BALB/c and C57BL/6 (20 animals from each strain), were used and randomly divided into 4 groups: control group; $AlCl_3$ group; $AlCl_3$+saffron group; and $AlCl_3$+honey group. Changes in liver biochemical markers such as gamma-glutamyl transpeptidase (GGT), alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin and lipid peroxidation levels were estimated. Induced and suppressed mRNA in the liver homogenate was scanned followed by up- and down-regulated genes were isolated, cloned, and sequenced.

**Results:** There was a significant increase in the cholesterol levels, triglycerides, GGT, ALT, AST, ALP, lipid peroxidation, and presence of hyperglycemia in the $AlCl_3$ group compared to the control. However, treating those animals exposed to AlCl3 by saffron and honey improved the disrupted liver biochemical markers and alleviated the increase of lipid peroxidation. Seven down-regulated genes (3 BALB/c and 4 C57BL/6) and 5 up-regulated genes (2 BALB/c and 3 C57BL/6) were observed. Aa2-245 gene was observed as being up-regulated in $AlCl_3$+saffron and $AlCl_3$+honey groups in the BALB/c strain.

**Conclusion:** The use of saffron and honey minimized the toxic effect of $AlCl_3$ in the liver by alleviating its disruptive effect on the biochemical and molecular levels.


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Aluminum (Al) is a constituent of cooking utensils, medicines, deodorants, and food additives. This has led to its easy access into the human body.[1] Sources of Al include corn, yellow cheese, salt, herbs, spices, tea, cosmetics, cookwares, and containers. Exposure to Al can disrupt many enzymes. For example, salts of Al may inhibit the action of acid and alkaline phosphatases, phosphodiesterase, and phosphooxydase.[2] In addition, exposure to Al can affect the triglyceride metabolism and triglyceride concentrations in the body.[3,4] It has been reported that Al exposure causes impairments in glucose utilization, free radical-mediated cytotoxicity, lipid peroxidation, reduced cholinergic function, impact on gene expression, and altered protein phosphorylation.[5] Aluminum has been found to cause oxidative stress in the plasma and the tissues of male rabbits.[6] The mechanism of Al induced toxicity has been attributed to its ability to potentiate the activity of iron ion [$Fe(_2^+)$] and $Fe_3^+$ ions in such a way as to cause oxidative damage.[7] Molecular changes such as DNA damages and gene suppression or expression may happen to hepatic cells when the model animal exposed to toxic materials.[8] Recently, ribonucleolytic activity, which is fundamental for the biological activities of proteins, has been found in many proteins with known biological activities.[9] Aluminum has been reported to affect the activities of translation machinery components in mouse liver in vivo and in vitro as Al can bind to phosphorylated bases on DNA, induce considerable changes in chromatin structure and disrupt protein synthesis, and catabolism.[5,9] Herbal/ natural products represent one of the most common forms of complementary and alternative medicines.[10] They are readily available and can be purchased from supermarkets and pharmacies. As these products can be taken without supervision or medical prescription, they must be safe for human health.[11,12] Many natural product extracts have been found to have a variety of pharmacological and antioxidant effects.[13,14] For example, *Crocus sativus L.,* commonly known as saffron, is a plant cultivated in various parts of the world including Iran, China, Spain, Italy, and Greece. Phytochemical studies of saffron have shown that the main chemicals responsible for its color are crocins, which are a series of mono and di-glucosyl esters of crocetin, a polyene dicarboxylic acid (8,8-diapocarotene-8, 8-dioic acid).[15] On the contrary to the majority of carotenoids' families, these compounds are extensively used in the food industries as food colorants due to their unique water-soluble behavior.[14,15] Many studies have indicated that crocins have various pharmacological effects such as protecting hepatocytes,[16] inhibition of tumor cell proliferation,[17] and protection against cardiovascular diseases.[18] Among the mechanisms underlying their various protective actions, the antioxidant activity has been hypothesized as being responsible for the various pharmacological effects of crocins.[19] Honey syrup of wild honey is another natural product produced by the honeybees and constitutes of a natural supersaturated sugar solution, which is mainly composed of a complex mixture of carbohydrates.[20] It also includes certain minor components such as enzymes, proteins, vitamins, flavonoids, phenolic acids, amino, and organic acids.[21] However, the composition of honey depends on the plant species visited by the honeybees and the environmental, processing and storage conditions.[22] Honey has been traditionally used for a range of different purposes and has a great potential to serve as a natural food antioxidant.[22,23] Enzymes in honey serve as antioxidants by helping the removal of free radicals and protection against oxidative damages.[24] Honey consumption has been reported to be effective in increasing the total plasma antioxidants in humans.[25] As there has been no study has investigated the biochemical and molecular roles of water saffron extract and honey syrup against hepatotoxicity induced by aluminium chloride ($AlCl_3$), this study aimed to explore the biochemical and molecular changes induced by $AlCl_3$ in the liver of 2 mice strains. In addition, the protective effects of water saffron extract and honey syrup on hepatotoxicity induced by $AlCl_3$ was investigated.

## Methods.

***Natural products extracts.*** Dried red stigmas of saffron were obtained from a local supermarket in the city of Abha, Saudi Arabia. Saffron was immersed in distilled water (500 mg $ml^{-1}$) for 24 hours, filtered, and prepared every 4 days. Wild honey was collected from the Aseer region, which is in the southern province of Saudi Arabia. It was diluted with distilled water (500 mg $ml^{-1}$) and made into syrup. It was also prepared every 4 days. Both saffron and honey extracts were intraperitoneally injected at 500 mg $kg^{-1}$ body weight once a day for the experimental period (45 days).

***Animal grouping.*** This study was carried out in the Department of Biology, College of Science, King Khalid University, Abha, Kingdom of Saudi Arabia between July and August 2009. Twenty BALB/c and 20 C57BL/6 mice weighing approximately 35-40 g were used in the experiment. Animals were obtained from the Animal House, College of Science, King Khalid University, Abha, Saudi Arabia. Ethical approval was obtained from the College of Science Ethical Committee, King Khalid University and the experiment was performed according to the International Guidelines for the Care and Use of Laboratory Animals. Animals of each mice strains were given food and water ad libitum and randomly divided into 4 groups (n=5 in each group). Control group: mice were given normal saline (0.9%

sodium chloride [NaCl]) for the experimental period. $AlCl_3$ group: mice were daily intraperitoneally injected with 40 mg $kg^{-1}$ body weight of $AlCl_3$ (pH, 6.8) freshly prepared every 4 days for the experimental period. $AlCl_3$+saffron group: mice were daily co-administered intraperitoneally with 40 mg $kg^{-1}$ body weight $AlCl_3$ and water extract of saffron (200 mg $kg^{-1}$ body weight) for the experimental period. $AlCl_3$+honey group: mice were daily co-administered intraperitoneally with 40 mg $kg^{-1}$ body weight $AlCl_3$ and honey syrup (500mg $kg^{-1}$ body weight) for the experimental period. At the end of the experimental period, the mice were slaughtered and blood samples were collected. Liver tissues were weighed and homogenized in phosphate buffer solution pH 7.4 and stored at -80°C until used.

***Samples and biochemical parameters:*** Butyryl coholinesterase and L-γ-glutamyltransferase activities were measured in sera according to Knedel and Bottger[26] and Szasz[27] respectively. Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in sera were assayed according to Reitman and Frankel.[28] Alkaline phosphatase (ALP) activity was measured at 405 nm by the formation of paranitrophenol from para-nitrophenyl-phosphate as a substrate using the method of Belfield and Goldberg.[29] Total billirubin was assayed using the method of Walter and Grade.[30] Furthermore, the contents of serum glucose, cholesterol, and triglycerides were measured using the methods of Trinder,[31] Wieland and Seidel,[32] and Fossati and Prencipe.[33] Liver thibarbituric acid reactive substances (TBARS) were assayed using the method of Ohkawa et al.[34]

***Isolation of total RNA from supernatant of liver homogenates and cDNA synthesis:*** A 100µl of animal supernatant of liver homogenate was subjected to RNA extraction using QIAGEN-QIAamp RNA blood mini kit according to the manufacturer's procedures (QIAGEN, Hilden, Germany). Reverse transcription reactions were performed using an oligo dT primer. The 25µl reaction mixture was prepared with 2.5µl of 5X buffer with magnesium chloride ($MgCl_2$), 2.5µl of 2.5mM deoxynucleosides (dNTPs), 1µl of 10pmol primer, 2.5µl RNA and 0.2µl reverse transcriptase enzyme (100U µl-1). Polymerase chain reaction (PCR) amplification was performed in a thermalcycler (Eppendorf) program at 95°C for 5 minutes, 42°C for one hour, 72°C for 10 minutes and soak at 4°C.[35]

***Differential display for detection of up-down regulated genes:*** The cDNA was subjected to a second PCR using 18S rRNA forward primer as an arbitrary primer (5'-GCA AGT CTG GTG CCA GCC-'3). The PCR reaction was performed as follows: a 25µl reaction mixture was prepared with 2.5µl 10x Taq DNA polymerase buffer (10 mM Tris HCl [pH 8.3]), 25mM potassium chloride [KCI]), 2.5µl 50mM $MgCl_2$, 2µl primer (40 pmol $\mu l^{-1}$), and 0.25µl of Taq polymerase (AmpliTaq, Perkin- Elmer, 5u $\mu l^{-1}$), 2.5µl from the cDNA, 2.5µl dNTPs 4mM, and 12.75µl of $dH_2O$. The PCR reaction was performed in 9700 thermal cycler (Perkin-Elmer) and the PCR conditions were performed as follows: initial denaturation at 95°C for 5 minutes, followed by 40-cycles (94°C for 1 minute, 53°C for 1 minute and 72°C for 2 minutes; final extension, 72°C for 10 minutes). Electrophoresis was performed at 80 Volts with 0.5x TBE buffer in 1.5% agarose gel.[36] Gel was stained in 0.5µg $ml^{-1}$ (w/v) ethidium bromide solution and destained in deionized water. Finally, gel was visualized and photographed by using a gel documentation system.

***Cloning of the up and down regulated DNA band:*** The resultant PCR product was excised from the gel and purified using a QIA quick gel extraction kit (QIAGEN Inc., Germany). The purified PCR product was cloned using TA-Cloning kit (Invitrogen, USA). The screened recombinant plasmid was subjected to DNA sequencing using M13 universal primer (Macrogen Company, Korea).

***Sequence analysis and GenBank accession numbers:*** A blast search was performed for the DNA sequence obtained and this sequence was then submitted to the GenBank under the accession number, HM042681.

***Phylogenic analysis for the obtained gene:*** Pair-wise and multiple DNA sequence alignments were carried out using CLUSTALW (1.82).[37] A bootstrap neighbor-joining tree was generated using MEGA438 from CLUSTALW alignments.[37]

***Statistical analysis.*** Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA). Data are presented as means with their standard deviations. Statistical evaluation of the data was performed using one-way Analysis of Variance followed by a post-hoc least significant difference (LSD) test. P-values less than 0.05 were considered to be statistically significant.

**Results.** ***Biochemical tests:*** A significant increase in buturyl cholinesterase, ALP, GGT, AST and bilirubin levels was found in the $AlCl_3$ groups compared to their matched control groups in both mice strains (**Table 1**). The aqueous extract of saffron and the honey syrup showed a significant alleviation in the increased levels of buturyl cholinesterase, ALP, GGT, AST and bilirubin levels in both mice strains (**Table 1**). There was a hyperglycemia and a significant increase in both total cholesterol and triglycerides levels in the $AlCl_3$ groups compared to their matched control groups in both mice strains. The aqueous extract of saffron and honey syrup showed a significant improvement in the levels of

**Table 1 -** Liver function enzymes, butryl cholinestrease ($UL^{-1}$), L-γ-glutamyltransferase (GGT) ($U L^{-1}$), aspartate transferase (AST), alanine aminotransferase (ALT) ($UL^{-1}$) activities, alkaline phosphatase (ALP) ($UL^{-1}$), and bilirubin level ($mg\ dl^{-1}$) in different mice groups (BALB/c and C57BL/6).

| Parameters | BALB/c | | | | C57BL/6 | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | $AlCl_3$ | $AlCl_3$+honey | $AlCl_3$+saffron | Control | $AlCl_3$ | $AlCl_3$+honey | $AlCl_3$+saffron |
| Butryl cholinesterase | 1282.7 ± 72.5 | $7167.3^{\ddagger}$ ± 43.8 | $2370^{\ddagger,a}$ ± 91.6 | $2470.8^{\ddagger,a}$ ± 72.2 | 706.7 ± 3.6 | $1215.9^{\ddagger}$ ± 6.6 | $1109.7^{\ddagger,b}$ ± 4.9 | $964.9^{\ddagger,a}$ ± 11.2 |
| GGT | 5.6 ± 0.5 | $14.6^{\ddagger}$ ± 2.1 | $9.2^{*b}$ ± 0.6 | $8.1^{b}$ ± 0.8 | 16.3 ± 0.9 | $32^{\ddagger}$ ± 1.6 | $26.6^{\ddagger,b}$ ± 2 | $15.6^{c}$ ± 7.7 |
| ALT | 5.5 ± 0.5 | $19.8^{\ddagger}$ ± 1.9 | $11.6^{\ddagger,a}$ ± 1.1 | $9^{\ddagger,a}$ ± 1.2 | 15.8 ± 1.3 | $35.4^{\ddagger}$ ± 1.1 | $28.1^{\ddagger,a}$ ± 1.8 | $23.8^{\ddagger,a}$ ± 0.7 |
| AST | 9.6 ± 1.1 | $15.4^{\ddagger}$ ± 1.1 | $12.8^{\dagger,b}$ ± 0.8 | $11.6^{c}$ ± 1.1 | 20 ± 1.6 | $34.6^{\ddagger}$ ± 2.3 | $30.4^{\ddagger,a}$ ± 2.1 | $27^{\ddagger,a}$ ± 1.6 |
| Total bilirubin | 0.5 ± 0.1 | $2.2^{\ddagger}$ ± 0.1 | $1.3^{\ddagger,a}$ ± 0.1 | $0.9^{*a}$ ± 0.1 | 1.5 ± 0.3 | $2.8^{\ddagger}$ ± 0.2 | $2.6^{\ddagger}$ ± 0.4 | $2.2^{\ddagger,b}$ ± 0.4 |
| ALP | 63.1 ± 2.8 | $92.3^{\ddagger}$± 1.4 | $91.3^{\ddagger}$ ± 1.3 | $70.7^{\ddagger,a}$ ± 1.1 | 50.8 ± 1.1 | $61.2^{\ddagger}$ ± 1.4 | $59.8^{\ddagger}$ ± 0.9 | $57.3^{\ddagger,a}$ ± 0.9 |

Values are expressed as mean ± SD.
Control group compared with $AlCl_3$, $AlCl_3$+honey, and $AlCl_3$+saffron groups, $^{*}p<0.05$, $^{\dagger}p\leq0.01$, $^{\ddagger}p\leq0.001$.
$AlCl_3$ group compared with $AlCl_3$+honey and $AlCl_3$+saffron groups; $^{a}p\leq0.001$, $^{b}p\leq0.01$.

**Table 2 -** Serum glucose ($mg\ dl^{-1}$), total cholesterol ($mg\ dl^{-1}$), and triglycerides ($mg\ dl^{-1}$) levels in different mice groups (BALB/c and C57BL/6).

| Parameters | BALB/c | | | | C57 | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | AlCl3 | $AlCl_3$+honey | $AlCl_3$+saffron | Control | AlCl3 | $AlCl_3$+honey | $AlCl_3$+saffron |
| Glucose | 39.7± 2.9 | $100.2^{\ddagger}$ ± 13.4 | $87.8^{\ddagger,a}$ ± 4.4 | $81.6^{\ddagger,a}$ ± 6.2 | 44.4 ± 2.8 | $101.4^{\ddagger}$ ± 8.4 | $65.2^{\ddagger,a}$ ± 3.2 | $85.2^{\ddagger,a}$ ± 3.2 |
| Cholesterol | 98.6 ± 1.3 | $121.2^{\ddagger}$ ± 1.2 | $110^{\ddagger,a}$ ± 0.7 | $104.4^{*,a}$ ± 3.6 | 103 ± 1.6 | $153.6^{\ddagger}$ ± 2.1 | $134.8^{\ddagger,a}$± 0.8 | $127.6^{\ddagger,a}$ ± 9.7 |
| Triglyceride | 69.8 ± 1.4 | $113.7^{\ddagger}$ ± 3.4 | $85.2^{\ddagger,a}$ ± 3.3 | $73.6^{*,a}$ ± 3.4 | 112.2 ± 0.83 | $178.4^{\ddagger}$ ± 2.1 | $129.2^{\ddagger,a}$ ± 3.7 | $117.8^{\dagger,a}$ ± 1.9 |

Values are expressed as mean ± SD.
Control group compared with $AlCl_3$, $AlCl_3$+honey, and $AlCl_3$+saffron groups, $^{*}p<0.05$, $^{\dagger}p\leq0.01$, $^{\ddagger}p\leq0.001$.
$AlCl_3$ group compared with $AlCl_3$ +honey and $AlCl_3$+saffron groups $^{a}$, $p\leq0.001$.

**Table 3 -** Concentration of liver thiobarbituric acid reactive substances (TBARS) ($nmole\ mg^{-1}$) in different mice groups (z/c and C57BL/6).

| Parameters | BALB/c | | | | C57BL/6 | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | $AlCl_3$ | $AlCl_3$+honey | $AlCl_3$+saffron | Control | $AlCl_3$ | $AlCl_3$+honey | $AlCl_3$+saffron |
| TBARS | 125.9 ± 12.7 | $218.2^{\ddagger}$ ± 5.3 | $153.4^{\ddagger,a}$ ± 11.7 | $165.2^{\ddagger,a}$ ± 6.8 | 126.8 ± 9.4 | $207.1^{\ddagger}$ ± 7.2 | $130.1^{a}$ ± 10.1 | $134.4^{a}$ ± 15.5 |

Values are expressed as mean ± SD.
Control group compared with $AlCl_3$, $AlCl_3$+honey, and $AlCl_3$+saffron groups, $^{*}p<0.05$, $^{\dagger}p\leq0.01$, $^{\ddagger}p\leq0.001$.
$AlCl_3$ group compared with $AlCl_3$+honey and $AlCl_3$+saffron groups $^{a}p\leq0.001$

glucose, triglycerides and total cholesterol in both mice strains (**Table 2**). An increase in lipid peroxidation in liver homogenates of the $AlCl_3$ groups in both mice strains was indicated by the high significant increase in TBARS (**Table 3**). The protective effect of the aqueous extract of saffron and honey syrup shows a decrease in the level of TBARS of both mice strains in the $AlCl_3$+saffron and the $AlCl_3$+honey group in both mice strains (**Table 3**).

***DNA-based molecular techniques:*** The up and down regulated genes in the examined animal liver homogenates showed that more than 36 band patterns were obtained by using the 18S primer as an arbitrary primer. Approximately 12 different up- and down-regulated genes were observed among the 36 genes (**Figure 1**). The up and down regulated genes had different molecular weights, ranging from 1.1Kbp to 50bp. Five different genes were observed within the BALB/c mice strain (3 down-regulated and 2 up-regulated) and 7 different genes were demonstrated in the C57BL/6 mice strain (4 down-regulated and 3 up-regulated) (**Figure 1**). A band with a molecular weight of 0.5kbp (up-regulated) from the $AlCl_3$+honey group in the BALB/c mice strain was selected to be a representative gene for all the up-regulated genes obtained. The sequence analysis revealed that the selected up-regulated gene was Aa2-245 liver regenerative gene (ribonuclease, RNase

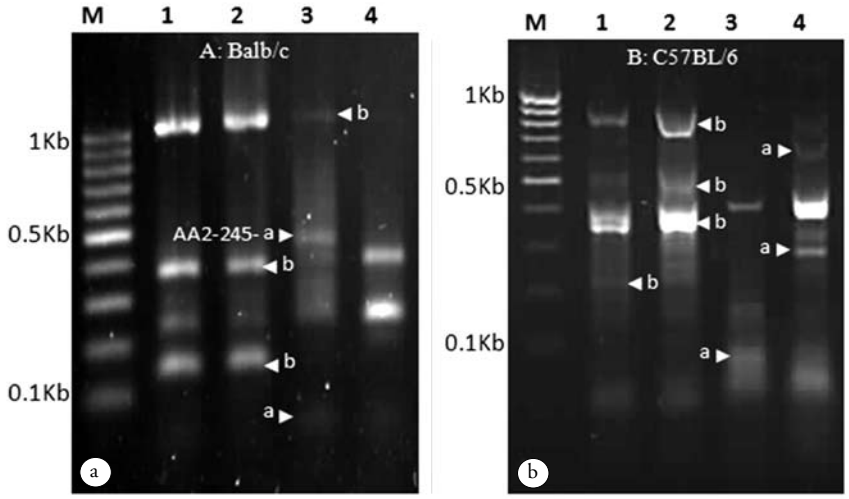


**Figure 1 -** Panels **a** and **b**) represents differential display for the BALB/c and C57BL/6 mice treated with $AlCl_3$ and with administrating of saffron extract and honey syrup. Lanes M: 1Kb ladder DNA marker, lane 1: control, lane 2: $AlCl_3$, lane 3: $AlCl_3$+saffron and lane 4: $AlCl_3$+honey. a - indicates up-regulated, b - indicates for the down-regulated genes.

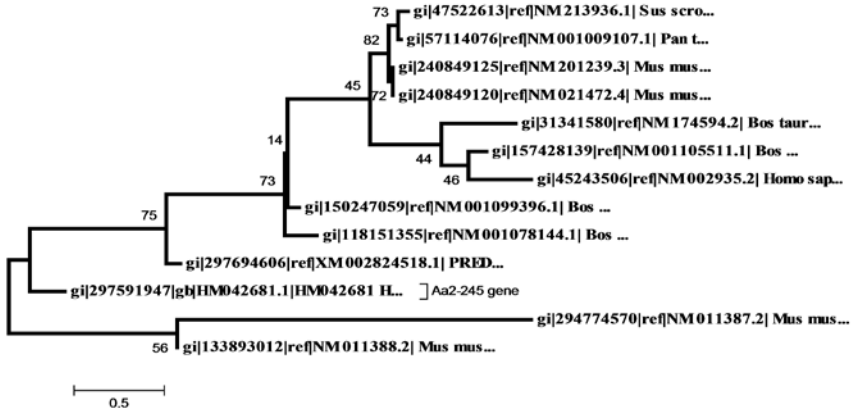


**Figure 2 -** Phylogenetic tree showing the evolutionary relationship between Aa2-245 liver regenerative gene nucleotide sequence and the other ribonuclease, RNAase family 4. The neighbor-joining method was used to construct the tree. The numbers on the branches represent bootstrap support for 1,000 replicates. Names refer to the accession number of the nucleotide sequences that encode the corresponding reverse transcriptase genes. Sus scro. - *Sus scrofa*, Pan t. - *Pan troglodytes*, Mus mus. - *Mus musculus*, Bos taur. - *Bos Taurus*, Homo sap. - *Homo sapiens*, PRED - predicted as *Pongo abelii*

A family 4). When the Aa2-245 was compared with other ribonuclease genes, the results postulated that the Aa2-245 was closely related to the ribonuclease RNAase gene isolated from Mus musculus (294774570 and 133893012) with a similarity of 65% (**Figure 2**).

**Discussion.** Aluminum exposure can result in its accumulation in the liver and hence it can be toxic to hepatic tissue at high concentrations.[39] Changes in aminotransferase activities could be expected to occur in association with a pathology involving the necrosis of the liver. Serum AST and total bilirubin increases in such cases, and escape to the serum from the injured hepatic cells. The serum ALT level is also useful in indicating the existence of liver diseases as it is present in the liver in large quantities. It increases in the serum when cellular degeneration or destruction of the liver happens.[40] The obtained results are consistent with previous findings by El-Demerdash[5] and Moshtaghie et al.[41] That ALP levels were significantly decreased in the liver while increased in serum following Al exposure. The decrease of the liver ALP may be due to binding of Al to DNA and RNA and inhibiting the activity of ALP.[42] Moreover, Rahman et al.[43] and Gaskill et al[44] suggested that the increase in the activities of ALP in different tissues might also be due to the increased

permeability of plasma membrane or cellular necrosis, indicating the stress condition of the treated animals. Gaskill et al[44] reported that the release of ALT and GGT from the cytoplasm of hepatic cells could have occurred secondary to cellular necrosis. In the present study, the activities of ALT and AST and GGT significantly increased in the serum of $AlCl_3$ groups in both mice strains. This may be due to the leakage of these enzymes from the cytoplasm of the liver cells into the general circulation and/or liver dysfunction and disturbance in the biosynthesis of these enzymes, with an alteration in the permeability of the liver membrane. The high increase of the bilirubin levels in $AlCl_3$ groups in both mice strains may be due to the fact that Al exposure can result in Al accumulation in the liver and this metal can be toxic to the hepatic tissue at high concentrations and may lead to high increase of the bilirubin level.[45]

Differences in activity of plasma cholinesterase are correlated with variations in the risk factors for cardiovascular and metabolic disease and may be under genetic control.[4] Plasma cholinesterase (EC 3.1.1.8; butyryl-cholinesterase, pseudocholinesterase) has been widely investigated due to being a marker of exposure to organophosphate chemicals[4] and the relationships between low activity and the delayed metabolism of the muscle relaxant succinylocholine.[46] The decrease in butyryl cholinesterase may be associated with the dysfunction of the liver after the administration of $AlCl_3$. Hyperglycemia may be induced as a result of the increased glucose production and decreased glucose utilization. The increase in blood glucose may be a sign of disrupted carbohydrate metabolism owing to increased breakdown of liver glycogen.[6,7] In addition, oxidative stress has been proposed as a major pathogenic link to both insulin resistance and the dysfunction of the pancreatic beta cell by the formation of amyloid proteins, which not only prevents the release of insulin into the circulation, but also destroys the insulin-secreting beta cells.[47] Growing evidence indicates that chronic or acute overproduction of reactive oxygen species plays an important causal or contributing role in the development of many diseases 48 and the protective effects of crocin or crocetin against cardiovascular, liver and tumor diseases has been repeatedly demonstrated in various studies.[18,49,50] This effect may explain the antioxidant capacities of saffron that improved the disrupted liver biochemical markers happened in $AlCl_3$+saffron groups in this study.

Lipid peroxidation, as one of the major signs of oxidative damage, has been found to have an essential role in the toxicity of numerous xenobiotics.[51] Furthermore, various chronic diseases, especially cardiovascular disease and cancer are associated with oxidative damage to biomolecules such as lipids, DNA, and proteins.[52] It was found that Al at sub-lethal levels induced lipid peroxidation and thus altered the physiological and biochemical properties of many biological systems.[6] The antioxidant properties of honey syrup[25,53] and the water extract of saffron[18,49,50] may have lead to alleviation of the disturbances in all biochemical parameters. In addition, the antioxidant effect of saffron and honey led to scavenging the free radical associated with the injection of $AlCl_3$ in both mice strains, and a decrease in the oxidative stress as shown by the increase of thiobarbituric acid reactive substances. The antioxidant properties of both tested natural treatments may have prevented the leakage of hepatic enzymes as shown by the decrease in butryl cholinesterase, GGT, AST, ALT, ALP and bilirubin.

The regenerative liver gene or the ribonuclease genes ribonuclease (RNAase 4) was induced in $AlCl_3$+ honey and $AlCl_3$+saffron groups in BALB/c mice. Zaho et al[54] reported that the presence of 4 members of the pyrimidine-specific ribonuclease superfamily was demonstrated in rat liver. The up-regulation of RNAase 4 gene may lead to resisting the toxic effect of the Al on the liver cells.[55] Ardelt et al[56] have also stated that cytotoxic RNAases, as exemplified by Onconase, represent a new class of antitumor agents with an entirely different mechanism of action than the drugs currently used in clinics. Further studies on animal models including human tumors grafted onto severe combined immune deficient (SCID) mice, and clinical trials are needed to explore the clinical potential of cytotoxic RNAases. It has been reported that the excretion of Al in urine was significantly increased after the intake of analgesics containing Al, which confirming the increased absorption and hence increased exposure to Al as a result of such medication.[57,58] The up-regulation of the renin expression by Al is a strong indication of the influence of Al on the renin–angiotensin–aldosterone-system, resulting in possible induction of essential hypertension.[57,58]

One limitation of this study is that we tested the effect of co-administration of saffron extract and honey syrup with AlCl3. Therefore, further studies should test pre- and post- administration of those natural products with AlCl3 in order to discover whether they have therapeutic or protective effect against Al hepatotoxicity.

In conclusion, Al has adverse effects on human health. Our results reported that $AlCl_3$ is capable of causing marked alterations in some biochemical parameters and inducing oxidative damage in the liver. The administration of the water extract of saffron and honey syrup with $AlCl_3$ has minimized its hazards. In addition, the water extract of saffron was proved to be beneficial in decreasing the levels of free radicals in the livers of both mice strains. The molecular changes

showed that Al causes a great deal of liver cell damage and both saffron and honey syrup resist these degenerative changes by induction the structure and function genes. One of these genes is Aa2-245, the ribonuclease, RNAase A family 4, ribonucleases which is widely found in living organisms and has been suggested to be involved in RNA metabolism and the regulation of gene expression.

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