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Pomegranate Molasses and its Peels as Anti-agent Cardiotoxicity Induced by Azithromycin in Male Rats

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Abstract

Azithromycin (AZ) is an effective macrolide antibiotic that has been used in the treatment of various types of serious bacterial infections. However, the cardiovascular adverse effects associated with (AZ) have recently attracted attention.The current study aimed to evaluate the protective effect of bioactive compounds in pomegranate molasses (pm) and its peels as anti-agent cardiotoxicity induced by azithromycin in male rats. The rats were divided into two main groups .The first group (6 rats) was fed on a basal diet and served as a negative control. The second group (30 rats) received Azithromycin (AZ) from the 14th to the 28th day to induce cardiotoxicity. The second group was divided into five subgroups. Group (1) was fed on basal diet only (and served as positive control),Group (2) was fed on a basal diet + a daily oral dose of Pomegranate molasses (2 ml /kg B.W/day), Group (3) was fed on a basal diet + a daily oral dose of Pomegranate molasses (4 ml /kg B.W/day), Group (4) was fed on a basal diet + a daily oral dose of Pomegranate peel extract (200 mg/kg), Group (5)was fed on a basal diet + a daily oral dose of Pomegranate peel extract (400 mg/kg) for 28 days .At the end of the experiment, feed intake (FI), body weight gain (BWG), feed efficiency ratio (FER), and relative heart weight were calculated. Assessment of some serum biochemical parameters, heart tissues were analyzed for antioxidant/oxidant markers and histopathology of hearts was assessed. The results revealed that pomegranate molasses and its peels improved the biological evaluation, heart functions, antioxidant enzyme activity, and histopathology of hearts compared to the positive group. In conclusion, administering pomegranate molasses and its peel extract can lower the impacts of azithromycin on the heart.

Keywords: cardiotoxicity – Azithromycin _ heart functions – pomegranate.

Introduction

Azithromycin (AZ) is an effective macrolide antibiotic that has been used in the treatment of various types of serious bacterial infections. However, the cardiovascular adverse effects associated with (AZ) have recently attracted attention ***(Rao et al.,2014).***Azithromycin administration for two weeks caused a significant elevation of serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH), which indicated the release of these cardiac biomarkers from the damaged myocardium into the circulation. In addition, AZ administration increased the oxidative stress and inflammatory response, as signified by increased plasma malondialdehyde (MDA) and tumor necrosis factor-alpha (TNFα), respectively ***(Mohamed and Kasse, 2018). El-Naeem et al., (2022)*** mentioned that azithromycin drug-

induced cardiotoxicity should be used in limited cases. The toxic effects of AZ on the heart can be potentially reduced by treatment with vit.C.

The pomegranate (*punica granatum L*.) is a deciduous tree distributed everywhere in the world. It has been used in various regions and folk or traditional medical systems as a food supplement or medicine because of its enormous compounds with lots of activities and without toxicity. Nowadays, it is widely approved that the beneficial health effects of fruits and vegetables in disease prevention are due to their bioactive component ***(Vijayalakshmi and Sangeetha, 2012).*** A significant amount of bioactive compounds such as phenolic acids, flavonoids, and tannins in pomegranate fruit assures them great nutritional value. It is a rich source of polyphenols such as anthocyanins, ellagitannins, and other phenolic compounds confirmed to have antioxidant and antitumoral activities ***(Al-Moraie et al., 2013).***

Pomegranate molasse (PM) is a large compound of Eastern diets, yet limited research has been performed on this product. It is highly nutritious because it is more concentrated and has high mineral content. Traditional methods boiling are still used to produce (PM). It is concentrated by boiling without adding other sugar or additives ***(Sumathy et al.,2013).*** Typical processing requires cleaning, crushing, extraction, filtration, clarification, and evaporation in an open container or under a vacuum. A recent study indicated that high temperature does not alter the antioxidant activity of (PM) against (ROS). On the other hand, it seems that the high temperature helps to release polyphenols from pomegranate fruit cells as there is no extraction with a solvent in the preparation of pomegranate molasses ***(Al-Malki and Sayed, 2014).***

The pomegranate fruit peel extract (PPE) can be processed for some products having medicinal, cosmetic, and industrial value ***(Luque et al., 2017).***The pomegranate peel extract (PPEE) has an effective free radical scavenging and antioxidant capacity ***(Zakaria et al., 2018).*** It exhibits marked antioxidant properties. It has been shown to reduce oxidative stress mediators, indicating its antioxidant capacity, attributed to diverse phenolic compounds, such as gallic acids, ellagic acids, ellagitannins, catechins, gallotan- nins, anthocyanins, quercetins, and ferulic acids. These polyphenols possess antioxidant properties in scavenging free radicals and inhibiting lipid oxidation ***(Abdel-Daim et al., 2020).***For this reason, the present research was carried out to study the protective effect of Pomegranate molasses and peels against azithromycin-induced cardiotoxicity in male rats.

Material and Methods

Materials

Pomegranate was obtained from the big market in Tanta, Egypt. Corn oil and starch were purchased from the local market. Casein, cellulose, vitamins, minerals, dextrin, L-cysteine, and choline chloride were obtained from the Cairo Company for Chemical Trading in Cairo, Egypt. Azithromycin powder was purchased from a local pharmacy. Manufactured in Egypt, Rats received AZ (30 mg/ kg/ day) intragastrically for two weeks. The solution was prepared at a concentration of 20 mg/1ml by dissolving 300 mg Azithromycin powder in 15 ml distilled water, according to ***Abd El-kader, (2019).*** Thirty-six male albino rats (*Sprague Dawley* strain) were obtained from the Laboratory Animal Colony, Helwan, Cairo – Egypt, weighing approximately (150± 10g). Kits were purchased from Egyptian American Company for Laboratory Service .

Methods

Preparation of Pomegranate molasses

Pomegranate molasses was prepared from the components found in the supermarket by adding sugar and lemon juice to Pomegranate juice (4 cup pomegranate juice +1/2 cup lemon juice+ 1/2 cup sugar) and boiling for 6 hours, according to **Abd Elmonem, (2014).**

Preparation of Pomegranate peel extracts (PPE)

Pomegranate peels were washed thoroughly under running tap water and dried at (45°C) for 12 hours in the air oven. The dried samples were milled using an electric stainless still mill (Braun, Model 537, Germany) to give a homogenous sample and kept in polyethylene bags at (-20°C) until use. A total of (1)kg peel powder was added to 1L boiling water, after which the mixture was kept in a bolted vessel for cooling. The solvent was filtered and concentrated in a water bath until the extract was reduced to a volume of 100 ml. The herb/extract ratio was(10/1). PPE was finally diluted to 10%, according to ***Kamali et al.,(2015).***

Experimental design

Thirty-six adult male albino rats *Sprague Dawley* strain weighting (150± 10g) were housed in well-aerated cages under a hygienic condition and were fed on a basal diet according to ***Reeves et al., (1993)*** for one week for adaptation. After this week, the rats were divided into two main groups:

The first group (6 rats) was fed on a basal diet and served as a negative control.
The second group (30 rats) received Azithromycin (AZ) from the 14th to the 28th to induce cardiotoxicity, according to ***Abd El-kader, (2019).*** The second group was divided into five subgroups: **Group (1):** was fed on a basal diet only (and served as positive control). **Group (2):** was fed on basal diet + a daily oral dose of Pomegranate molasses (2 ml /kg). **Group (3):** was fed on basal diet + a daily oral dose of Pomegranate molasses (4 ml /kg), according to ***Chalfoun-Mounayar et al.,(2012).***
Group (4): was fed on basal diet + a daily oral dose of PPE (200 mg/kg).**Group (5):** was fed on basal diet + a daily oral dose of PPE (400 mg/kg) for 28 days, according to ***Emam et al., (2020)***. At the end of the experiment (28) days, the animals were deprived of food and water overnight before being sacrificed then animals were weighed and sacrificed under light ether anesthesia.

Blood samples were collected in dry centrifuge tubes from hepatic portal veins. Serum samples were separated by centrifugation at 3000 rpm for 10 minutes and kept in the plastic vial at -20 till analysis. The heart was removed, washed with isotonic saline, dried with filter paper, and weighed. Two samples of the heart were taken. The first sample was kept in formalin saline 10% for histopathological examination. The second sample of the heart was maintained at -80°C for tissue homogenate preparation to determine antioxidant parameters. The homogenate was centrifuged at 1000 rpm for 10 min. The supernatant was used for the assay of some laboratory analyses.

Biological evaluation

At the end of the experiment, feed intake, body weight gain, relative organs weight, and feed efficiency ratio were calculated according to ***Chapman et al., (1959).***

Biochemical analysis of serum

Serum samples were used for the determination of phenolic compound in pomegranate molasses and its peels by HPLC, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), and

creatine kinase myocardial bound (CK-MB) markers of cardiotoxicity measured using automated biochemistry analyzer (SPIN120-Spin react-benchtop 8 wavelengths: 340 and 670 nm). Albumin was measured according to ***Drupt, (1974) .***Total protein was estimated according to ***Sonnenwirth and Jaret, (1980).*** Globulin was calculated according to ***Busher et al., (1990)*** using the following equation: Globulin = Total protein – Albumin. Serum total lipids, total cholesterol, and triglycerides were determined in the serum according to ***(knight et al., 1972; Allain et al., 1974; Fossati and Prencipe, 1982),*** respectively. HDL-c, LDL-c, and VLDL-c were determined in the serum according to **(Lopes-*Virella et al., 1977 and Henriksen et al., 1981).***

Assessment of antioxidant activities in the heart tissue

Antioxidant indications were assessed such as Superoxide dismutase (SOD), were assessed according to ***(Nishikimi et al., 1972),*** and Catalase (CAT) was assessed by colorimetric assay ***(Sinha, 1972).*** Lipid peroxide (LPO) as malondialdehyde (MDA) was assessed by colorimetric assay ***(Buege and Aust, 1978),*** and Nitric oxide (NO) was determined according to ***(Cortas and Wakid, 1990).***

Histopathological examination

The heart of each sacrificed rat was removed and fixed in a 10% neutral buffering formaldehyde solution with a pH of 7.5, then cleaned in xylol before being fixed in paraffin. For histological analysis, a 4-5 µm thick piece was cut and spotted with Hematoxylin and Eosin (H&E) ***(Bancroft and Gamble, 2008).***

Statistical analysis

One-way analysis of variance (ANOVA) was used, followed by the Duncan test, in SPSS software (18) to know the difference between means at $P < 0.05$. The data were presented as a mean ± standard deviation (SD) ***(Snedecor and Cochran, 1989).***

Results

Biological evaluation

Feed intake (FI), body weight gain % (BWG), and feed efficiency ratio (FER), shown in Table 1, have significantly decreased in control +ve group compared to the normal group. However, the other treated groups have revealed a significant increase in all of them compared with the control+ve group, and the best result found in group Pomegranate peels (400 ml/kg) in (FI, BWG) and the best results for FER was recorded for Pomegranate peels (200 &400 mg /kg).(table1)

Table (1)

The protective effect of Pomegranate molasses and peel on feed intake (FI), body weight gain (BWG %), and feed efficiency ratio (FER) in rats with cardiotoxicity **(**mean ± SD)

Parameters / Groups	FI (g) for 28 days	BWG (%)	FER
Control –ve	565.20 ± 3.89 [a]	40 ± .04 [a]	0.07±0 .001 [a]
Control +ve	508.60 ± 1.47 [f]	16 ± 1.04 [f]	0.03 ±0.007 [d]
Pomegranate molasses2ml	524 ± 1.20 [e]	25 ± 0.01 [e]	0.05 ±0 .002 [c]
Pomegranate molasses 4ml	530 ± 0.51 [d]	28 ±0 .02 [d]	0.05 ± 0.003 [c]
Pomegranate peels200ml	536 ± 3.50 [c]	31 ± 1.64 [c]	0.06 ± 0.002 [b]
Pomegranate peels 400 ml	554 ± 1.57 [b]	35 ± 1.34 [b]	0.06 ± 0.004 [b]

Means in the same column with completely different letters are significantly different at p<0.05.

Relative Heart weight

As shown in (Table 2), relative heart weight has increased in the control+ve group compared to the control–ve group. However, it was significantly decreased in all treated groups compared with the control +ve Group. The best result was recorded in Pomegranate peels (400 mg/kg), which recorded a significant increase in relative heart weight compared with other investigated groups.

Table(2)

The protective effect of Pomegranate molasses and peels on relative Heart weight in rats with cardiotoxicity **(**mean ± SD)

Parameters / Groups	Relative Heart weight%
Control –ve	0.32±0.02[c]
Control +ve	0.53 ± 0.06[a]
Pomegranate molasses2ml	0.43 ±0 .01[b]
Pomegranate molasses 4ml	0.42 ±0 .03[b]
Pomegranate peels200ml	0.40 ±0 .05[b]
Pomegranate peels 400 ml	0.35 ±0 .04[c]

Means in the same column with completely different letters are significantly different at p<0.05.

Heart functions

The data in Table 3 indicated that mean values of LDH, CPK, and CKMB in control +ve group were significantly higher than in the Control –ve group. All parameters in treatment groups significantly decreased (P<0.05) compared to the control +ve group. The best findings in LDH, CPK, and CKMB were found in Pomegranate peels (400 mg/kg).

Table(3)

The protective effect of Pomegranate molasses and peel on heart functionsin rats with cardiotoxicity (mean ± SD)

Parameters / Groups	LDH (U/L)	CPK (U/L)	CKMB (ng/ ml)
Control –ve	442 ± 2.73[f]	428 ± 2.37 [f]	0.3080 ± 0.01 [f]
Control +ve	1745 ± 3.41[a]	930 ± 3.37 [a]	0.5540 ± 0.03 [a]
Pomegranate molasses2ml	1620 ± 2.26 [b]	820 ± 1.87 [b]	0.4340 ± 0.02 [b]
Pomegranate molasses 4ml	1400 ±4.23 [c]	730 ± 3.32 [c]	0.3960 ± .01 [c]
Pomegranate peels200ml	1160 ± 5.41 [d]	628± 2.25 [d]	0.3440 ± 0.02 [d]
Pomegranate peels 400 ml	750 ± 4.35 [e]	530 ± 1.95 [e]	0.3400 ± 0.02 [e]

Means in the same column with completely different letters are significantly different at p<0.05.

Serum Total protein, Albumin, and Globulin

The data in Table 4 demonstrated that the mean value of total protein, albumin, and globulin in (the control+ve group) was significantly declined compared with the control –ve group. However, all other examined groups recorded a significant increase compared to the control +ve group. The best result was found in the group of Pomegranate peels (400 ml/kg).

Table(4)

The protective effect of Pomegranate molasses and peel on total protein, albumin, and globulin in rats with cardiotoxicity (mean ± SD)

Parameters / Groups	T. Protein (g/dl)	Albumin (g/dl)	Globulin(g/dl)
Control –ve	7.82 ± 0.20 [a]	4.62 ± 0.15 [a]	3.20 ± 0.14 [a]
Control +ve	5.2 4 ± 0.15 [e]	2.84 ± 018 [d]	2.40 ±0 .17 [d]
Pomegranatemolasses2ml	5.72 ± 0.49 [c]	3.12 ± 0.46 [d]	2.60 ±0 .16 [c]
Pomegranatemolasses4ml	6.4 2 ± 0.16 [c]	3.6 2 ± 0.29 [c]	2.80 ± 0.15[b]
Pomegranate peels200ml	6.5 2 ± 0.40 [c]	3.7 2 ± 0.26 [c]	2.80 ±0 .15[b]
Pomegranate peels 400 ml	7.42 ± 0.16[a]	4.22 ±0 .08 [b]	3.20 ± 0.14 [a]

Means in the same column with completely different letters are significantly different at p<0.05.

Antioxidant enzymes (CAT, SOD), malondialdehyde (MDA), and nitric oxide (NO) in heart tissue

Table 5 illustrates the activities of catalase (CAT), and superoxide dismutase (SOD) significantly declined in the control+ve group compared to the control –ve. At the same time, they rose in other groups compared with the control+ve group. The best result was found in the group of Pomegranate peels (400 ml/kg). Also, the same table revealed that the mean value of MDA and NO were significantly higher in the control +ve group increase compared with Control –ve. However, their values were significantly lower in other groups compared to the control+ve group. The best findings in MDA and NO were recorded in Pomegranate peels (400 mg/kg).

Table(5)

The protective effect of Pomegranate molasses and peel on CAT, SOD, MDA, and NO in heart tissue of rats with cardiotoxicity (mean ± SD)

Parameters / Groups	CAT (U/g.t)	SOD (U/g.t)	MDA (nmol/g.t)	NO (µmol/g.t)
Control –ve	3.5 ±0.10[a]	306.75 ±1.67 [a]	22.40 ± 3.26 [f]	0.79 ± 0.04 [f]
Control +ve	1.80 ±0.08[f]	124. 70 ±1.92 [f]	52.20 ± 1.83 [a]	1.91 ± .05 [a]
Pomegranate molasses 2ml	2.32 ±0.09[e]	179.40 ±2.88 [e]	41.60 ± 2.40 [b]	1.45 ± 0.10 [b]
Pomegranate molasses 4ml	2.53 ±0.15 [d]	204.45 ±4.32 [d]	37.60 ± 0.54 [c]	1.89 ± 0.05 [d]
Pomegranate peels 200ml	2.78 ±0.18 [c]	232.75 ±4.90 [c]	34.72 ± 1.10 [d]	1.27 ±0 .13 [e]
Pomegranate peels 400 ml	3.05 ±0.10 [b]	263.50 ±2.56 [b]	27.20 ± 1.62 [e]	0.92 ±0 .03 [e]

Means in the same column with completely different letters are significantly different at p<0.05.

Serum total cholesterol, triglycerides, high, low, and very low-density lipoprotein-cholesterol

The data in Table 6 demonstrated that the mean value of total cholesterol (TC), triglycerides (TG), low and very low-density lipoprotein-cholesterol (LDL-c-VLDL-c) was significantly increased in the positive control group when compared with the negative control. In contrast, the high-density lipoprotein-cholesterol (HDL-c) level decreased in the (+ve) group compared to the normal control. The best result of total lipids, total cholesterol (TC), triglycerides (TG), (LDL-c-VLDL-c), and (HDL-c) was found in the group administrated with pomegranate peels (400 ml/kg) as compared with the negative control group.

Table (6)

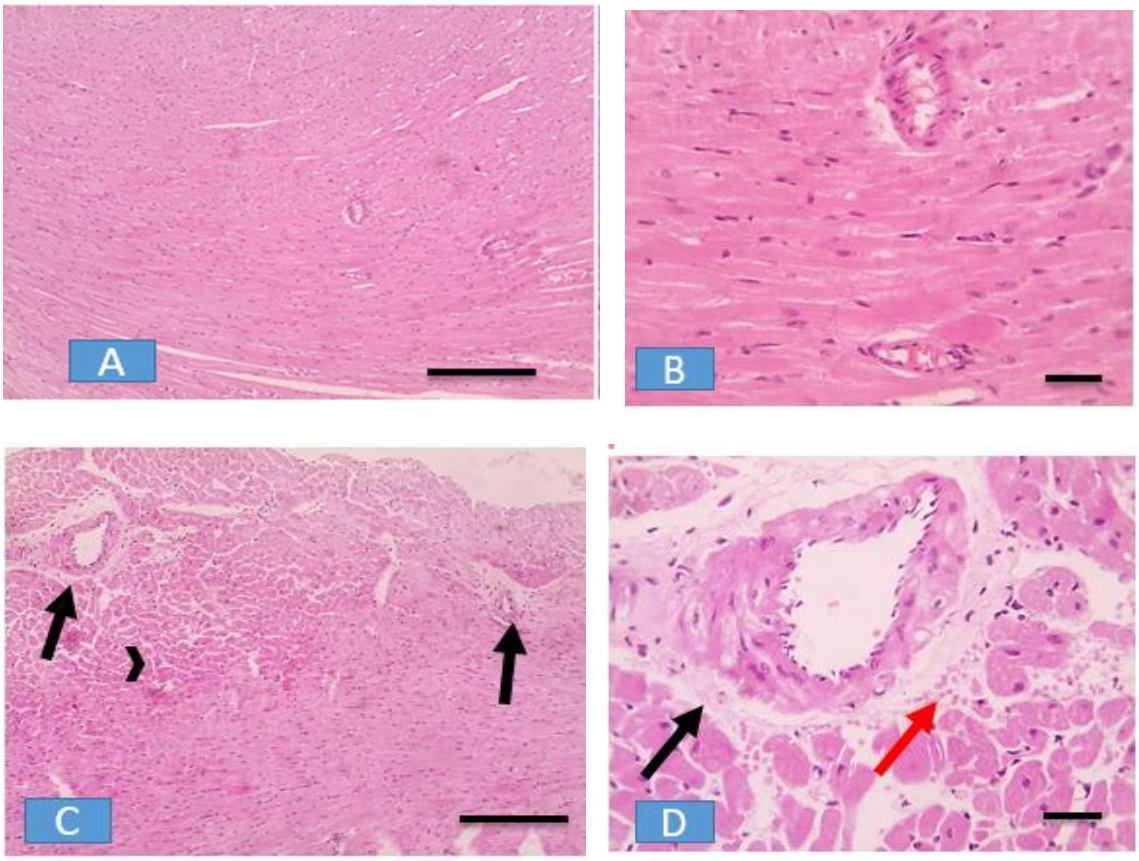
The protective effect of Pomegranate molasses and peel on total cholesterol, triglycerides, high, low, and very low density lipoprotein cholesterol in rats with cardiotoxicity (mean±SD)

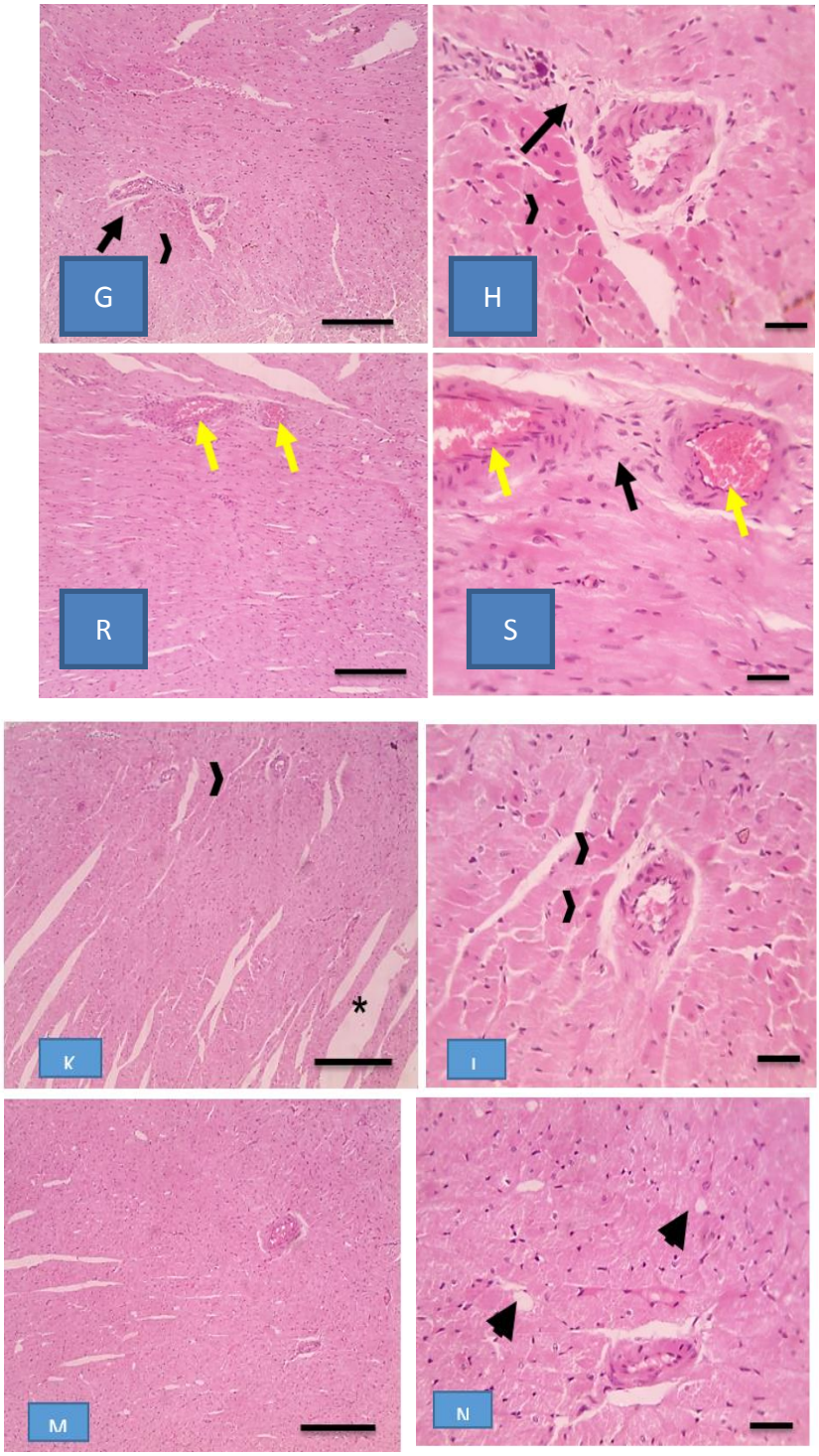
Parameters / Groups	T.C(mg/dl)	T.G(mg/dl)	HDL-c(mg/dl)	LDL-c(mg/dl)	VLDL-c(mg/dl)
Control –ve	91.40 ± 2.15 [f]	70 ± 1.15 [e]	49 ± 1.09 [a]	28.20 ± 2.04 [f]	14 ± 0.92 [e]
Control +ve	194.12± 2.85 [a]	164.80± 2.75 [a]	31 ± 1.64 [e]	130 ± 0.01 [a]	32.96 ± 2.06 [a]
Pomegranate molasses 2ml	184.96 ±1.89 [b]	129.80± 2.04 [b]	36 ± 1.22 [d]	123.20± 2.04 [b]	25.96 ± 1.63 [b]
Pomegranate molasses 4ml	171.16± 2.32 [c]	110.40± 1.08 [c]	38 ± 1.09 [c]	111.80± 1.84 [c]	22.10 ± 2.10 [c]
Pomegranate peels 200ml	151.30 ±1.53 [d]	104.80 ± 1.72 [c]	39 ± .45 [c]	90 ± 1.76 [d]	20.96 ± 2.76 [c]
Pomegranate peels 400 ml	118 ± 1.44 [e]	85 ± 1.23 [d]	46 ± 1.09 [b]	54 ± 1.35 [e]	17.20 ±0 .69 [d]

Means in the same column with completely different letters are significantly different at p<0.05.

Histopathological Results:

Histological sections of rat heart showing the control group **(A&B)** with the normal muscle fibers, interstitial tissue and blood vessels **.**The positive group **(c&d)**shows severe pathological changes including: multifocal large areas of hyalination (deep eosionophilic sarcoplasm with pyknotic nuclei) (opened arrowheads), vacuolation (closed arrowheads), congestion (yellow arrows), marked perivascular fibrosis (black arrows) with extravasated RBCs (red arrows). The pomegranate molasses (2ml/kg) **(G&H)** group showing mild perivascular fibrosis (black arrows), very few mononuclear and mast cells infiltration, small perivascular areas of hyalinatition (opened arrowheads)**.** The pomegranate molasses (4ml/kg) **(R&S)** group showing milder congestion (yellow arrows) with very mild perivascular fibrosis (black arrows)**.** The pomegranate peels (200ml/kg) **(K&I)** group showing interstitial edema (*), hyalinatition in few cardiac muscles (opened arrow heads). The pomegranate peels (400ml/kg) **(M&N)** group showing mild vacuolation (closed arrowheads) in few cardiac muscles .

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Discussion

Azithromycin (AZ) is an effective macrolide antibiotic that has been used in the treatment of various types of serious bacterial infections. However, the cardiovascular adverse effects associated with AZ have attracted attention recently. Prolonged Q-T interval, malignant arrhythmia (torsade de pointes), and even sudden deaths due to ventricular arrhythmia ***(Lu et al., 2015).*** Therefore, the present work aimed to detect the pathological changes induced by AZ in albino rats and the possible protective effect of Pomegranate molasses and peel using biochemical and histological methods.

The present work's findings agree with ***Banihani.,(2013),*** Who observed that AZ caused a marked reduction in feed intake and mean body weight. On the other hand, in treated groups with (Pomegranate molasses and peels), feed intake, BWG, and FER were significantly increased compared to the AZ group (+ve). Reduced body weight gain may be attributed to the direct toxic effects of this chemotherapy (AZ) on the intestinal mucosa and the subsequent action on the gastrointestinal tract. This influences the appetite, eating behavior, consumption, and assimilation of food, and a deterioration in the metabolism of glucose and fatty acids, leading to malnutrition. Also, these results agree with ***Al-Malki,, (2014),*** who noticed that weight gain observed in the animals treated with Pomegranate molasses and peels might be because the extract did not disrupt the hormonal-metabolic processes of the tested animals for weight gain and loss regulations. ***Al-Moraie., (2013)*** reported that the diets using Pomegranate molasses and peels at different levels increased the body weight gain of rats compared with a positive control group. In this respect, ***Colombo., (2013)*** found that oral administration of Pomegranate molasses and peels significantly increased body weight gain, feed intake, and FER.

Our results support that by ***Moneim et al., (2011),*** who found that rats fed with Pomegranate molasses and peels showed a significant decrease in relative heart weight compared with the untreated rats. Pomegranate juice is an important source of phenolic compounds, particularly anthocyanins, especially the 3-glucosides and 3,5-diglucosides of delphinidin, cyanidin, and pelargonidin. These components, along with gallagyl-type tannins, ellagic acid derivatives and other hydrolyzable tannins could contribute to the antioxidant activity of pomegranate juice. On the other hand, methanolic pomegranate peel extract (PPE) has been shown to have high antioxidant activity ***(Rosenblat et al., 2011).***In the current study, AZ administration for two weeks caused a significant elevation of serum LDH, CPK, and CK-MB, which can be interpreted as acute myocardial damage caused by azithromycin via inducing the ischemia development in the myocardial tissue, indicating these cardiac biomarkers' release from the damaged myocardium into the circulation. Myocardial damage occurs mostly in cases with an abrupt decline in coronary blood flow. Then cardiac biomarkers are released from the damaged myocardium into the circulation. LDH, CK-MB, and aspartate aminotransferase (AST) are the biomarkers to determine such damage. The rise of cardiac biomarkers in the blood is interpreted as the messenger of myocardial damage ***(Walker, 2006).*** Results are supported by ***Ozlem et al., (2015)*** found that the Plasma CK-MB and LDH levels were increased in Azithromycin -administered group significantly when compared to the normal control group. These results also agree with ***Abd El-kader,(2019),*** who found that the azithromycin-treated group showed marked elevation in creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). The findings of the present work are in agreement with ***Imran et al., (2019),*** who found that the treatment with ethanolic peel extract of *Punica granatum* (EPEPG) reduced the activity of both creatine kinase (CK-MB) and lactate dehydrogenase (LDH) enzymes. The cardioprotective response is also supported by increased cardiac antioxidant enzyme activity and reduced extent of lipid peroxidation. The treatment

with ethanolic peel extract is better than PM in improving cardiac biomarkers. This may be due to PM having less antioxidant effect due to heating, as previously mentioned ***(Abd Elmonem, 2014).***Lipids play an important role in virtually all aspects of biological processes in the body. Disturbances of their level in tissues and serum are usually associated with many abnormalities, including gallstone formation, atherosclerosis, and coronary artery disease***(Abd Elmonem, 2014).*** Lipid profile analysis revealed a significant increase in the plasma level of total cholesterol, LDL-cholesterol, and triglycerides by Azithromycin ***(Olayinka and Ore, 2014).*** It has been suggested that cholesterol is a general indicator of the level of lipids in circulation***(Thurnham et al., 1990)*** and, together with polyunsaturated fatty acids (PUFA), are LDL's main components. PUFA is the substrate required for MDA formation. The amount of peroxidized lipid formed may be related to the substrate amount and the level of lipid peroxidation (LPO). Oxidation of LDL-cholesterol is known to result in the depletion of lipoprotein antioxidants and subsequent accumulation of cholesterol esters***(Gutteridge,1995).***

The findings of the present work agree with ***Kochar et al., (2019)*** found that pomegranate molasses statistically recovered the lipid profiles, especially serum T.G, VLDL, HDL, and LDL but not total cholesterol. ***Shimaa (2022)*** Found that pomegranate peel powder (POP) administrations significantly diminished the elevation of serum TC, TG, VLDL-C, and LDL-C values and elevated serum HDL. Some previous studies have demonstrated that pomegranate fights cardiovascular disease by different mechanisms, such as reducing oxidative stress, inhibiting the oxidation of potentially harmful LDL, and quenching free radicals***(Gouda et al., 2016).***AZ administration increased the oxidative stress, as signified by increased plasma MDA and NO, and decreased catalase (CAT), and superoxide dismutase (SOD) in rats, respectively. These findings are in agreement with ***Ebenezer and Ayokanmi,(2014)*** found that there was a significant decrease in the activities of heart catalase (CAT), and superoxide dismutase (SOD) treated group with azithromycin. ***Ozlem et al., (2015)*** showed that MDA levels were significantly increased in azithromycin-administered groups compared to the control group.

On the other hand, ***Abd El-kader, (2019)*** showed that AZ treated group showed a marked elevation in Malondialdehyde (MDA). ***Chalfoun et al., (2012)*** found that superoxide dismutase(SOD) activity increased in pomegranate molasses compared with the (+ve***). Kochar et al., (2019)*** found that the concentration of serum MDA (a well-known marker of the degree of lipid peroxidation) was significantly($P<0.05$) improved in PM supplemented group. Molasses or juice were added to the drinking water of mice for 11 weeks leading to a significant increase in superoxide dismutase activity ***(Mounayar et al., 2012).*** Studies in rats and mice have confirmed that the antioxidant properties of a pomegranate by-product extract made from whole fruit without juice showed a decrease in cellular lipid peroxide content and an increase in reduced glutathione levels ***(Rosenblat et al., 2006). Nabil et al.,(2022)***found an increase in CAT and SOD.

On the other hand, there is decreasing in MDA in Peel Pomegranate compared with the (+v e) group. ***Imran et al., (2019)*** found that the treatment with ethanolic peel extract of *Punica granatum* (EPEPG)decreased the levels of malondialdehyde (MDA). It also increased superoxide dismutase (SOD) and catalase (CAT) in tissue and blood glutathione**.** Results are supported by ***Shimaa, (2022),*** who found that pomegranate peels restored the activities of antioxidant enzymes SOD and GSH, which declined after CCl_4 treatment and decreased MDA levels significantly. Pomegranate peel extract (PPE) is also rich in polyphenolic class antioxidants, including flavonoids like gallotannins, ellagitannins, ellagic, ferulic and gallic acids, anthocyanins, quercetins, and catechins. The polyphenols show

important biological activities, including oxidation inhibition, free radical elimination, and reducing the risks of cardiovascular diseases. Ellagitannins may be responsible for PPE's anti-mutagenic and good antioxidant activities. PPE exhibits strong antioxidant activities ***(Wang et al., 2015).***Histopathological examination of the AZ group revealed marked distortion, fragmentation, and loss of cardiac muscle striation. Signs of myocardial necrosis in the form of hypereosinophilia, cytoplasmic vacuolation, and peripheral pyknotic nuclei were evident. There was a marked increase in the tissue spaces with interstitial edema and inflammatory cellular infiltration. Dilated, congested, and even ruptured blood vessels were noticed ***(Abd El-kader, 2019).Kumar and Pushpa, (2016*)** found that rats treated with Pomegranate extract showed no alterations in the architecture in the heart section. The myocardium was normal, with no chronic inflammation. Histopathological examination of heart tissue showed that treatment (before and after) with Pomegranate peel extract ameliorated the effect of Azithromycin administration on cardiac tissue; cardiac myocytes looked more or less similar to those of control

Conclusion

This study concluded that elevated plasma cardiac biomarkers and histopathological abnormalities represent AZ-induced cardiotoxicity. This cardiac adverse side effect may be related to oxidative stress.Pomegranate molasses and its peel extract can lower the impact of azithromycin on the heart because of its high antioxidant activity

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دبس الرمان وقشوره كمضاد للسموم القلبية التي يسببها أزيثروميسين في ذكور الجرزان

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الملخص العربي

أجريت الدراسة الحالية لمعرفة دور دبس الرمان وقشوره كمضاد للسموم القلبية التي يسببهاأزيثروميسين في ذكور الجرزان. تم استخدام ستة وثلاثون من ذكور الفئران تتراوح أوزانهم بين (150 ± 10 جم) قسمت إلي مجموعتين رئيسيتين. المجموعة الأولي (6فئران) تغذت علي الغذاء القياسي كمجموعة ضابطه سالبه المجموعة الثانية (30 فأر) أخذت الأزيثروميسين معويا بمقدار 30 مجم / كجم / يوم من اليوم الرابع عشر إلي اليوم الثامن والعشرون وتم تقسيم هذه المجموعة إلي خمس مجموعات فرعية . مجموعه (1) تتغذى على الغذاء القياسي كمجموعة ضابطة موجبة . مجموعة (3,2) تغذت علي الغذاء القياسي بالإضافة إلي جرعات من دبس الرمان(2 و 4 مل / كجم من وزن الجسم) عن طريق الفم لمدة 28 يوما .مجموعة (5,4) تغذت علي الغذاء القياسي بالإضافة إلي جرعات من قشور الرمان (200 و 400 ملجم / كجم من وزن الجسم) عن طريق الفم لمدة 28 يوما . في نهاية التجربة تم حساب المأخوذ الغذائي, الزيادة المكتسبة في الوزن, معدل كفاءة الغذاء و الوزن النسبي للقلب .كما تم تقدير بعض المتغيرات البيوكيميائية في السيرم و مضادات الأكسدة والعوامل المؤكسدة في نسيج القلب كما أجري الفحص الهيستوباثولوجي لأنسجة القلب . أظهرت النتائج أن دبس وقشور الرمان أدت إلى تحسين في التقييم البيولوجي وظائف القلب ووظائف الكبد و الإنزيمات المضادة للأكسدة و الفحص الهستوباثولوجى لأنسجة القلب مقارنة بالمجموعة الضابطة الموجبة لذلك يمكن أن نستنتج أن تناول دبس وقشور الرمان يمكن أن يقلل من تأثير اللأزيثروميسين على القلب.

الكلمات المفتاحية: دبس الرمان ، قشور الرمان, أزيثروميسين، وظائف القلب,الأنزيمات المضادة للأكسدة.