

POSSIBLE CARDIOPROTECTIVE ACTION OF POMEGRANATE JUICE *PUNICA GRANATUM* AND  
PROPOLIS AGAINST MYOCARDIAL INFARCTION INDUCED IN RATS.

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**Abstract**

**Background:** The present study was conducted to evaluate the protective role of pomegranate juice alone or in combination with propolis extract against isoproterenol (ISO) induced myocardial infarction in rats.

**Material and Methods:** Male Wister albino rats (n=60) weighing 220-280g were divided into six groups each group contain ten rats; group I: negative control fed standard diet. Group (II-VI): Rats were injected subcutaneously with isoproterenol (150 mg/kg) for 3 days. Group III: Rats were given pomegranate juice 1ml /rat / day. Group IV: Rats were given propolis extract (50 mg/kg BW). Group V: Rats were given pomegranate juice + propolis extract. Group VI: Rats were given glyceronitrate (2.6mg/kg.bw/day).

**Results:** The ISO-induced myocardial infarction in untreated rats has indicated by significant (p < 0.001) elevation of cardiac marker enzymes such as aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), creatine kinase (CK), and cardiac troponin I (CnI ) when compared with control group. Rats treated with pomegranate juice and propolis extracts showed highly significant (p<0.001) improvement in cardiac marker enzymes activities when compared with untreated myocardial infarction group. Histological investigation showed features of damaged muscles (atrophy, necrosis) which while it was ameliorated in treated groups with superior effect with pomegranate juice.

**Conclusion:** It was concluded that, pomegranate juice in combination with propolis have a potential to alleviate the oxidative stress that induce myocardial infarction and therefore reduce the incidence of cardiovascular diseases.

**Key words:** Pomegranate juice, Propolis, Myocardial infarction, Rats.

**Introduction**

Acute myocardial infarction (AMI) is the second main cause of mortality after cancer (Yusuf et al.2001). Previous experimental and clinical studies concerning the enhanced free radical generation and/or interrupted endogenous antioxidant enzymes production in ischemic heart diseases have been described (De Biase et al., 2003). Antioxidant defenses include a variety of biological systems, including vitamins as ascorbic acid (vitamin C),  $\alpha$ -tocopherol (vitamin E), reduced glutathione (GSH), coenzyme Q10, cysteine, carotenoids, flavonoids, polyphenols, and other various exogenous antioxidants (Venardos et al., 2007; Rodrigo et al., 2013). Polyphenols act as antioxidants both through the prevention of damage from ROS and their iron chelating ability (Perron and Brumaghim, 2009). Pomegranate, *Punicagranatum*, contain high bioactive compounds and is known to be high in antioxidant activity (Gil et al., 2000; Esmailzadeh et al., 2004; Rosenblat and Aviram, 2006; Tzulker et al., 2007; Rock et al., 2008). Propolis contains more than 300 active constituents, with flavonoids, phenolic acids, phenolic acid esters, steroids and amino acids being among the main components (Gómez Caravaca et al., 2006).

Propolis contains more than 300 active constituents, with flavonoids, phenolic acids, phenolic acid esters, steroids and amino acids being among the main components (Viuda-Martos et al., 2008). Propolis has several biological and pharmacological properties, as antimicrobial (Nassar et al., 2013), antioxidant (Marquele et al., 2005;

Pari and Gnanasoundari 2006; Salah et al.,2006; Kanbur et al., 2009). Propolis considered as anti-tumor, anti-bacterial, anti-fungal, anti-viral, anti-inflammatory anti-oxidant, anti-cancer and immune-stimulant (Santos et al., 2003; Banskota et al., 2001; Valente et al.,2011; Sforcin 2007). The aim of current to evaluate the protective effect of propolis extract, PJ or a combined with propolis extract against incidence of MI in rats.

## **Materials and Methods**

Isoproterenol hydrochloride was purchased from Sigma chemical company, St. Louis, MO, USA. Nitroglycerine was purchased from Ranbaxy limited company, India. Cardiac troponin I kits, Lactate dehydrogenase kits, creatine Kinase kits and Aspartate aminotransferase kits were obtained from Dimension Vista™ Flex® reagent cartridge.

### **Preparation of pomegranate juice and propolis extraction**

Pomegranate fruits were cut in to pieces, Seeds were grinded in mixture grinder and 10% (w/v) extract (juice) was prepared. Juice was filtered to get clear juice. PJ were prepared fresh every day until the end of experiment. Propolis dissolved with (50ml of 70% ethanol) at room temperature. Then, the supernatant was evaporated in the rotary evaporator device under vacuum at 50°C until dryness. Dried ethanoic extract of propolis (28 g) was suspended in phosphate buffered saline (PBS) (pH 7.2) to obtain 1% stock solution (Hegazi et al., 2004). The dose of propolis used in this experiment was 50 mg/kg B.W according to Turkez et al, (2010).

### **Experimental animals**

The handling of animals was done according to ethical committee of the university. Male Wister albino rats (n=60) weighing 220-280 g were obtained from the animal experimental unit of King Fahd Center for Medical research, King Abdulaziz University. After the adaption period, animals divided into sex main groups each 10 rats, as follows: Group I: Rats considered as Negative control. Group (II-VI): Rats were injected with isoproterenol (150 mg/kg, S.C) for 3days. Group III: Rats were protected with pomegranate juice 1ml /rat / day. Group IV: Rats will be protected with propolis extract (50 mg/kg BW). Group V: Rats were protected with pomegranate juice + propolis extract. Group VI: Rats were protected with glyceronitrate (2.6mg/kg.bw/day). At the end of the experimental period (30 days) blood sample was collected then centrifuged at 3000 rpm for 15 min to separate serum and stored at -80° C until biochemical analysis.

### **Histopathological examination**

Parts of heart tissue were placed in 10% formalin. The wax impregnated tissues were embedded in paraffin blocks using the same grade wax, the paraffin blocks were cut with rotary microtome at 3-5µ thickness. Sections were stained with Hematoxylin and Eosin (H&E) and examined microscopically.

### **Immunohistochemistry for Desmin and CD4**

Immunohistochemistry was performed using Vantaa Lifesciences Benchmark XT© Staining module. The paraffin-embedded slides were de-paraffin zed with 3 changes of xylene, then rehydrated in a series of graded ethanol. Slides were placed in a preheated retrieval buffer, 0.1 mmol EDTA, pH 8.0, for 30 min, then cooled in the buffer for 5 min, followed by a 5-min rinse under running distilled water. After heat-induced epitope retrieval slides were placed on an auto-stainer. Sections were incubated with 3% hydrogen peroxide in ethanol for 5 min to inactivate the endogenous peroxides and incubated in C4d (dilution 1:50) for 30 min, followed by rinsing with Tris-buffered saline solution with Tween 20 (TBST) wash buffer. Secondary incubation was with DUAL-labeled polymer horseradish peroxidase for 15 min. Sections were then incubated in 3,3-diaminobenzidine for 5 min, counterstained with modified Schmidt hematoxylin for 5 min and rinsed for 3 min in tap water to blue sections, dehydrated with graded alcohols and cleared in 3 changes of xylene before mounting. In desmin Aby used uterus tissue from female albino rat as positive control, while in C4d Ab. used tonsillar tissue also from albino rat as positive control.

### **Statistical analysis**

Results were expressed as a (mean ±SD). Evaluation of results and statistical analysis was carried out using descriptive, correlation and regression analysis. In all the above-mentioned tests, P < 0.05 was taken to be statistically significant by using one way ANOVA,

## **Results**

The plasma levels of AST in myocardial infarction rats (G2), rats treated with mixture pomegranate juice and propolis extract (G5) and group treated with glycerol nitrate (G6) were significantly lowered than negative control

group (G1) (P <0.0001, P <0.029, P <0.0001, respectively). The plasma levels of AST in rats treated with pomegranate juice (G3) and treated with propolis extract (G4) were significantly lower than MI diseased group (G2), (P<0.0001 and P <0.0001). The group treated with glycerol nitrate (G6) was significantly higher (P <0.001) than ISO-treated group.

The plasma levels of LDH in myocardial infarction rats (G2) treated with mixture pomegranate juice and propolis extract (G5) and treated with glycerol nitrate (G6) were significantly higher than control group (P <0.010, P <0.002 and P <0.0001) respectively. Plasma levels of CK were significantly higher in MI diseased group (G2), rats which treated with propolis extract (G4), group which treated with mixture pomegranate juice and propolis extract (G5) and group treated with glycerol nitrate (G6) than negative control group (G1) (P =0.0001, P =0.046, P =0.011, and P =0.0001 respectively). Plasma levels of CK were significantly lower in groups which treated with pomegranate juice, rats treated with propolis extract, also in group which treated with mixture pomegranate juice and propolis extract than untreated MI rats (P <0.0001) but was significantly higher in group treated with glycerol nitrate (G6) (P<0.0001) than ISO(G2). Plasma levels of TnI were significantly higher in MI diseased group and rats which treated with glycerol-nitrate (P<0.004 and P<0.008) when compared to negative control, while the Plasma levels of C1N1 were significant reduction in the groups which treated with pomegranate juice (G3), rats treated with propolis extract (G4) and in group which treated with mixture pomegranate juice and propolis extract (G5) (P <0.001, P <0.021 and P <0.010, respectively) when compared to ISO diseased group (G2) (Table 1).

**Table 1:** Comparison of plasma levels of measured parameters in different studied groups versus control and isoproterenol groups using the ANOVA one way test.

Parameters	(G1) Control	(G2)ISO. diseased group	(G3) PJ treated group	(G4) Propolis extract treated group	(G5)Mix. Treated group	(G6)GTN treated group
AST	111.20±12.96	156.80±18.01	114.70±16.26	102.90±14.31	138.20±43.22	184.88±39.98
P1 value	-	<b>0.0001</b>	NS	NS	<b>0.029</b>	<b>0.0001</b>
P2 value	-	-	<b>0.001</b>	<b>0.0001</b>	NS	<b>0.027</b>
LDH	154.80±45.67	271.90±40.13	169.80±42.44	180.40±60.87	298.90±119.96	664.11±190.69
P1 value	-	<b>0.010</b>	NS	NS	<b>0.002</b>	<b>0.0001</b>
P2 value	-	-	0.023	0.041	NS	<b>0.0001</b>
CK	169.90±57.97	424.50±66.13	242.20±56.77	247.80±50.11	270.50±51.94	770.33±173.06
P1 value	-	<b>0.0001</b>	NS	0.046	<b>0.011</b>	<b>0.0001</b>
P2 value	-	-	0.0001	0.0001	<b>0.0001</b>	<b>0.0001</b>
CTNI	0.025±0.005	0.035±0.011	0.023±0.005	0.027±0.008	0.026±0.007	0.034±0.007
P1 value	-	<b>0.004</b>	NS	NS	NS	<b>0.008</b>
P2 value	-	-	0.001	0.021	<b>0.010</b>	NS

**P1:** Significance versus control; **P2:** Significance versus Isoproterenol. **NS:** Non significant (**P-value** ≥ **0.05**). **P-value** ≤ **0.05** mean there are **significant**, **P-value** ≤ **0.001** mean there are **highly significant**, **P-value** ≤ **0.0001** mean there are **very highly significant**.

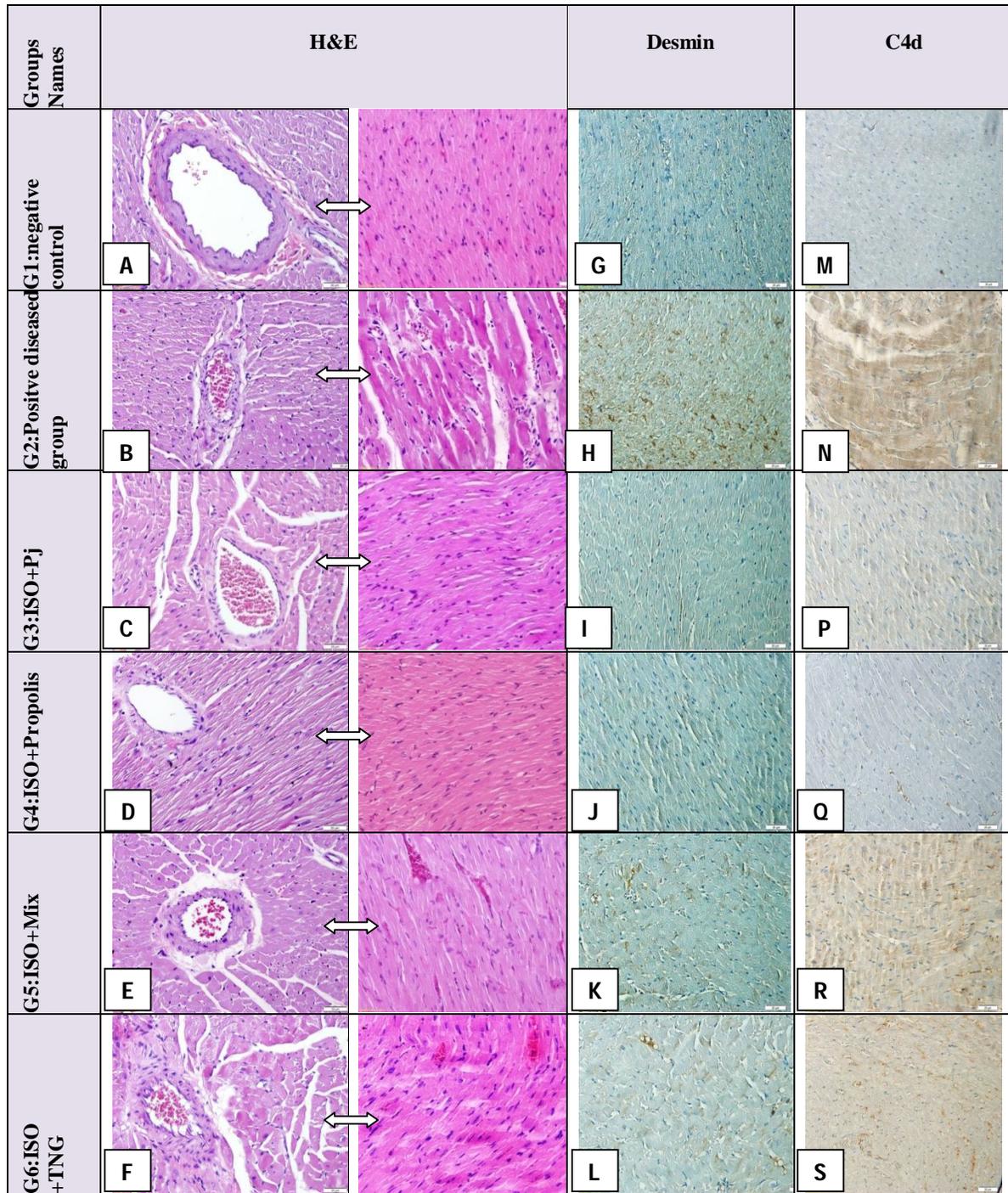
### Histopathological examination

The histological examination of the heart tissue of normal healthy rats showed normal architecture manifested by normal coronary artery wall thickness, normal size and appearance of cardiac muscles with oval central nuclei as illustrated in (Fig2A). In rats treated with ISO, it was revealed irregular thickening of coronary wall and narrow of lumen due to buildup of cholesterol, disorganization of cardiac muscle structure with acidophilic dark regions indicating apoptosis as demonstrated in (Fig.2B). The heart sections of the rats treated with PJ and also in rats which treated with propolis extract showed highly improvement of pathological structure and showed normal thickness of coronary artery with wide lumen without any narrowing, preservation of normal structure of cardiac muscles with absence of degenerated dark bands (Fig2C&D). Oral administration of mixture of PJ and propolis extract revealed a marked improvement in the structure of the heart tissue and perfective thickness of coronary artery with moderate lumen without cholesterol deposition (Fig2E). Meanwhile, in rats treated with glyceronitrate showed preservation of degenerative changes induced by ISO with increased in infarction size, also observed increased in apoptotic dark cells numbers in the cardiac muscle. Also, observed irregular thickening of coronary wall of artery with narrow of lumen (Fig2F).

### Immunohistochemistry by Desmin and CD4

The desmin (marker for hypertrophy) and C4d (marker of cell apoptosis) antibodies used in this project were specific for muscle tissue. In the control group were negative for desmin and C4d staining in myocardium cells (Fig2G&M). The infarcted areas in ISO treated rats showed intense immunoreactivity for desmin and C4d staining (Fig2H&N). Myocardium cells in rats which treated with PJ and in group treated with propolis extract exhibited reduced immunoreactivity for desmin and C4d staining (Fig2I&P/ J&Q) this mean, myocardium cells of these two groups became near to cardiac myocytes of control group, while in rats treated with mixture of PJ and propolis extract

were mild immunoreactivity for desmin and C4d staining (Fig2K&R). Meanwhile in rats which treated with GTN showed relatively high desmin and C4d staining in the tissue of myocardium (Fig 2L&S).



**Figure2:# Sections for cardiac muscle and wall of left ventricle in: Group 1-1:** showing Cardiac muscles with normal size, appearance no degeneration with normal wall thickness of coronary artery with wide lumen without any narrowing. **In group 2-2:** showing disorganization of cardiac muscle structure with apoptosis and irregular thickening of coronary wall with narrow of lumen due to buildup of cholesterol, **while In groups (3-3 & 4-4):** showing preservation of normal structure of cardiac muscles with absence of degenerated dark bands and normal thickness of wall with wide lumen, **In group 5-5:** showing also a marked improvement in structure of the heart tissue with perfective thickness of the wall with moderate lumen without cholesterol deposition. **In group 6-6:** showing preservation of degenerative changes induced by ISO. With increased in infarction size with irregular thickening of coronary wall of artery with narrow of lumen. **(H & E x 40)# Sections for rats cardiac muscle from the left ventricle immune-stained for desmin** (marker for hypertrophy) **and C4d** (marker of cell apoptosis). **G1:** showing negative expression, **In group 2** showing increased expression in ischemic cardiac fibers, **In group 3 and group 4** showing

decreased expression in these two groups. **Ingroup 5** showing Mild expression, while in **group 6** high expression. (**Desmin& C4d x 400**).

## Discussion

The current protocol aimed to evaluate the cardio protective effect PJ and propolis against ISO induced MI in rats. The hypothesis was focused on possible prophylactic role of pretreatment with PJ propolis and its mixture could prevent MI induced by ISO in the rats. ISO is well known cardio toxic agent is widely used as an agent to evaluate the effect of drugs in the myocardial consequences of ischemic disorders (Sheela and Shyamaladevi 2000).

Isoproterenol is well known cardio toxic agent due to its ability to destruct myocardial cells. ISO is widely used as an agent to evaluate the effect of drugs in the myocardial consequences of ischemic disorders (Sabeena et al., 2004). ISO-induced MI has been reported to show many metabolic and morphologic aberrations in experimental animals similar to those of MI in humans (Karthikeyan et al., 2007). The herbal drugs have been prescribed widely because of their effectiveness, fewer side effects and relatively low cost (Venkates et al., 2003). Several studies revealed the benefits of medical plants like propolis and pomegranate which showed a wide protection against cardiovascular disease which may be attributable to its antioxidant properties (Lee and Shibamoto 2003; Chopra et al., 1995). In the current study, observed significant increase in the activities of enzymes such as AST, LDH, CTNI and CK on isoproterenol induced myocardial infarction rats due to the damage in the heart muscle, rendering the leakage of enzymes into the serum when compared with control group. The obtained results were in agreement with (Haresh and Jitendra 2012) whose reported that, in ISO-injected rats significant elevation in marker enzymes in serum compared with control rats (Farvin et al., 2004).

A similar result was reported by (Murugesan et al., 2012 and Vaibhav et al., 2010) whose reported that, ISO injected rats showed significant elevation in of those markers was observed. In the present study, rats treated with PJ showed highly improvement and ameliorated reduction of serum concentrations of AST, LDH, CTNI and CK level with respect to ISO positive group. This could be attributed primarily to the action of ellagic acid, polyphenol extracted from many natural products (i.e. pomegranate) and has been identified to have potent antioxidant activity (Lanny 2000 ; Pountsi et al., 2005). Researches indicate that Ellagic acid can prevent, treat, or cure specific disease as cancer, heart disease, liver disease, high blood pressure and bone disease (Stoner and Gupta 2001). The present results are aligned with the result of (Mai et al., 2014; Bakheet et al., 2014) whose reported that pretreatment with gallic acid significantly decreased the activity of AST, ALT, LDH and CK and the levels of CTNI in serum of ISO treated rats. Similar results was reported by (Mahalaxmi et al., 2010) who found that Pre-treatment with PJ extract significantly decreased LDH and CK levels. The present results showed also that, propolis treated group showed significant improvement and ameliorated reduction of serum concentrations of AST, LDH, CTNI and CK level with respect to ISO positive group. Caffeic acid phenethyl ester (CAPE) is one of medicinal component propolis (a honey-bee hive extract). Similar result was reported by (İlhan et al., 2014) who found that CAPE prevented the increased AST, CTNI and LDH levels induced by ISO treatment rat.

On other hand, Co-administration of pomegranate juice and propolis extract showed no significant difference in AST and LDH serum levels while there were highly significant reduction in the levels of CTNI and CK levels when compared with positive group. This may be due to pro-oxidant toxicity effects of these two antioxidants which defined as any endobiotic or xenobiotic that induces oxidative stress either by generation of ROS or by inhibiting antioxidant systems. Some of the popular and well known antioxidant flavonoids have been reported to act as pro-oxidant also when a transition metal is available (Halli-well, 2008). The result in these group exhibited less effected than PJ or propolis extract both separately. In the present experiment, in the rats treated with nitroglycerin observed that there were highly significant increase in serum levels of AST, LDH and CK but there were no significant difference ( $P > 0.05$ ) in the CTNI when compared with positive group. According to this result we showed negative effect of GTN due to sustained administration of it. Sustained GTN administration causes tolerance and is associated with pro-oxidant effects, endothelial dysfunction and increased sensitivity to vasoconstrictors (Munzel et al., 2005; Klemenska and Beresewicz 2009; Kosugi et al., 2011).

Atherosclerosis is an inflammatory disease (Lusis, 2000), is the buildup of cholesterol and LDL oxidation (Navab et al., 2004) and fatty deposits (called plaques) on the inner walls of the arteries. These plaques can restrict blood flow to the heart muscle by physically clogging the artery or by causing abnormal artery tone and function.

Isoproterenol (ISO), a  $\beta$ -adrenergic agonist, causes severe stress in the myocardium, resulting in gross and microscopic infarct-like necrosis of the heart muscle. Some of the mechanisms proposed to explain ISO-induced damage to cardiac myocytes (Patel et al., 2010)

In the current study, heart tissues in ISO treated rats showed disorganization of cardiac muscle structure with acidophilic dark regions indicating apoptosis and irregular thickening of coronary wall with narrow of lumen due to buildup of cholesterol when compared with control group. Thus the lipids play an important role in the pathogenesis of MI. An increase in the concentration of LDL cholesterol and a decrease in HDL cholesterol are associated with raised risk of MI (Mediene-Benchekor et al., 2001). This result was agreement with (Sushama et al., 1989; Deepa and Varalakshmi 2005) who's reported that ISO produces free radicals which is a causative factor for irreversible damage to the myocardial membrane and may cause cellular cholesterol accumulation. In the present study, heart tissue in MI rats treated with PJ showed apparent normal histological structure cardiac muscles with absence of degenerated dark bands and normal thickness of coronary artery with wide lumen without any narrowing when compared with positive

group PJ is rich in polyphenols and showed high ability as free radicals inhibition and lowered LDL oxidation (Gil et al., 2000; Aviram et al., 2002; Aviram 2002) and may have anti atherosclerotic properties in mice and humans (Aviram et al., 2000). Many flavonoids have extensive biological properties that reduce the risk of heart disease. Ellagic acid has antioxidant property, which indirectly helps to decrease the levels of lipids by preventing the membrane damage, they protect LDL cholesterol from oxidation, inhibit the formation of blood clots and have hypolipidemic effects and anti-inflammatory action (Manach et al., 1996). Thus, as a rich source of polyphenols, propolis represents a potential alternative strategy for the prevention of cardiovascular disorders. The results revealed that the heart tissues in MI rats treated with propolis extract showed preservation of normal structure of cardiac muscles and normal coronary artery without any narrowing by cholesterol. Propolis is a bee product which presents in its composition mainly flavonoids and other compounds and has been used in folk medicine in many countries since ancient times, because it has antioxidant, antimicrobial and anti-inflammatory properties.

Myocardial amelioration was seen in rats that received propolis, suggesting a protective effect of propolis in myocardial damage. This is probably due to increase in antioxidant enzymes, non-enzymatic antioxidants, and decrease of ROS as suggested by (Al-Amoudi 2015).

Accordance to the study of (Daleprane and Abdalla 2013), the authors hypothesized that propolis may aid in the prevention rather than treatment of atherosclerosis. In addition, Co-administration of PJ and propolis extract protect ISO induced MI in rats model, which they revealed a marked significant improvement in histological architecture of the heart tissue and perfective thickness of coronary artery with moderate lumen without any cholesterol deposition. The combination of various types of polyphenols makes the pomegranate antioxidants unique and different from other antioxidants by having a much wider spectrum of action against several and not just one type of free radicals (Aviram et al., 2005). The antioxidant activity of propolis represented by increase of antioxidant enzymes activities and/ or decrease ROS as documented by many researchers (Newairy et al., 2009; Koyu et al., 2009; Yousef and Salama 2009).

Immuno-histochemically (IMHC) cardiac markers are important for AMI (Ortmann et al., 2000). (IMHC) markers have been used to detect MI, including desmin and C4d. The main aim of this study was to assess the concentration of desmin and C4d in cardiomyocytes of patients with chronic heart failure. One of the important observation in this study, the examined sections of heart of group treated with ISO showed dark brown desmin stain which they was intense immunoreactivity for desmin staining in the area of infarcted when compared with control group. A similar result was reported by (Agnieszka et al., 2009) who indicated that increased accumulation of desmin in cardiomyocytes was associated with more abnormal clinical parameters as compared with a group of patients with normal expression of this protein. This may be a result of damage of the desmin network in cardiomyocytes. This result is at variance with that reported by (Somma et al., 2004) who reported that a significant decrease in the amount of desmin in diseased myocardial compared to normal tissue.

Moreover, the present results differ from those of (Ouyang et al., 2010) who showed that the ischemic/infarcted areas showed decreased immunoreactivity for desmin antibody when compared to negative control group. On other hand, heart section of rats treated with PJ showed reduction or no stain of desmin antibody (Ab), this indicate that there were no antigen (Ag) present in cells of heart tissue section by exploiting the principle of (Ab) binding specifically to (Ag) in biological tissue. As well as in group which treated with propolis extract showed a negative immunoreactivity for desmin staining, this mean; myocardium cells of these two groups became near to cardiac myocytes of normal group when compared with positive group. The analysis of results showed that co-administration of PJ and propolis extract showed mild immune-reactivity for desmin staining. We notice very highly significant reduction in (Ag) present in heart section of rats. Also, it was reported that rats treated with GTN showed relatively high desmin staining in the tissue, this mean, sections for rat cardiac muscle from the left ventricle treated with sustained GNT have large numbers of (Ag) present in the cells.

In addition, immunohistochemically staining techniques by C4d may also be helpful in establishing a diagnosis of early acute MI by highlighting myocytes with ischemic injury or necrosis.

In the current study, the examined sections of heart of group treated with ISO showed dark brown C4d stain which they were intense immunoreactivity for C4d staining in the area of infarcted, sensitivity for delineating early infarctions but no influx of inflammatory cells. A similar result was reported by (Rachel et al., 2012) who showed that strong positivity in necrotic myocytes in areas of ischemic injury/infarction, with stronger staining at the periphery of larger areas of infarction. Moreover, (Crystal et al., 2010) demonstrated that there was a strong immunoreactivity to C4d, confirming that these areas of injury did represent true myocardial infarctions. In this study, we demonstrated that C4d is a powerful indicator of the impact of MI. In the current study, Myocardium cells in rats which treated with pomegranate juice showed very highly reduced immunoreactivity for C4d staining due to decline in the area of infarction.

On another hand, rats treated with propolis extract exhibited no C4d staining. This indicate disappearance of antigen of necrosis in heart section of myocardium cells, this resulting in nonreactive of this antibody (C4d). The presence of C4d has 100% specificity for necrotic myocytes as histologically normal myocardium was nonreactive. Meanwhile, Combination of PJ and propolis extract showed moderate immunoreactivity for C4d staining. In addition, at autopsy, strong C4d staining was still present in rats which treated with GNT when compared with positive group (Nijmeijer et al., 2003; Jenkins et al., 2008). It was recommended to use those natural products to alleviate the oxidative stress that induce myocardial infarction.

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## Conflict of Interest

Authors declare that there is no conflict of interests.

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