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# The Protective Effect of Ethanolic Extract of Olive Leaf on Aluminum Phosphide-Induced Cardiac Toxicity in Rats

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***ABSTRACT***

**Background:** The purpose of this study is to evaluate a treatment for aluminum phosphide (ALP) poisoning, which is known as "rice tablet". **Methods:** In the present study, the impact of different doses of ethanolic extract of olive leaves (100, 200, and 400 mg/kg) on ALP (120 mg/kg)-induced cardiotoxicity was evaluated in anesthetized-gastrotomized adult male Wistar rats. Thirty-five rats were randomly assigned into five groups (n=7) as follows: control (AC) and treatment groups [ALP+Olea100 (AO100), ALP+Olea 200 (AO200), and ALP+Olea 400 (AO400)]. Physiological data including blood pressure, heart rate, electrocardiogram (ECG), as well as oxidative stress markers were measured in heart tissues. **Results:** ALP-intoxication led to perturbed normal ECG and increased oxidative stress. Administration of olive leaf extract at various concentrations, however, mitigated bradycardia after 90 minutes, following ALP-intoxication (in AO200), hypotension (in AO100), and cardiac conduction disturbances (decreased QTC in the AO200 30 ($P<0.05$), 60 ($P<0.001$), and 90 ($P<0.05$) minutes after intoxication) and decreased PR 60 ($P<0.05$) and 90 minutes ($P<0.01$) after intoxication. This was compared with baseline as well as detrimental changes in cardiac electrophysiology [mitigated ST-segment elevation in AO200 and depressed T-wave in the AO200 ($P<0.05$) and AO400 ($P<0.01$) groups 90 minutes after intoxication] **Conclusion**: Based on these authentic results, it seems that olive leaf extracts can be useful in reducing the severity of symptoms in ALP-poisoned individuals and could be utilized in a poisoning emergency.

**Keywords:** *Aluminum Compounds; Cardiotoxicity; Electrocardiography; Gastric lavage; Oxidative stress; Pesticides.*

## Introduction

A vast majority of scientific texts have addressed a wide range of beneficial properties of olive leaves and their active ingredient called oleuropein. Based on traditional information, the most known medicinal feature of olive leaf is cardioprotection. Similarly, anti-inflammatory,

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antioxidant, antiarrhythmic, hypotensive, and antiatherosclerotic effects of olive leaf extract have been reported by different experimental and clinical studies. Also, recent advancecs demonstrated some potent and protective effect of this leaf extract aginst lead poisoning and carbendazim, diazinon, doxorubicin, busulfan, thioacetamide intoxications, and so on.

The toxicity of aluminum phosphide (ALP) also called "rice tablet", which is broadly used for fumigation process of grains, is mainly due to the presence of the phosphorous compound that could be released as phosphine gas, which is categorized as protoplasmic toxin (Azad *et al.*, 2001). The ultimate biological complications of phosphine are numerous cellular enzymatic and organelle dysfunctions and cell death (Singh *et al.*, 1996). The intensity of poisoning is very high when ALP is absorbed by either gastrointestinal tract or respiratory tract, whereas contamination of skin with ALP does not cause a significant problem for human beings (Chaudhry, 1997, Moffat *et al.*, 2011). Following the digestion of ALP in gastric acid in the stomach, it releases phosphine gas by which a variety of symptoms can occur within a few minutes (Anger *et al.*, 2000, Christophers *et al.*, 2002). In case of toxicity, a high chemical oxidation process may induce vomiting, abdominal pain, restlessness, tachycardia, tachypnea, acidosis, and hypotension. Finally, cardiovascular and renal failure and severe acidosis are the most lethal complications of aluminum phosphide poisoning (Singh *et al.*, 1996).

Various observations indicated that medicinal plants can be useful in the emergency room due to their effectiveness against drug poisoning (Gurjar *et al.*, 2011). Because of lower side effects of herbs in comparison with synthetic drugs, the use of medicinal herbs has increased in recent years (Nasri and Shirzad, 2013, Rafieia-Kopaei, 2011, Sewell and Rafieian-Kopaei, 2014). Myriad studies showed that olive has many medicinal properties. Olive oil is useful for treatment of gallstones, dry cough, and pyorrhea, and it acts as an aphrodisiac substance to increase libido (Somova *et al.*, 2003, Trovato *et al.*, 1993).

## Materials and Methods

### *Study design and animal procedures*

In this study, 35 adult male Wistar rats weighing 290-310 g were randomly divided into four groups (n=7) ,including a control group (AC), and ALP+Olea100 (AO100), ALP+Olea200 (AO200), and ALP+Olea400 (AO400) groups (**Figure 1**). The animals were housed in cages containing four animals each in an air-conditioned environment (24±4 °C) with a humidity of 65±5% and an artificial lighting with a light-dark cycle of 12:12 h. All the animals had free access to standard rat chow and tap water. To perform the experiment, animals were anesthetized with sodium thiopental. Afterwards, neck and abdomen were completely shaved and placed on a surgical bed. A small bulb was placed on the floor of the surgical bed to maintain the temperature of the animal's body at 37 °C. After preparation of the animals, their organs were fixed on the surgical bed. To perform tracheostomy, a longitudinal incision was made in the midline of neck about 1 cm from sternal manubrium to the top. After removing the muscles of cervical region, the trachea was detached and intubated. By placing a small cushion (for example, an insulin syringe barrel) under the neck of the animal, the head of the rat was placed in the right position, and after the removal of the cervical muscles, the right carotid artery was exposed, and the vagus nerve was isolated inside the carotid sheet. A ligature was passed beneath the vessel at the site of a carotid bifurcation, and then, the ends of ligature were clamped. After that, the vessel was closed by bulldog clamps at the proximity of the sternal manubrium, and the second knot (a loose knot) was made after the clamps on the first knot. A small incision was obliquely made about 1 cm above the site of clamps. A heparinized catheter (PE-50) was retrogradely inserted towards the midline to the heart. Next, the loose knot located on the top of the vessel and catheter was tightened. The catheter was then pushed down (should not enter the heart) after the removal of the clamps. Therefore, the carotid artery was cannulated to record blood pressure and was connected to the Power Lab® apparatus to record arterial pressure fluctuations. To



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perform gastrostomy, an incision was made in the abdominal region of the rats where the stomach was readily accessible (the left side of the abdomen beneath the ribs). Afterward, the animals' stomach was exposed by blunt disection, and immediately, a small incision was made in the stomach to insert a special tube in it. The tube was connected and fixed to the stomach by a suture, and ALP was administered at a dose of 120 mg/kg in the stomach of each rat via gastrostomy tube. In treatment groups, the ethanolic extract of olive leaves was also administered via gastrostomy tube for 20 minutes at concentrations of 100, 200, and 400 mg/kg per body weight. Three sub-dermal needle electrodes from ECG recorder were connected to both forelimbs and left hind limb and monitored lead II of ECG during the experiment.

***The olive leaves extraction procedure***

At first, one kilogram of the aerial parts of olive leaves was collected from Razi Herbal Medicine Research Center affiliated with the University of Lorestan, in Lorestan, Iran. In order to obtain the extract of olive leaves, one liter of 80% ethanol was added to dried leaves and then was vigorously vortexed for 12 minutes. This procedure was carried out twice. The resulting extract was concentrated by utilization of a rotary vacuum evaporator. The final extract was completely freeze-dried and was kept at 4 °C until later use. The extraction efficiency was 11.4%, and the amount of oleuropein concentration was reported as 18.45%.

***Measuring oxidative stress***

In order to study oxidative stress, reactivity of catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) was measured in heart tissues of the rats. So, at the end of each protocol, the heart was excised under deep anesthesia, was immediately frozen in liquid nitrogen, and was stored at −70 °C; this was done to estimate MDA (Sigma, USA), CAT (ZelBio), and GPx (Pars Azmoon, Iran) using spectrophotometric methods of Ohkawa *et al.* and Khaper and Singal using diagnostic kits (Khaper and Singal, 1997, Ohkawa *et al.*, 1979).

Measuring hemodynamic parameters: After cannulation of carotid artery, the data of arterial blood pressure and heart rate were transmitted to power lab apparatus by a pressure transducer. In this protocol, different doses of olive extract were examined. The measured indices included systolic blood pressure (SBP), mean arterial pressure (MAP), heart rate and Electrocardiografic (ECG) parameters (RR, PR, QRS, QTC, ST Height, T-amplitude). Hemodynamic information was continuously recorded from the baseline and after 30, 60, and 90 minutes following different treatments.

***Ethical concideration***

Rats were obtained from Lorestan University of Medical Sciences, and all the experimental procedures were performed according to the protocols authorized by the Animal Care Committee of Lorestan University of Medical Sciences (IR. LUMS.REC.1396.323).

***Data analysis***

The Kolmogorov–Smirnov test was used to determine whether the data were distributed normally. Mean ± SEM was calculated for all groups. Hemodynamic parameters within the groups were measured using analysis of variance (ANOVA) and Tukey's post hoc test and Bonferroni comparison between all groups. A value of P-value < 0.05 was considered significant.

## Results

***Hemodynamic parameters and cardiac function***

Alterations in heart rate in AC, AO100, AO200, and AO400 groups during the various experiment periods were presented in **Table 1**. Comparing the heart rates at baseline, 30, 60, and 90 minutes after intoxication with ALP showed that in the AC group, after 60 ($P$<0.001) and 90 minutes ($P$<0.05), the heart rate was significantly decreased compared with the baseline. Moreover, in the AO100 group, the heart rate was significantly reduced following poisoning with ALP after 60 ($P$<0.001) and 90 minutes ($P$<0.05), compared with the baseline. On the other hand, in AO200 group, the heart rate of rats was significantly reduced ($P$<0.05) in the 30$^{th}$ and 60$^{th}$ minutes; however, the decrease in the heart rate of the animals was decreased in the 90$^{th}$ minuteof intoxication, compared with the baseline.



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In the AO400 group, the heart rate did not show any significant increase after poisoning with ALP and in comparison with the baseline; but, leaf extract could not halt the unbound decrease in the animals' heart rate at this dosage.

***Comparing the MAP changes***

Changes in MAP values in the AC, AO100, AO200, and AO400 groups in the baseline and 30, 60, 90 minutes after intoxication with ALP were presented in **Table 1**. The results showed that MAP was significantly ($P$<0.01) diminished at 60 and 90 minutes following the poisoning, compared with the baseline. In AO100 group, the reduction of MAP was not statistically significant, compared with the baseline values and at any period of time. However, in the AO200 group, MAP was significantly diminished, compared with the baseline when the comparison was made at the 60$^{th}$ ($P$<0.01) and 90$^{th}$ minute ($P$<0.001) following the administration of ALP. Finally, in AO400 group, MAP level also showed a significant decrease after 60 and 90 minutes of intoxication with ALP (P <0.001), when compared with the baseline values.

***Comparing systolic blood pressure***

As shown in **Table 1**, changes in systolic arterial pressure during various periods were evaluated in all the experimental groups. Comparison of changes in systolic blood pressure (SBP) at baseline, 30, 60 and 90 minutes after intoxication with ALP showed that SBP was significantly reduced in the 90$^{th}$ minute ($P$<0.01) in the AC group when compared with the baseline (before ALP intoxication). Nonetheless, in AO100 group, SBP was not significantly reduced at any period of time when compared with the baseline value. Furthermore, SBP values were significantly reduced in AO200 ($P$<0.01) and AO400 ($P$<0.01) groups in the 60th and 90th minutes in comparison with baseline values.

***ECG parameters***

Changes in QRS intervals in experimental groups during the various periods were presented in **Table 1**. Comparison of this variable at baseline and 30$^{th}$, 60$^{th}$, and 90$^{th}$ minutes following ALP intoxication did not show any significant difference in any of the groups (**Figure 2**).

*Comparing changes in QTC intervals:* The values regarding QTC intervals at various periods of time were demonstrated in **Table 1**. Comparison of changes in QTC intervals at baseline and in 30, 60, and 90 minutes after ALP intoxication showed a significant reduction of QTC in the control group 60 and 90 minutes after ALP intoxication compared to the baseline ($P$<0.05). Compared with the baseline, QTC interval was significantly reduced in AO200 group after 30 ($P$<0.05), 60 ($P$<0.001), and 90 ($P$<0.05) minutes . QTC was also considerably lowered in AO400 group compared with baseline value when the comparison was made in the 30$^{th}$ ($P$<0.01), 60$^{th}$ ($P$<0.01), and 90$^{th}$ ($P$<0.05) minutes following the intoxication.

*Comparing changes in RR intervals:* Variations in RR intervals of all the experimental groups at the different periods were presented in **Table 1**. Comparing the changes in RR intervals at baseline and 30$^{th}$, 60$^{th}$, and 90$^{th}$ minutes of intoxication showed a significant reduction of RR in the control group ($P$<0.05). RR interval was significantly reduced in AO200 group after 30 ($P$<0.05), 60 ($P$<0.001), and 90 ($P$<0.05) minutes compared with the baseline values. RR was also significantly lowered in AO400 group, compared with the baseline when the comparison was made in the 30$^{th}$ ($P$<0.01), 60$^{th}$ ($P$<0.01), and 90$^{th}$ ($P$<0.05) minutes of ALP intoxication. The findings of this study demonstrated that RR interval was significantly increased in the AO100 group in comparison to the AC group when compared with the 60$^{th}$ ($P$<0.001) and 90$^{th}$ ($P$<0.05) minutes of intoxication. Accordingly, RR interval was further increased in the 30$^{th}$ minute when compared with 60$^{th}$ minute ($P$<0.01). In AO200 group, with respect to the baseline value, the increase in RR interval was significant only in the 60$^{th}$ minute, while AO200 group inhibited the increase in RR interval at this dose. In AO400 group, in comparison with baseline value, no significant difference was observed in RR interval at different periods. So, olive leaf extract suppressed ALP-induced increase of RR interval at this concentration.

*Comparison changes in PR intervals:* Alterations



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of PR intervals in all the experimental groups were presented in **Table 1** in different periods of time. Comparing changes in PR interval at baseline and after 30, 60, and 90 minutes following ALP intoxication showed that in AO100 group, PR interval was significantly decreased in the 60$^{th}$ ($P$<0.05) and 90$^{th}$ minutes ($P$<0.01) compared with the baseline value. Such a decrease in PR interval was more ($P$<0.01) in the 60$^{th}$ and 90$^{th}$ minutes when compared with the 30$^{th}$ minute of intoxication. No significant changes were observed in PR intervals in other groups.

*Comparing changes in T wave height:* Differences in T wave height regarding all the experimental groups are depicted in **Table 1** at the different periods of time. Comparison of changes in T wave height at baseline, and after 30, 60 and 90 minutes of ALP intoxication demonstrated that in the control group, the height of T wave was significantly ($P$<0.05) lower in the 90$^{th}$ minute compared with the 60$^{th}$ minute. Olive leaf extracts inhibited ALP-induced decrease of T wave height at different periods in AO100 group, compared with the baseline value. In the AO200 group, the height of T wave was significantly ($P$<0.05) lower in the 90$^{th}$ minute in comparison with baseline and 30$^{th}$ minute. In AO400 group, the height of T wave was significantly decreased in the 90$^{th}$ ($P$<0.01) and 60$^{th}$ minutes ($P$<0.05) when compared with the baseline value.

*Comparison of changes in the height of ST segment:* Differences in ST segment height regarding all the studied groups at various time intervals were shown in **Table 1**. Comparison of alterations in ST height at baseline, and 30, 60, and 90 minutes after ALP intoxication showed that in AO200, the height of ST wave was significantly increased inthe 30$^{th}$ minutes compared with the baseline value. On the other hand, the height of deviation was significantly ($P$<0.05) reduced in 90$^{th}$ minute in comparison with 30$^{th}$ minute. The height of ST segment did not change substantially at different periods of time in other groups.

***Comparison of CAT activity in 90$^{th}$ minute after ALP intoxication***

Changes in CAT enzyme activity after 90$^{th}$ minutes of intoxication were illustrated in **Figure 3**. The level of myocardial enzyme activity in AO200 group was significantly lower than the control group. In this dose, olive extract prevented the increase in catalase activity in the state of ALP-induced toxicity ($P$<0.05).

***Comparing GPx activity in the 90$^{th}$ minute after ALP intoxication***

Alterations in the level of GPx enzyme activity 90 minutes after ALP poisoning was shown in **Figure 4**. The results indicated that the activity did not significantly change among the studied groups when compared in the different time intervals.

***Comparing MDA concentration in the 90$^{th}$ minute after ALP intoxication***

According to **Figure 5**, variations in the levels of MDA were compared regarding all the groups 90 minutes after poisoning with ALP. The results showed that the concentration of MDA was significantly elevated in all the groups treated with different doses of olive leaf extract.



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**Table 1.** Average changes in the mean of studied variables by terms of groups.

| Groups | Number | Base line | Minutes after intoxication | | |
|---|---|---|---|---|---|
| | | | 30 | 60 | 90 |
| Arterial pressure (mmHg). | | | | | |
| Control | 10 | 108.00±44.46[a] | 85.40±40.79 | 55.72±25.63[c] | 52.12±19.55[c] |
| ALP+Olea100 | 8 | 90.29±48.35 | 84.34±44.64 | 73.07±36.41 | 57.61±19.31 |
| ALP+Olea200 | 8 | 135.00±26.80 | 120.21±30.82 | 76.88±20.39[c] | 71.81±21.28[c] |
| ALP+Olea400 | 8 | 148.94±33.72 | 118.53±73.33 | 77.33±20.66[c] | 197.00±47.06[c] |
| Heart rate (beat/min) | | | | | |
| Control | 10 | 329.80±48.06 | 227.54±103.02 | 134.70±77.32 | 196.37±128.68[b] |
| ALP+Olea100 | 8 | 292.00±53.86 | 241.00±78.54 | 130.74±61.57[c] | 195.43±77.39 |
| ALP+Olea200 | 8 | 306.00±52.56 | 183.21±81.06 [b] | 171.28±87.42 [b] | 243.48±77.05 |
| ALP+Olea400 | 8 | 253.54±114.39 | 125.90±85.53 | 147.25±90.94 | 227.14±137.19 |
| Systolic blood pressure (mmHg). | | | | | |
| Control | 10 | 114.81±45.48 | 98.05±48.82 | 65.27±32.99 | 56.77±22.51[b] |
| ALP+Olea100 | 8 | 69.11±51.62 | 88.10±47.45 | 76.37±38.31 | 59.27±19.74 |
| ALP+Olea200 | 8 | 145.63±32.44 | 129.80±35.33 | 88.44±11.21[c] | 83.26±22.25[c] |
| ALP+Olea400 | 8 | 160.30±33.28 | 127.86±32.54 | 94.51±16.70[c] | 67.53±17.23[c] |
| The QRS duration (ms). | | | | | |
| Control | 10 | 18.06±3.15 | 18.7±3.93 | 21.13±2.93 | 22.33±4.38 |
| ALP+Olea100 | 8 | 20.3±3.21 | 19.91±2.71 | 21.10±3.8 | 22.83±2.65 |
| ALP+Olea200 | 8 | 20.47±6.98 | 18.95±3.14 | 19.98±2.07 | 22.03±2.62 |
| ALP+Olea400 | 8 | 30.56±21.76 | 27.63±15.68 | 33.51±25.30 | 34.69±29.50 |
| The QTC interval (ms). | | | | | |
| Control | 10 | 149.00±44.89 | 125.80±48.53 | 98.43±26.61[b] | 91.60±23.47[b] |
| ALP+Olea100 | 8 | 148.96±35.76 | 131.61±40.90 | 110.98±33.39 | 110.58±19.27 |
| ALP+Olea200 | 8 | 163.58±35.56 | 105.19±37.21[c] | 107.08±17.05[b] | 114.47±20.47[b] |
| ALP+Olea400 | 8 | 183.67±37.99[c] | 191.98±124.46[c] | 119.36±78.67 | 137.21±102.47 |
| The RR interval (ms). | | | | | |
| Control | 10 | 185.65±27.66 | 336.12±186.60 | 654.94±297.94[c] | 574.12±386.22[b] |
| ALP+Olea100 | 8 | 211.08±38.85 | 307.32±216.73 | 672.46±282.77[c,d] | 491.57±186.23[b] |
| ALP+Olea200 | 8 | 200.91±37.14 | 459.67±294.35 | 540.78±246.97[b] | 383.88±167.35 |
| ALP+Olea400 | 8 | 264.37±177.83 | 681.06±299.52 | 723.96±345.07 | 576.90±475.6 |
| The PR interval (ms). | | | | | |
| Control | 10 | 47.29±5.27 | 48.90±5.21 | 45.08±5.85 | 41.92±4.20 |
| ALP+Olea100 | 8 | 52.73±5.39 | 53.98±6.89 | 43.79±7.35[b,d] | 42.58±2.74[c] |
| ALP+Olea200 | 8 | 51.43±7.95 | 47.06±6.71 | 45.87±4.36 | 43.68±2.72 |
| ALP+Olea400 | 8 | 76.42±20.99 | 58.69±22.76 | 78.5±21.03 | 70.56±30.56 |
| The height of T waves (µv). | | | | | |
| Control | 10 | 207.89±73.95 | 157.35±67.85 | 212.24±119.45 | 101.72±92.84[b,d] |
| ALP+Olea100 | 8 | 167.90±74.07 | 176.13±78.10 | 194.96±75.00 | 108.53±84.31 |
| ALP+Olea200 | 8 | 142.9±077.72 | 170.66±87.78 | 138.69±99.24 | 55.05±39.92[b,e] |
| ALP+Olea400 | 8 | 263.69±154.73 | 198.49±144.84 | 89.16±76.99[b] | 40.78±36.71[c] |
| The ST segment deviations (µv). | | | | | |
| Control | 10 | 69.65±60.98 | 92.23±57.94 | 65.46±63.04 | 42.08±32.69 |
| ALP+Olea100 | 8 | 62.89±58.79 | 72.67±60.59 | 75.62±39.15 | 51.88±41.31 |
| ALP+Olea200 | 8 | 87.87±50.88[b] | 117.07±88.76 | 47.03±30.91 | 30.94±23.45[d] |
| ALP+Olea400 | 8 | 105.00±84.41 | 41.37±29.06 | 89.76±67.66 | 52.77±40.73 |

Parameters within the groups were measured using ANOVA, Tukey's post-hoc, and Bonferroni test. [a]: Mean±SEM; b: P<0.05 versus its baseline; [c]: P<0.001 versus its baseline; [d]: P<0.01 versus 30 minutes; [e]: P<0.05 versus 60 minutes.



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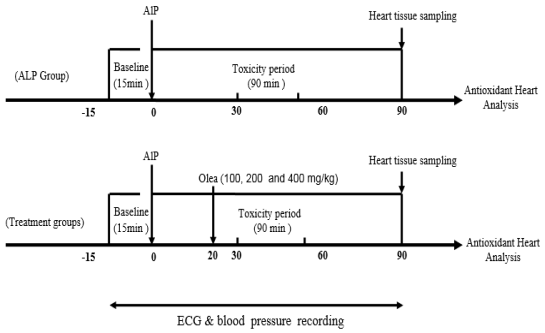


**Figure. 1.** Illustration of the experimental groups involved in toxicity of ALP (120.mg/kg). Different doses of ethanol olive leaf extract (100, 200 and 400 mg/kg) was administered into stomach 20 minutes after ALP exposure.

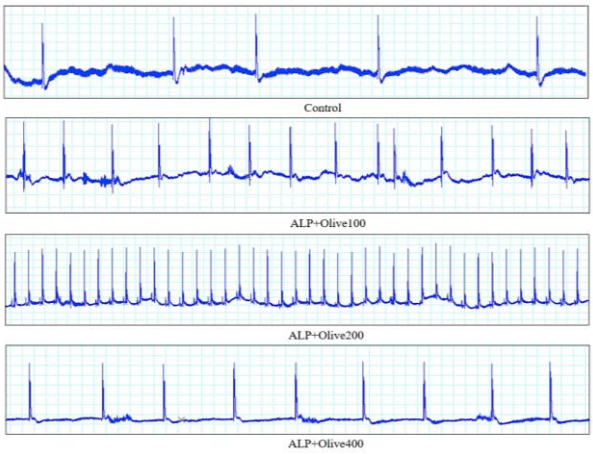


**Figure. 2.** Changes in electrocardiogram parameters in the experimental groups involved in toxicity of ALP (120 mg/kg). Different doses of ethanol olive leaves extract (100, 200 and 400 mg/kg) was administered 20 minutes after ALP exposure.

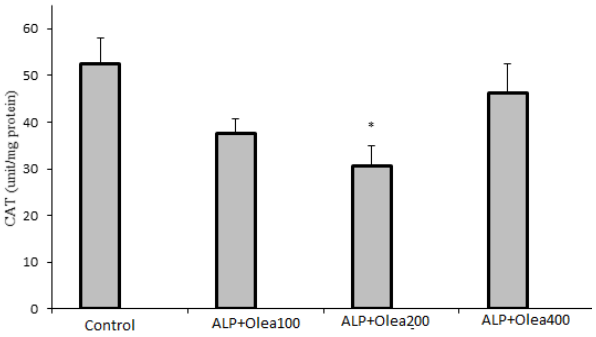


**Figure 3.** Average amount of enzyme activity in cardiac tissue catalase in AC, AO100, AO200, and AO400 groups 20 minutes after ALP (120 mg/kg) exposure and olive leaves extract (100, 200 and 400 mg/kg) administration at baseline and after 30, 60 and 90 minutes.Data are presented as mean±SEM. * P<0.05 versus control group

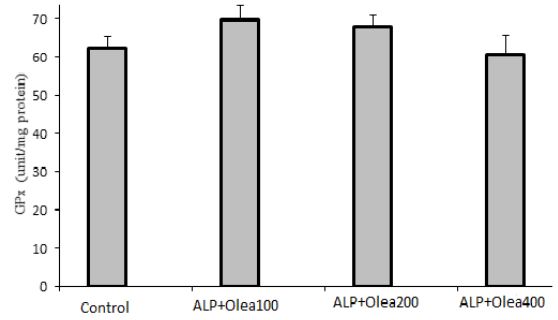


**Figure 4.** The average enzyme activity of cardiac tissue glutathione peroxidase (GPx) in AC, AO100, AO200, and AO400 groups, 20 minutes after ALP (120 mg/kg) exposure at baseline and after 30, 60 and 90 minutes.Data were presented as mean±SEM.

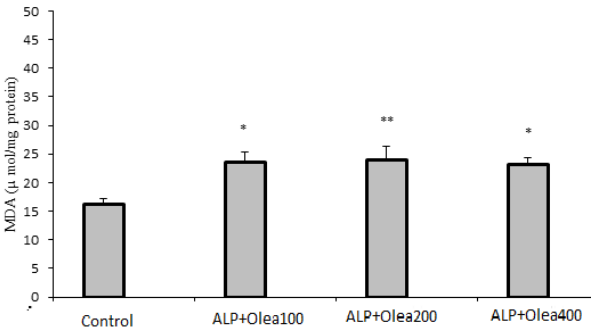


Figure. 5. Tissue content of malondialdehyde in AC, AO100, AO 200, and AO400 groups 20 minutes after ALP (120 mg/kg) exposure during baseline and after 30, 60 and 90 minutes.Data were presented as mean±SEM. * P<0.05 versus control group; ** P<0.01 versus control group.

## Discussion

The present study showed the beneficial effects of olive leaf extract on ALP-induced cardiotoxicity. The findings indicated that intoxication with ALP led to a progressive reduction in heart rate and blood pressure. Severe hypotension and shock are serious complications of ALP toxicity which usually results in death (Bayazit *et al.*, 2000). In the rats intoxicated with ALP, electrocardiographic findings showed that pathological injuries were similar to the damages occurred in myocardial ischemia, causing perturbations in electrical conduction system of the heart. Apparently, a reduction in cardiac output is one of the leading cause of ALP-induced hypotension. In the current study, in comparison with the control group, olive leaf extract, at a dose



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of 100 mg/kg prevented the reduction of MAP and SBP significantly after 60 and 90 minutes of intoxication. As shown in the results, in AO400 group, olive leaf extract significantly prevented the decrese in heart rate after 90 minutes in comparison with the control group. In similar studies conducted on murine models, at concentrations of 10, 20, 40 mg/kg, ALP induced detrimental changes in ECG, similar to what happens in myocardial ischemia. Such alterations usually lead to changes in biochemical factors (Lall *et al.*, 1997). Based on the studies, olive leaf compounds have protective impacts against cardiovascular diseases (Baharvand-Ahmadi *et al.*, 2015, Khosravi-Boroujeni *et al.*, 2013, Madihi *et al.*, 2013b, Shayganni *et al.*, 2016). In 2004, Manna et al. for the first timefound that olive extract had protective effects against acute reperfusion ischemic injuries in an isolated heart model (Manna *et al.*, 2004). In line with this study, in 2006, Andreadou et al. demonstrated protective effects of oleuropein on cardiovascular ischemic injuries through the reduction in oxidative stress and the size of infarction (Andreadou *et al.*, 2006). As an inferation, several studies revealed that the administration of 100-200 mg/kg oleuropein attenuated doxorubicin-induced cardiotoxicity in a rat model (Andreadou *et al.*, 2007). Therefore, olive leaf extract or its active components can be useful in reducing blood pressure and preserving the heart contractility. The changes in ECG which were mentioned can be reversed within 10-14 days in patients who survived from ALP toxicity (Singh *et al.*, 1985). However, the mortality rate is very high in poisoned patients, and their survival rates vary, depending on the severity of intoxication and the half life of ALP (Chugh *et al.*, 1991). Following intoxication with ALP, the concentration of MDA, as a biomarker of lipid peroxidation, is increased in cardiac tissue and the levels of CAT and GPx are decreased in parallel fashion. In the present study, CAT levels were significantly lower in AO200 compared with the control group, indicating that olive leaf extract prevented the increased turn-over of antioxidant enzymes which might be due to the decrease in the rate of oxidative stress (Lall *et al.*, 1997). Animal and human studies have revealed that intoxication with ALP leads to the increase in the rate of oxidative stress as monitored by measuring MDA reactivity (Baradaran *et al.*, 2013, Dua and Gill, 2004, Rafieian-Kopaei *et al.*, 2014). Several papers also indicated that ALP-induced oxidative stress could impair electron transport chain, leading to excessive generation of oxygen free radicals and the changes in antioxidant defense system, including the decrease in CAT levels (Madihi *et al.*, 2013a, Rafieian-Kopaei, 2014, Shirzad *et al.*, 2011). Regarding this study, it was shown that increase in ST segment and decrease in T wave height were not statistically significant in the control group. Also, administration of olive leaf extract at a dose of 200 mg/kg resulted in a decrease in ST segment deviations after 90 minutes, and administration of 100 mg/kg inhibited the decrease in T wave height, compared with the baseline values. Based on pharmacological research, olive extract has antiarrhythmic properties against arrhythmogenic potentials of calcium chloride and aconitine (Esmailidehaj *et al.*, 2016, Mipando, 2004). In 1978, Petkow and Manolov showed that a single intravenous injection of oleuropein within 10-40 mg/kg possesses antiarrhythmic properties against barium chloride in rabbits. In ECG, ST segment implies ventricular depolarization and the initiation of repolarization (Petkov and Manolov, 1978). Previous studies indicated that ALP could paradoxically elevate/depress ST segment, which shows pericardial/myocardial injuries (Baghaei *et al.*, 2014). The findings, however, demonstrated that intoxication of rats with ALP could elevate ST segment in ECG while the administration of olive leaf extract prevented such elevation. A growing body of evidence shows that there is a positive correlation between increase in the ST segment and mortality rate. This parameter is capable of predicting the intensity and the effectiveness of therapeutic strategies in acute toxicity with ALP. PR and QTC intervals are indices of electrical conductance of heart which are both increased in response to intoxication with ALP. QTC is calculated based on Bazett formula in which QT is divided by the square root of RR interval (Ahnve, 1985). This perturbation in electrical conductance



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mimics cardiac arrest, or ischemic injuries occurred following ALP toxicity (Baghaei *et al.*, 2014, Soltaninejad *et al.*, 2012). In the present research, the administration of olive leaf extract at a dose of 100 mg/kg diminished PR interval in 60$^{th}$ and 90$^{th}$ minutes after intoxication of rats with ALP, which suggested that olive extract can neutralize deleterious impact of ALP on electrocardiograph. In ECG, QT interval pertains to depolarization and repolarization of ventricles. QTC is normally decreased when heart rate is increased. In clinics, QTC is usually used (instead of QT) for demonstrating perturbations in electrical conductance in ventricles (Nazemi *et al.*, 2016). In the control group of this research, which was treated only with ALP, QTC was significantly decreased after 60 and 90 minutes in comparison with the baseline. Furthermore, olive leaf extract prevented ALP-induced decrease of QTC at a dose of 100 mg/kg after 60 and 90 minutes. Considering that QTC has an inverse association with square root of RR interval, an increase in RR interval implies the decrease in heart rate following intoxication with ALP. It should be noted that olive leaf extract prevented the decrease in heart rate caused by ALP intoxication through reducing RR interval. Hence, it is concluded that olive leaf extract at a concentration of 100 mg/kg improved heart function and increased QTC; this showed the protective effects against ALP-induced oxidative stress. The levels of MDA and ROS were rapidly increased following intoxication with ALP. Conversely, the activity of CAT, GSH, and thiol-containing compounds were decreased in response to the ALP intoxication of rats. Reports indicated that poisoning with ALP resulted in elevation of the rate of oxidative stress as measured by the concentration of MDA, which was considered a biomarker of lipid peroxidation. However, the authors could not show any significant difference between control and treatment groups concerning the levels of MDA in heart tissue of rats. The main limitation of this study was the risk of using rice tablets for the researcher.

Based on the results obtained in this study, the authors recommendthe following points for future studies: 1-Evaluating olive leaf extract in mitochondrial oxidative stress; 2-Investigating other formulations of olive extract in ALP-induced cardiovascular diseases and 3-increasing the periods of time for assessing the effect of olive leaf extract on ALP-induced cardiotoxicity.

## Conclusion

According to the findings, intoxication with rice tablets is associated with electrocardiographic abnormalities and oxidative stress. In addition, administration of olive leaf extract at different doses primarily improves bradycardia, hypotension, and conduction disturbances of the heart (QTC increased and PR decreased) caused by poisoning with ALP ,prevents increase of ST segment, and decreases T- wave height.

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## Authors' contributions

Moazami M, Sedighi M, Haydari S, Rashidipour M, and Mahmoudi GA were involved to mesurements ad data collection. Moghimian M, Nazari A, Ghaderpour S, and Bakhshesh M were parcitipted to processed data, performed analysis and designed figures. Moghimian M and Nazari A drafted the manuscript and supervised the work. All authors read the final manuscript and verified for publication.

## Conflict of interest

Authors declared no conflict of interest.

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