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Research paper

Can rutin ameliorate aluminum phosphide-induced acute cardiac toxicity in adult albino rats?

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Abstract

Current management of rice tablet or aluminium phosphide (AlP) poisoning has remained mostly supportive despite of its fatal outcome and unfortunately no antidote is found yet, therefore, there is a need to search for a treatment that can adequately protect against its toxicity. To the best of our knowledge, there are no studies until now concerning the cardioprotective effect of rutin against AlP cardiac toxicity in rats. For this purpose, this study was carried out to investigate the possible protective effect of rutin against AlP induced cardiotoxicity in rats. Forty male albino rats were randomly divided into four groups. Group I: normal control group was served as untreated rats and received distilled water orally through a gastric tube. Group II: Rutin treated group received a dose of 100 mg/kg rutin dissolved in distilled water and given orally through a gastric tube. Group III: AlP intoxicated rats received AlP oral single sub-lethal dose (2 mg/Kg body weight) dissolved in distilled water and given through a gastric tube. Group IV: AlP intoxicated rats + Rutin treated one hour after receiving AlP in doses as mentioned above. After that we tested the following parameters: ECG changes including HR and ST-segment elevation, serum level of TNF-α, IL-6 and H-FABP (pg/ml), antioxidant and Oxidant parameters in cardiac tissue as GSH, SOD, and MDA, apoptotic factor caspase-3 and histopathological examination of cardiac tissue was also included. The results showed that treatment with rutin caused a significant decrease in heart rate and ST segment elevation, a significant decrease in activity of TNF-α and IL-6 and levels of H-FABP also a significant decrease in the activity of SOD with decreased levels of MDA and caspase-3 level and a significant increase in the level of GSH compared to (AlP) intoxicated group, also histopathological changes induced by AlP improved after treatment with rutin. It is concluded that AlP intoxication caused ECG, biochemical and histopathological changes which were potentially improved with rutin.

Keywords: *Acute; Aluminum Phosphide (Alp); Cardiac Toxicity; Rats; Rutin.*

1. Introduction

Rice tablet or aluminium phosphide (AlP) is a potentially lethal pesticide that is used extensively for grain preservation. AlP has currently drawn attention because of an increasing number of deaths in the past four decades due to its low cost and availability in the markets that led to its increased misuse to commit suicide (Singh et al., 2014).

AlP mechanism(s) of action not well exactly known until now but previous studies suggest that like other solid phosphides, AlP produces its toxic effects through the liberation of deadly toxic gas, phosphine (PH_3), which is liberated when come in contact with water, moisture or gastric acid. This deadly toxic gas cause inhibition of cytochrome oxidase c and oxidative stress leading to generation of reactive oxygen species (ROS) decreasing the activities of both catalase and glutathione peroxidase and stimulates the activity of superoxide dismutase (SOD) and malondialdehyde (MDA) (Afolabi et al., 2018).

The toxic clinical effects of aluminium phosphide are usually instantaneous, non-specific (Mokhtar et al., 2015) and multisystemic including cardiotoxicity, neurotoxicity, electrolyte imbalance, hepatotoxicity, metabolic disturbances, haematological toxicity, renal toxicity and others (Afolabi et al., 2018) but cardiac tissue is more affected by oxidative stress produced by AlP than other human tissues, as it has an elevated oxidative metabolic activity and an increased polyunsaturated fatty acids content making seventy percent of AlP related deaths attributable to cardiovascular complication (Gouda et al., 2018).

To date, the treatment mostly supportive and there is no treatment that can successfully cure AlP poisoning and unfortunately, no specific antidote is found yet, so there is a need to search for a treatment that can adequately protect against it (Afolabi et al.,2018).

Rutin is one of flavonoid glycosides that are found in herbs and plant foods and has different protective effects in vitro and in vivo (Imam et al., 2017) against lipid peroxidation and oxidative stress-mediated diseases (Lopez-Revuelta et al., 2006). Rutin is an immunomodulator has antioxidant, anti-diarrheal, anti-tumor, and anti-inflammatory effect and myocardial protection (Herrmann and Janke, 2001). Rutin is an important anti lipoperoxidant agent and has been found to be a strong scavenger of superoxide and hydroxyl radicals (Nègre-Slvayre et al., 1991). Furthermore, rutin is a scavenger of the superoxide dismutase–sensitive free radicals (Robak and Gryglewski, 1988; Korkmaz and Kolankaya, 2010). Investigation of the preventive effects of rutin, and various isoflavones against ischemia-reperfusion (I/R) injury –induced hemodynamic alterations revealed that rutin possessed the highest ability to attenuate cardiac I/R asso-

ciated hemodynamic alterations (Bhandary et al., 2012). In addition, rutin increased anti-oxidant molecules and reduced cells necrosis and apoptosis and overall cardiac dysfunction in rats exposed to sodium fluoride-induced oxidative stress (Umarani et al., 2015).
To the best of our knowledge, there are no studies concerning the possible protective role of rutin against cardiac toxicity of AlP in rats, therefore, this study tried to investigate this protective effect of rutin against this toxicity.

2. Materials and methods

2.1. Drugs and chemicals

Aluminium phosphide in form of Tablet (3gm) was purchased from the local market for agricultural products and pesticides in Benha, Qaluybia governorate, Egypt.
Rutin in form of powder was purchased from Sigma-Aldrich (MO, USA).

2.2. Animals

Forty male adult albino rats weighing 200-250 g were obtained from Experimental Animal Breeding Farm, (Helwan-Cairo). They were caged 10 per cage in well-ventilated place at room temperature in the animal house at the department of pharmacology, Benha Faculty of Medicine. They allowed free water and standard food (pellets specific for rat feeding obtained from the animal breeding farm) for 2 weeks of acclimatization. During the whole period of experiment, animals were treated humanely according to the protocol of handling of experimental animals of Benha Faculty of Medicine.

2.3. Study design

All experimental protocol and procedures were done under ethical guidelines for animal use. After 2 weeks of acclimatization, forty rats were randomly divided into 4 groups of 10 animals each as following:
Group I: normal control group was served as untreated rats and received distilled water orally through a gastric tube.
Group II: Rutin treated group received a dose of 100 mg/kg rutin dissolved in distilled water and given orally through a gastric tube (Gelen et al., 2017).
Group III: Aluminium phosphide intoxicated rats received aluminium phosphide oral single sub-lethal dose (2 mg/Kg body weight) dissolved in distilled water and given orally through a gastric tube (Dua and Gill 2004).
Group IV: Aluminium phosphide intoxicated rats + Rutin treated (one hour after receiving AlP dose as mentioned above) and given orally through a gastric tube.
Doses of tested drugs used in this study were with the equivalent therapeutic doses of human according to (paget and Barnes, 1964).
After the last treatment, the animals were anesthetized and ECG records were done using needle electrodes. After that, the blood samples were taken from the heart by the technique described by Parasuraman et al., 2010. Then all rats were sacrificed under anesthesia by decapitation and tissue samples were obtained from the heart of each animal and used for the preparation of tissue homogenates and histopathological examination.
In this study we were investigate the following parameters:

1) ECG changes including HR, ST-segment elevation.
2) Serum level of TNF-α and IL-6.
3) Serum level of H-FABP (pg/ml).
4) Antioxidant and Oxidant parameters in cardiac tissue: GSH, SOD, MDA.
5) Cardiac tissue level of Apoptotic factor caspase 3.
6) Histopathological examination of cardiac tissue.

2.4. Procedural details

2.4.1. Induction of cardiotoxicity

Rats received Aluminium phosphides (AlP intoxicated rats) were given an oral single sub-lethal dose of ALP (2 mg/Kg body weight) through a gastric tube (Dua and Gill 2004).

2.4.2. Determination of ECG changes

Adult male albino rats, weighing about 200-250 gm were used. The animals were anesthetized with urethane in a dose of 1.5- 1.75 gm/kg body weight. Half of the dose was injected intraperitoneally, to induce rapid onset and the other half subcutaneously, to ensure long maintenance of the anesthetic effect. After complete anesthesia, the rats were led on their back. ECG records were done using needle electrodes. The four limb electrodes were fixed to the animal's four limbs and records were done using the standard lead II at a rate of 25m/min (Nirmala et al., 1994).

2.5. Sampling

2.5.1. Blood samples

After ECG records were done, blood samples were taken from the heart by the technique described by Parasuraman et al., 2010 and incubated at 37^0C until blood clotted and then centrifuged at 3000 revolutions per minute (rpm) for 15 min for separation of serum which was stored at 4ºC for future analysis of tested biochemical parameters including serum levels of TNF-α and IL-6 and serum level of H-FABP.

2.5.2. Preparation of the cardiac tissue homogenate

Heart specimens were minced and homogenized (10%) in ice-cold 1.155 KCl-0.01M sodium and potassium phosphate buffer (pH 7.4) in a Potter–Elvehjem glass homogenizer. Then the homogenate was centrifuged at 10,000rpm for 20min at 4°C, and the resultant supernatant was separated and analyzed to measure oxidative, inflammatory and apoptotic parameters (Bradford, 1976).

2.6. Analysis

2.6.1. Serum levels of TNF-α and IL-6

Were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits according to the manufacturer's instructions (Quantikine HS; R & D Systems, Minneapolis, MN, USA). The color intensity of the enzymatic indicator reaction was measured photometrically at 450 nm in an ELISA plate reader, with a minimum detectable level 0.5 pg/mL.

2.6.2. Serum level of H-FABP

Rat heart fatty acid binding protein (h-FABP) ELISA kit. Catalog number: CSB-E16184r.

2.6.3. Measurement of superoxide dismutase (SOD) and malondialdehyde (MDA) in the cardiac tissue

To measure the SOD (E.C. 1.15.1.1) enzyme activity the reduction of the $O_2^{\bullet-}$ with nitrobluetetrazolium (NBT) was assessed spectrophotometrically at 560 nm. Inhibition of the enzyme activity 50% was taken and expressed as U/mg protein (Sun et al., 1988).
MDA level was measured according to the method of Esterbauer and Cheeseman spectrophotometrically at 532 nm at –95 °C52. Results were calculated according to the results of the standard graph and expressed as pmol/mg protein (Esterbauer & Cheeseman, 1990).

2.6.4. Measurement of reduced glutathione (GSH) in cardiac tissue

GSH level was measured by a colorimetric method based on the reduction of 5, 5'- dithionitrobenzoic acid (DTNB) with GSH to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405nm. Values were expressed as nmol/mg protein (Browne and Armstrong, 1998).

2.6.5. Measurement of caspase -3 concentration in cardiac tissue homogenate

Quantitative determination of tissue caspase-3 was measured using Correlate-Assay, Caspase-3 Colorimetric Assay Kit, (Catalog no. 907-013) (Ali and Wahid, 2013).

2.6.6. Histopathological examination of cardiac tissue

The chest was opened and the heart was excised as a whole, put on cold (8°C) 30 mM KCl to achieve diastolic arrest (Hochman et al., 1987). The heart tissues were preserved in neutral formalin 10% and referred for histopathological examination. The hearts were cut into transverse sections from the ventricle and interventricular septum, each section was fixed with methanol and ethanol (1:1), processed with paraffin wax, sectioned at 5µm and stained with hematoxylin and eosin (H&E). The cardiac sections were examined for morphological alterations.

2.7. Statistical analysis

Results are presented as mean ± standard deviation (mean ± SD). Statistical analysis was performed by SPSS version 16 using one-way analysis of variance (ANOVA) to detect overall significant differences between the group means followed by t-test to detect the difference between individual groups. Probability (P) values of < 0.05 were considered as statistically significant.

3. Results

3.1. ECG changes

Experimental induction of cardiotoxicity by aluminium phosphide (AlP) in rats resulted in a significant increase in heart rate and ST segment elevation compared to the normal control group (Table 1).
While treatment with rutin after aluminium phosphide toxicity caused a significant decrease in heart rate and ST segment elevation compared to (AlP) intoxicated group but still significantly higher than the normal control group (Table 1).

3.2. Biochemical results

In this study experimental induction of cardiotoxicity by aluminium phosphide (AlP) in rats resulted in a significant increase in serum levels of TNF-α and IL-6 and increase in levels of H-FABP compared to the normal control group (Table2).
While treatment with rutin after aluminium phosphide toxicity caused a significant decrease in activity of TNF-α and IL-6 and a significant decrease in levels of H-FABP compared to (AlP) intoxicated group but still significantly higher than the normal control group (Table 2).
In this study, experimental induction of cardiotoxicity by aluminium phosphide (AlP) in rats resulted in a significant increase in cardiac tissue activity of SOD, MDA and a significant increase in cardiac tissue level of caspase-3 in association with a significantly decreased level of GSH compared to the normal control group (Table 3).
Treatment with rutin after aluminium phosphide toxicity caused a significant decrease in the activity of SOD, with decreased levels of MDA and caspase-3 level compared to (AlP) intoxicated group but still significantly higher than normal control group, in association

with significant increased level of GSH compared to (AlP) intoxicated group but still significantly lower than normal control group (Table 3).

3.3. Histopathological study of cardiac tissue

In this study, histopathological results of the control group show normal cardiac muscle fibers with normal structure of branching striated cardiac tissue forming parallel fibers and centrally located nucleus (Fig 1). Rutin treated group, the slide shows near normal structure of viable myocardium (Fig.2). In AlP intoxicated group cardiac muscles the slide shows goasts of myocardial cells indicating infarction (Fig. 3) while in AlP intoxicated rats and rutin treated group the slide shows moderate inflammatory cells infiltration mainly lymphocytes with limitation of infarction area (Fig.4).

Table 1: Effect of Oral Administration of Aluminium Phosphide (AlP) and or Rutin in A Rat Model of Aluminium Phosphide (AlP)-Induced Cardiotoxicity on Average (Mean ± SD) on Heart Rate (B/Min), ST-Segment Elevation (Mm)

Groups (n=10)	Parameter	
	Heart rate (b/min)	ST segment elevation (mm)
normal control	301±8	0
Rutin	299±15.17	0
AlP	594±17 [a, b]	12.6 ± 0.39 [a, b]
AlP + Rutin	427±12 [a, b, c]	4.15 ±0.19 [a, b, c]

a: Significant compared with normal control group at $P < 0.05$.
b: Significant compared with Rutin group at $P < 0.05$.
c: Significant compared with AlP + Rutin group at $P < 0.05$.

Table 2: Effect of Oral Administration of Aluminium Phosphide (AlP) and or Rutin in A Rat Model of Aluminium Phosphide (AlP)-Induced Cardiotoxicity on Average (Mean ± SD) Serum Level of TNF-A (Pg/Ml), IL-6 (Pg/Ml) and H-FABP (Pg/Ml).

Groups (n=10)	Parameter		
	TNF-α (pg/ml)	IL-6 (pg/ml)	H-FABP (Pg/ml)
normal control	83±14.1	19±5.2	102±6.12
Rutin	85±20.1	20±5.8	119±8.4
AlP	390±19.6 [a, b]	75±8.4 [a, b]	7401±22.13 [a, b]
AlP + Rutin	145±12.1[a, b, c]	38±3.6 [a, b, c]	2590±14.1[a, b, c]

a: Significant compared with normal control group at $P < 0.05$.
b: Significant compared with Rutin group at $P < 0.05$.
c: Significant compared with AlP + Rutin group at $P < 0.05$.

Table (3): Effect of oral administration of aluminium phosphide (AlP) and or rutin in A rat model of Aluminium Phosphide (AlP)-induced cardiotoxicity on average (Mean ± SD) of SOD activity (U/mg protein), MDA (pmol/mg protein), GSH (nmol/mg protein) and Caspase-3 (Pg/ml) in cardiac tissue.

Groups(n=10)	Parameter			
	SOD (U/mg protein)	MDA (pmol/mg protein)	GSH (nmol/mg protein)	Caspase-3 (Pg/ml)
normal control	2.8±1.1	48±8.1	65±1.4	1452±18.6
Rutin	2.9±1.5	51.41±7.2	63.17±3.4	1480±16.1
AlP	9.8±2.0 [a, b]	175.2±13.2 [a, b]	12.15±2.1[a, b]	3170±21.2 [a, b]
AlP + Rutin	4.3±1.1[a, b, c]	78.6±8.6[a, b, c]	40±6.8[a, b, c]	1945±13.6 [a, b, c]

a: Significant compared with normal control group at $P < 0.05$.
b: Significant compared with Rutin group at $P < 0.05$.
c: Significant compared with AlP + Rutin group at $P < 0.05$.

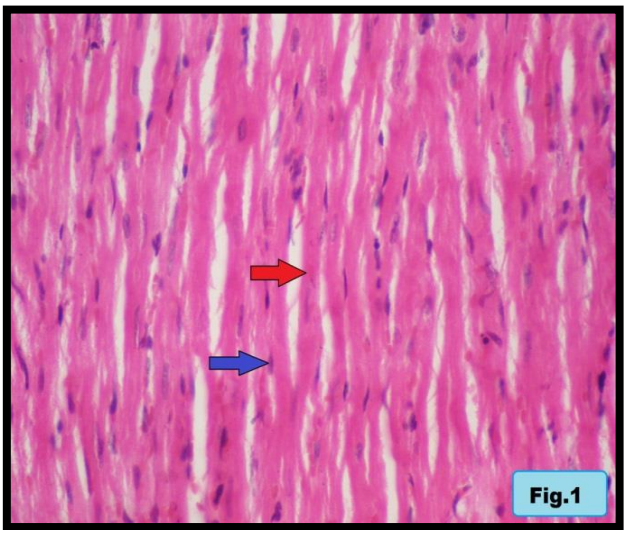

Fig. 1: Histopathlogical Slide of Longitudinal Section of the Heart Stained with Hematoxylin and Eosin (40X) in the Rats of Control Group Shows Cylindrical Muscle Fibers Have Acidophilic Sarcoplasm (Red Arrow) and Centrally Located Oval Nucleus (Blue Arrow).

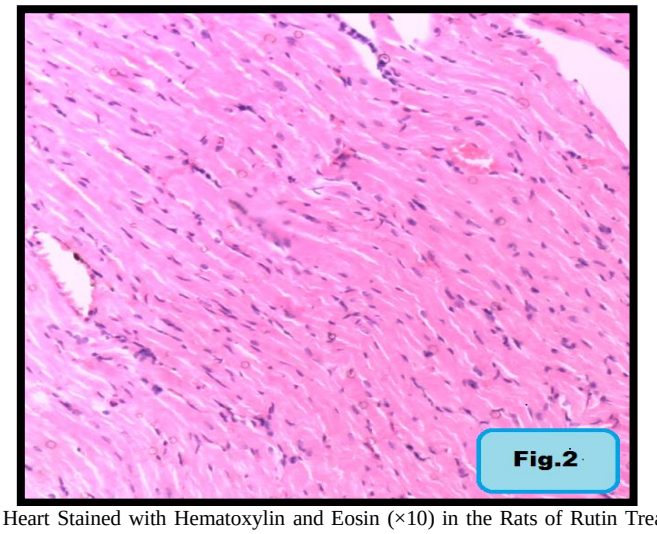

Fig. 2: Histopathological Slide of the Heart Stained with Hematoxylin and Eosin (×10) in the Rats of Rutin Treated Group (100 Mg/Kg) Shows Near Normal Structure of Viable Myocardium.

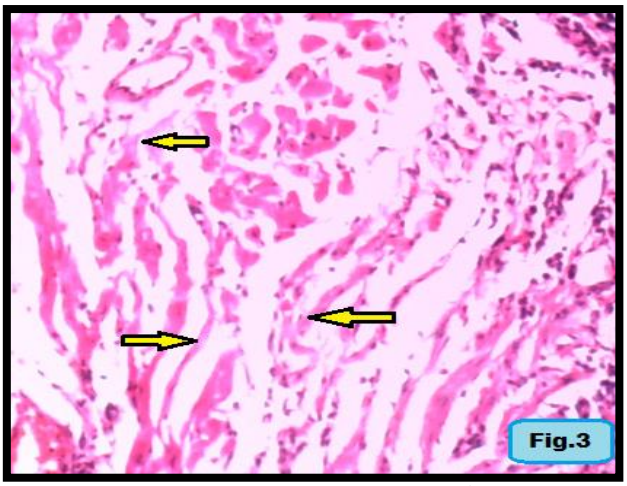

Fig. 3: Histopathological Slide of the Heart Stained with Hematoxylin and Eosin in the Rats of AlP Intoxicated Group (2 Mg/Kg) Shows Goasts of Myocardial Cells Indicating Infarction (Yellow Arrow) (40X).

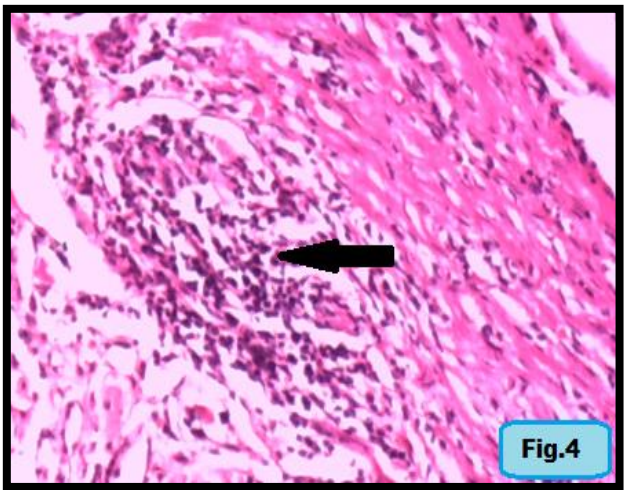

Fig. 4: Histopathological Slide of the Heart Stained with Hematoxylin and Eosin (×10) in (AlP 2mg/Kg + Rutin 100mg/Kg) Group Shows Myocardial Muscle Fibers with Moderate Inflammatory Cells Infiltration Mainly Lymphocytes (Black Arrow) with limitation of infarction area.

4. Discussion

In this study, the adjuvant therapeutic effect of rutin on the cardiotoxicity of aluminium phosphide (AlP) on rats was evaluated on some basis including ECG records as (heart rate and ST segment elevation), biochemical basis including (serum levels of TNF-α, IL-6 and H-FABP), cardiac tissue levels of (SOD, MDA, GSH and caspase -3 concentration) and histopathological basis.

Data obtained in the present study revealed that aluminium phosphide (AlP) administration resulted in a significant increase in heart rate with ST-segment elevation compared to the normal control group.

It is well known that heart rate is affected by changes in oxygen levels and the body compensate for its decrease by increasing the cardiac output with consequent tachycardia and arrhythmia (Merone et al., 2017).

This is also in line with a recent report of Ahmadi et al. (2018) who founded that heart rate significantly increases in the AlP poisoning and tachycardia became significant 90 min after the poisoning and HR increased with time. Also reported that the ST elevation was first recorded 60 min after AlP poisoning and continued to rise with time until the rats died.

This is also in agreement with earlier studies founded that AlP poisoning leads to ST elevation (Soltaninejad et al., 2012; Baghaei et al., 2014).

Results of this study also revealed that treatment with rutin after aluminium phosphide toxicity resulted in a significant decrease in heart rate with improved ST-segment elevation compared to (AlP) treated group but still significantly higher than normal control group.

Ma et al. (2017) found that rutin showed improved cardiac functions recorded by echocardiography. Moreover, Kolchin et al. (1991) found that administration of rutin and quercetin solution was found to improve the contractile function of the left ventricular myocardium, decrease the incidence of heart rate and conductivity disorders, limit the ischaemic damage area, and improve coronary circulation and ST-segment elevation.

The present results showed that aluminium phosphide (AlP) caused a significantly increased serum level of TNF-α, IL-6 and H-FABP with a significant increase in activity of cardiac tissue of SOD, MDA, and caspase-3 in association with a significantly decreased level of GSH compared to the normal control group.

These data are in accordance with Sheweita et al. (2015) who reported increased activity of SOD and CAT antioxidant activities. Also, Gouda et al. (2018) found that the level of MDA was significantly increased in AlP intoxicated group while enzymatic antioxidant parameters were significantly decreased in AlP intoxicated group.

The present study revealed that treatment with rutin after aluminium phosphide toxicity leading to a significant decrease in activity of SOD, with decreased levels of MDA, IL-6, and TNF-α compared to (AlP) intoxicated group but still significantly higher than normal control group, in association with significant increased level of GSH compared to (AlP) intoxicated group but still significantly lower than normal control group.

These findings were supported by Lv et al. (2018) who demonstrated that treatment with rutin significantly reversed the induced reductions of SOD, CAT, GST and GSH-Px activities in chronic heart disease in the pig. In addition, it has been explained that rutin has an anti-inflammatory activity due to modulation of apoptosis-associated proteins (Aruna et al., 2013).

In this regard, Hsieh et al. (2014) explained that the treatment of isolated hearts with green tea extract rich in flavonoids as rutin showed marked protection of hearts from oxidative stress and avoid cell edema and apoptosis.

The present study also revealed that treatment with rutin after aluminium phosphide toxicity leading to a significant decrease in activity of serum level of HFABP compared to (AlP) intoxicated group but still significantly higher than the normal control group. Diagnosis and assessment of myocardial toxicity and injury require sensitive and specific biomarkers. The present results suggest that H-FABP may be an ideal option, highly sensitive and its plasma concentration increases even earlier than that of Mb after myocardial injury.

YE et al. (2018) also suggest that HFABP might be an ideal option to diagnose the myocardial injury. It is sensitive, excellent and can serve as a very good marker for myocardial injury. These results are augmented by Ma et al. (2017) who confirmed that rutin attenuates cardiotoxicity by regulating autophagy and apoptosis. Also, Topal et al. (2018) stated the possible protective role of rutin against cardiotoxicity. Wu et al. (2017) prove the beneficial effect of rutin on GSK-3β and TNF-α expression in lung cancer.

In this study, histopathological results show that AlP intoxicated group was characterized by marked histopathological abnormalities in cardiac tissues as infarction when compared to control group. In agreement with our finding, Shah et al. (2009) reported the same histopathological changes as necrosis and infarction in many of the myocardial fibers. Also, AlP effect was reported in Akkaoui et al. (2007) study. Fox et al. (2007) founded that tachycardia can resulted in cardiac ischemia, ventricular arrhythmias which ended in infarction and arrhythmias in AlP group. AlP cardiotoxic effect not reported in Anand et al. (2011) study may be because of their usage of a single lethal dose (20mg/kg body weight) that caused rapid animal death without causing an inflammatory response. In addition, rutin decreased muscle fiber disruption, necrosis, mononuclear cells infiltration, and myocytes swelling. This is in agreement with Hashemzaei et al. (2016) who observed that the polyphenol-containing compounds e.g. rutin can increase mitochondrial metabolism, increase the expression of respiratory chain members, and increase oxygen tissue utilization. Also, it can inhibit the main apoptotic pathway by modification of Akt protein expression.

5. Conclusion

From the results of current study, it is concluded that AlP intoxication caused ECG, biochemical and histopathological changes which were potentially improved with rutin. However, further studies evaluating the cardiotoxic effects of AlP at different concentrations on cardiac functions are needed.

6. Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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