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Cardiopreventive effect of ethanolic extract of Date Palm Pollen against isoproterenol induced myocardial infarction in rats through the inhibition of the angiotensin-converting enzyme.

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Abstract

The present study aimed to examine the putative preventive effect of the ethanolic extract of Date Palm Pollen (DPP, Phoenix dactylifera L., family Arecaceae) on isoproterenol-induced myocardial infarction (MI) in rats. Twenty four rats were randomly divided into four groups including control. They were treated with DPP extract (400 mg/kg) and clopidogrel (0.2 mg/kg) for 7 days followed by myocardial injury induction using subcutaneous isoproterenol (100 mg/kg) with an interval of 24h for two days (6th and 7th day). Administration of
isoproterenol exhibited indicative changes in the ECG pattern evidenced by significant
elevation of ST-segment and cardiac injury markers \textit{viz.}; troponin-T, creatine phosphokinase
(CPK), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) by 315\%, 71\%,
64\% and 170\%, respectively as compared to control. Additionally, the angiotensin-converting
enzyme (ACE) activity in plasma was increased by 33\% associated to histological myocardial
necrosis. However, pre-co-treatment with DPP extract improved the cardiac biomarkers
injury, normalized cardiac function indices and prevented the ventricular remodeling process
through inhibition of ACE activity by 34\% and the inhibition of the generation of radical
oxygen species. Extensive characterization of this DPP extract using LC-HRMS revealed
numerous flavonoids and phenols compounds which could be endowed with cardiopreventive
actions. Overall, these results proved that DPP extract has preventive effects on cardiac
remodeling process.

\textbf{Keywords}: «DPP ethanolic extract; cardiopreventive; myocardial infarction; ACE; ECG;
Electrospray Ionization Mass Spectrometry».

\textbf{Abbreviations}: DPP, Date Palm Pollen; MI, myocardial infarction; HRESIMS, high
resolution electrospray ionization mass spectrometry; ECG, electrocardiographic; LV, left
ventricular; CPK, creatine phosphokinase; ALT, alanine aminotransferase; LDH, lactate
dehydrogenase; ACE, angiotensin-converting enzyme; TC, total cholesterol; TG, triglycerides;
ECL, electrochemiluminescence; ROS, reactive oxygen species.

\section{1. Introduction}

Cardiovascular diseases remain the most important cause of mortality in both developed
and developing countries, accounting approximately 20\% of all annual deaths worldwide
(Ittagi et al., 2014). The cardiovascular system is susceptible to many chronic diseases such as
hypertension and myocardial infarction. The myocardial infarction (MI) reflects the death of
cardiac myocytes due to prolonged ischemia. It is considered an acute coronary syndrome that
may happen during the natural path of coronary atherosclerosis. This pathology could be
mediated to several factors affecting the arterial wall (Boersma et al., 2003). Hence, it is a
result of imbalance between coronary blood supply and cardiac demand (Mnafgui et al.,
2016). It increases myocardial necrosis which causes cardiac dysfunction including blood
pressure, heart rate and electrocardiographic (ECG) changes and left ventricular (LV)
dysfunction associated with an alteration in activities of cardiac-specific enzymes. Cardiac
troponins are frequently accompanied with inflammation-related proteins and myocardial
infarction in case of severe heart damage (Mnafgui et al., 2016).

Isoproterenol [1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol HCl] is a synthetic
catecholamine with β-adrenergic agonist effect which shown to cause severe stress in the
myocardium resulting in infarction-like necrosis of the heart muscles (Upaganlawar et al.,
2011). However, the administration of isoproterenol in supra-maximal doses could stimulate
subendocardial ischemia, necrosis, hypoxia followed by fibroblastic hyperplasia with
decreased myocardial compliance and inhibition of diastolic and systolic function
(Mehdizadeh et al., 2013). In veterinary and human medicine, numerous synthetic drugs were
designed for the management of heart attack but exhibit many side effects. Hence, several
attempts have been taken for the identification of new therapeutic approaches to prevent
myocardial infarction. A great attention has been given to the polyphenols as effective
bioactive compounds that protect cells from myocardial damage. Naturally-occurring
polyphenolic compounds with antioxidant properties are widely in vegetables, fruits, tea, etc
(Hertog et al., 1993).

Historically, date palm trees (Phoenix dactylifera L.) belonging to family Arecaceae were
extensively used in folk medicine as potential source for treatment of many human diseases.
Date Palm Pollen (DPP) has been reported as rich source of diverse secondary metabolites
that elucidate its potential uses in several disorders. Antioxidants play a significant action as
preventive agents via neutralization or inhibition of reactive oxygen species (ROS) that
suppress the development and progression of many diseases. Recent investigations reported
that date palm possesses a potent ability to neutralize free radical (Rahmani et al., 2014; Al-
Farsi et al., 2005). Accordingly, the DPP proved effective in many biological proprieties such
as aphrodisiac, anti-inflammatory, anti-coccidial, anti-apoptotic (Elberry et al., 2011;
Metwaly et al., 2014), anti-toxicant (Eraslan et al., 2008), and hepato-protective (Uzbekova et
al., 2003) activities.

Despite this large flow of data on the promising properties and attributes of DPP, no
studies have been performed to explore the preventive effect of DPP against experimentally-
induced myocardial infarction in rats. This encouraged us in the current study to explore this
effect with scientific evidence.

2. Materials and methods

2.1. Chemicals
Isoproterenol hydrochloride powder obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, USA). ACE kit was purchased from Trinity (Trinity, UK). All other chemicals used in this study were analytical grade.

2.2. DPP collection

DPP was collected from *Phoenix dactylifera* L., family Arecaceae in Tozeur (South-west, Tunisia) in April 2015. After collection, the pollen was air-dried and ground to fine powder using a grinder. The powdered material was stored at 4 °C until further use. Its botanical identification and authentication were confirmed at the Department of Botany of the University of Sfax (Tunisia).

2.3. Extraction of plant material

Sample of powdered plant material (200 g) was extracted twice with 800 mL of ethanol for 24 h. The macerate was then filtered through filter paper (Whatman, Sfax, Tunisia) in a Buchner funnel. The filtered solution was evaporated in a rotary evaporator under vacuum at 45 °C till complete dryness. The dry extract and stock solution were kept at 4 °C until further analysis.

2.4. Animals

A total of 24 adult male Wistar rats, weighing 170-190 ± 10 g, were obtained from the local Central Pharmacy (Tunisia) and used in the present study. The animals were housed in clean cages in an air conditioned room and kept under standard conditions of temperature (25 ± 2 °C), humidity (60 ± 5%) and light (12 h dark/12 h light cycle). They were kept on standard diets and free access to tap water. The experimental protocols were conducted in accordance with the guide for the care and use of laboratory animals issued by the University of Sfax (Tunisia), and approved by the Committee of Animal Ethics.

2.5. Induction of experimental myocardial infarction

Isoproterenol was dissolved in normal saline solution and injected to rats (100 mg/kg) at an interval of 24 h for 2 consecutive days to induce experimental myocardial infarction (Mnafgui et al., 2016). Animals were sacrificed 48 h after the first dose of isoproterenol.

2.6. Experimental protocols

After acclimatization, the animals were randomly divided into four groups of six rats each as following:
Group 1: (Control) rats, received standard laboratory diet and allowed to drink saline water *ad libitum*, served as a control;

Group 2: isoproterenol (Isop) rats, received saline water and standard diet for 7 days. At the 6th day these rats were subcutaneously injected with Isoproterenol (100 mg/kg), once at an interval of 24 h for two consecutive days to induce myocardial infarction;

Group 3: (Isop+Clop) rats received clopidogrel (trade name Plavix, 0.2 mg/kg by gastric gavages) for 7 days and were injected subcutaneously with isoproterenol (100 mg/kg) on days 6 and 7.

Group 4: (Isop+DPP) rats received the ethanolic extract of DPP (400 mg/kg) for 7 days and were injected subcutaneously with isoproterenol (100 mg/kg) on the 6th and 7th days. All rats were fasted overnight but had free access to water at the last administration of the drug. After the 7 days induction, the animals were weighted and sacrificed by decapitation in order to minimize the handling stress. The trunk blood and heart were collected. Plasma was obtained by cold centrifugation of the blood (1500 × g, 15 min), frozen and stored at -20 °C till further use for the biochemical assays. Immediately after sacrifice, the heart was excised out, rinsed with saline and fixed in a Bouin solution for 24 h and embedded in paraffin. The sections of 5 µm thickness were stained with Hematoxylin–Eosin (H&E). The slides were examined under light microscope and photomicrographs were taken by an Olympus U-TU1X-2 camera connected to an Olympus CX41 microscope (Tokyo, Japan) (Mnafgui et al., 2016).

2.7. **Electrocardiography**

The ECG patterns were recorded using veterinary electrocardiograph (ECG VET 110, Biocare, China). ECG recording were made in anesthetized with ketamine (100 mg/Kg) intraperitoneally, at the end of the experimental period (24 h after the second dose of isoproterenol). Needle electrodes were inserted under the skin of the animals under light ether anesthesia in lead II position. The ECG record period was between 15-30 seconds.

2.8. **Biochemical analysis**

Following blood collection, animals were sacrificed and mid abdominal incision was processed in order to dissect out the heart. It was weighed and further subjected for histopathological analysis. The collected plasma was used for the determination of ACE using the available commercial kit from Trinity (Trinity, UK). Cardiac dysfunction markers CPK, LDH and ALT were measured in frozen aliquots of plasma by standardized enzymatic procedures using commercial kits from Abbott (Abbott, USA). The levels of plasma cardiac troponin-T were measured using Roche's electrochemiluminescence (ECL) technology.
The lipid profile including total cholesterol (CT) and triglycerides (TG) were measured in frozen aliquots of serum by standardized enzymatic procedures using commercial kits from Abbott (Abbott, France) on an automatic biochemistry analyzer (Architect ci 4100, USA) at the clinical pathological laboratory of Sfax Hospital.

2.9. **LC/HRMS analysis**

High resolution mass spectral data were obtained on a Thermo Instruments ESI-MS system (LTQ XL/LTQ Orbitrap Discovery, UK) connected to a Thermo Instruments HPLC system (Accela PDA detector, Accela PDA autosampler and Accela Pump). A reversed-phase column (Pursuit XRs ULTRA 2.8, C18, 100 x 2 mm, Agilent Technologies, UK) was used to carry out the analyses. The volume of the injected sample was set at 20 µl and 30 °C was chosen for column temperature. Mobile phases A and B, consisted of 0.1% formic acid in water and MeOH, respectively. For separation at a flow rate of 1 ml/min, a gradient program was used. 100% solvent A was the initial mobile phase, followed by a gradient to 100% solvent B over 20 minutes, the mobile phase was then hold on 100% solvent B for 5 min and to 100% solvent A for 25 min. Drying gas flow rate was 1 ml/min at 320 °C. MS was operated in the positive ion mode in a mass range of m/z 100-2000.

2.10. **Statistical analysis**

Data are presented as mean ± standard deviation (SD). Values were derived from six animals per group, and differences were examined by a one-way analysis of variance (ANOVA) followed by the Fisher test (Stat View). *P < 0.05 was considered statistically significant.

3. **Results**

3.1. **Effect of DPP ethanolic extract on body and heart weight of experimental rats**

The effects of isoproterenol and DPP extract treatment on heart weight, body weight and heart weight to body weight ratio are shown in Table 1. There is no significant difference in the body weight between the groups observed. Isoproterenol treated rats showed a significant increase (P < 0.05) in heart weight and heart weight to body weight ratio by 41% as compared to control rats. However, rats pretreated with DPP extract followed by isoproterenol exhibited notable decrease (P < 0.05) in the heart weight by 22% compared to untreated myocardial infarcted rats. Moreover, no significant difference was observed in heart weight and heart weight to body ratio between animals treated with DPP extract and those treated with clopidogrel.
3.2. **Effect of DPP-T on ECG pattern**

Fig. 1 and Fig. 2 represented the electrocardiogram pattern of normal and experimental rats, respectively. Control animals exhibited normal ECG pattern. Rats treated only with isoproterenol showed significant increase of ST–segment \((P < 0.05)\) compared to control group, indicating infarcted myocardium. Also, isoproterenol treated rats illustrated the incidence of wave of Pardee and planing R-wave equivalent of the Q wave of necrosis. However, the treatment of infarcted rats with clopidogrel and DPP extract exhibited a remarkable decrease of ST-segment compared to untreated ones. Additionally, rats treated with DPP extract or clopidogrel showed a whole neutralization of the ST-segment elevation with normal QRS compared to Isoproterenol treated ones.

3.3. **Plasma markers of cardiac damage**

Table 2 indicated the effects of DPP extract on marker enzymes of cardiac function including CPK, ALT, LDH and tropornin-T in serum of control and experimental rats. The plasma enzyme activities of CPK, ALT, LDH and tropornin-T were significantly increased \((P < 0.05)\) in the isoproterenol-induced infarcted rats by 71, 64, 170 and 315\%, respectively compared to control rats. However, pre-co-treatment of infarcted rats with DPP extract significantly \((P < 0.05)\) normalized the cardiac function indices.

3.4. **Plasma lipid profile**

As shown in Table 3, isoproterenol-induced myocardial infarcted rats displayed significant increase in the plasma concentration of total cholesterol and triglycerides \((P < 0.05)\) compared to control group. DPP extract pre-co-treatment significantly decreased the plasma levels of cholesterol and triglycerides compared to isoproterenol group.

3.5. **Histopathological examination**

As shown in Fig. 3, control rats exhibited normal myocardium structure without any infarction edema. However, the isoproterenol-induced infarcted rats showed clear increase in myofibril thickness, necrosis, and loss of transverse striations compared to control group. However, pre-co-treatment of infarcted rats with DPP showed normal myocardial architectures with evident transverse striations.

3.6. **Effect of ethanolic extract of DPP on plasma ACE Activity**

As shown in Fig.4, the ACE activity in plasma of untreated infarcted rats showed a significant increase by 33\% as compared to control group of rats \((P < 0.05)\). Interestingly, the treatment of infarcted rats with DPP extract underwent a notable decrease of ACE activity by 34\% as compared to the untreated infarcted group.
3.7. *LC/MS analysis of bioactive compounds in DPP extract*

LC/HRESIMS analysis of the DPP extract showed a rich profile of 29 secondary metabolites belonging to two main chemical classes (Fig. 5). Phenolic compounds, including flavonoids, flavonoid derivatives, flavonoid glycosides, tannins, coumarins, and other phenolic derivatives, stand for approximately 60% of the total DPP metabolite profile. Additionally, terpenoid compounds, including carotenoid derivatives, steroids, fatty acids, and other terpene derivatives represent about 40% of the total DPP metabolite profile. About 60% of the detected metabolites proved to possess either a cardioprotective effect, protects against myocardial infarction or ACE enzyme inhibitors (Table 4). Even there was no reported activity for the remaining 40% of the detected metabolites, they could have potential antioxidant or free radical scavenging effect due to their phenolic scaffold.

4. **Discussion**

DPP has been reported as a rich source of diverse secondary metabolite possessing free radical scavenging potential that may overcome heart disease (Daoud et al., 2015). The present study was designed to investigate, for the first time, the preventive effect of DPP ethanolic extraction against isoproterenol-induced myocardial function in rats.

A subcutaneous injection of supra-maximal dose of isoproterenol has been reported to cause severe myocardial stress and induce infarction such as necrosis which is followed by increased release of cardiac enzymes, accumulation of lipid peroxidases, and impaired cardiac function (Jing et al., 2014). Rats treated with isoproterenol showed an obvious elevation of ST-segment. Accordingly, Rajadurai et al. (2007) recorded that modification of ST-segment is indicative of myocardial ischemia and infarction. The alteration of ECG pattern is related to the consecutive loss of integrity of cell membrane in injured myocardium (Mnafgui et al., 2016). However, administration of DPP ethanolic extract in a dose of 400 mg/kg reduced the abnormalities observed in the ECG of isoproterenol-induced rats. Therefore, DPP remarkably restored the alteration of ST-segment induced by isoproterenol, suggesting the preventive effects of DPP extract on cell membrane.

The evaluation of myocardial cell injury was performed by the determination of specific and sensitive biomarkers in plasma like troponin-T, CPK, ALT and LDH (O’Brien et al., 2006; Evran et al., 2014; Mnafgui et al., 2016). In the current study, the significant increase in plasma biomarkers activities have been recorded in isoproterenol group as compared to control. The high level of troponin-T and plasma cardiac markers predicts the risk of both cardiac death and subsequent infarction (Acikel et al., 2005; Rajadurai et al., 2007). Pre-co-
treatment with DPP extract showed an improvement in the levels of plasma cardiac enzymes in isoproterenol-induced rats. These results suggest that DPP could reduce the degree of damage in the myocardium by maintaining membrane integrity and therefore, restricting the leakage of these enzymes.

On the other hand, lipids play a crucial role in cardiovascular diseases not only in hyperlipidemia and the development of atherosclerosis, but also by modifying the structure, composition, and stability of the cellular membranes (Saxena and Panjwani, 2014; Shaik et al., 2012). An increase in total lipid levels (TG and CT) was detected in isoproterenol-injected rats that could enhance the induction of the atherosclerotic plaque, associated with myocardial infarction. The obtained results proved that the pre-co-treatment with DPP extract ameliorated the status of isoproterenol-induced cardio toxicity in rats. This underlines that DPP extract is responsible for protection of structural and architectural integrity of cardiomyocytes. The current findings showed that DPP provided a preventive effect to the myocardium by attenuation of ventricular dysfunction through maintaining the ECG pattern and cardiac markers enzymes near to normal condition in isoproterenol-treated rats.

Scientific evidences have suggested that the cardiac renin-angiotensin system (RAS) was activated during the remodeling process after acute myocardial infarction (Mnafgui et al., 2016; Harada et al., 1999; Borghi et al., 2006). The myocardial infarction induced by isoproterenol is often underwent a significant rise in ACE activity associated with elevation in heart weight ratio indicative of ventricular remodeling process. This mechanism improves the dilation of the non-infarcted left ventricular, the infarct expansion as well as the compensatory reactive hypertrophy (Mnafgui et al., 2016; Borghi et al., 2006). The increase in the ACE activity certainly report the inhibition of cardiac remodeling process by reducing the expression of cytokine transforming growth factor (TGF-β1) which is a mediator of the remodeling process and fibrosis tissues (Mnafgui et al., 2016). The oral administration of DPP extract to isoproterenol-induced infarcted rats contributed to a significant inhibition of plasma ACE activity with remarked decrease in heart weight ratio. Interestingly, our results highlight the cardioprotective effect of DPP preventing the increased risk of infarct expansion and LV remodeling following myocardial infarction. In fact, numerous clinical and experimental studies revealed that the activity of cardiac rennin-angiotensin system is started after myocardial infarction and failure (Teyssedou, 2007; Mnafgui et al., 2016). The current results evidenced that the DPP extract prevented the excessive heart fibrosis. It has been proved, for the first time that stimulates the systolic and diastolic improvement through increasing the pumping capacity and restoring the myocardial stiffness (Kannan et al., 2011).
LC/HRESIMS analysis of the DPP extract indicated a rich profile of many secondary metabolites belonging to two main chemical classes. Approximately 60% of the total DPP metabolite profile was accounted to phenolic compounds with different subclasses while terpenoid derivatives represent around 40% of the total DPP metabolite profile. Literature review of their biological activity revealed that 60% of the identified compounds have potential cardiopreventive, anti-myocardial infarction effects and ACE inhibition activities. For example, the terpenoids stigmasterol (Li et al., 2015), β-sitosterol (Lei et al., 2015) and estradiol (Lagranha et al., 2010), the carotenoids lutein (Zou et al., 2011 and 2014) and δ-tocotrienol (Wong et al., 2015), and flavonoids isorhamniten (Ibarra et al., 2002) exhibited cardiopreventive effect. Additionally, some metabolites reported to be protective agents against harmful effects of myocardial infarction including the steroid β-sitosterol acetate (Lei et al., 2015) and the phenolic derivatives catechin (Bhardwaj et al., 2014), apigenin (Du et al., 2015), and methyl-p-hydroxycinnamate (Jyoti Roy and Stanley Maynzen Prince, 2013). Moreover, our survey on the identified bioactive molecules in DPP extract revealed their in vivo ACE inhibitory activity such as estradiol (Dean et al., 2005), ellagic acid (Al Shukor et al., 2013), luteolin, quercitin, apigenin and rutin (Guerrero et al., 2012), quercitrin (Hackl et al., 2002), luteolin-7-O-glucoside (Simaratanamongkol et al., 2014) and ferulic acid (Geng et al., 2010). Finally, there was no reported activity for the remaining 40% of the detected metabolites, they could have potential antioxidant or free radical scavenging effect due to their phenolic scaffold. These compounds warrant urgent investigation of their cardiopreventive anti-myocardial infarction effects and ACE inhibition activities in the light of our results.

5. Conclusion

Herein, we represent the first experimental evidence that DPP exerted cardiopreventive effect from the acute myocardium infarction and cardiac remodeling process induced by isoproterenol through the inhibition of ACE activity. This was supported by the presence of different DPP metabolites belonging to different chemical scaffolds with documented cardiopreventive, anti-myocardial infarction effects and ACE inhibition activities. DPP could therefore be regarded as a promising cardiopreventive agent and rich source of bioactive pharmacological products.

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Figure captions

**Fig. 1.** Effect of DPP ethanolic extract on ST-segment elevation (mV) in the ECG (recorded from limb lead II) in normal control, isoproterenol alone injected and treated rats. Values are given as mean ± SD for group of six rats. Statistically, values are represented as follows: * P < 0.05 significant differences compared to controls. # P < 0.05 significant differences compared to isoproterenol group. @ P < 0.05 significant differences compared to isoproterenol-treated group with clopidogrel.

**Fig. 2.** Effect of DPP ethanolic extract on electrocardiographic (ECG) pattern in normal and experimental rats.

**Fig. 3.** Histopathological changes of myocardial tissue (H&E9500). Control group showing normal myocardial histology, clear transverse striations and no inflammatory cell infiltration. Isoproterenol (Isop) group showing myocardial cells necrosis, separation of cardiac myofibrillar and large inflammatory cells infiltration. Isop + Clop (0.2 mg/kg)-treated group showing few inflammatory cell infiltration and improvement of myocardium necrosis. Isop + DPP (400 mg/kg) showing normal myocardial architectures with evident transverse striations.

**Fig. 4.** ACE activity in serum of normal and experimental rats. Values are given as mean ± SD for group of six rats. Statistically, values are represented as follows: * P < 0.05 significant differences compared to controls. # P < 0.05 significant differences compared to isoproterenol group. @ P < 0.05 significant differences compared to isoproterenol-treated group with clopidogrel.

**Fig. 5.** LC-HRESIMS analysis of DPP ethanolic extract.
Fig. 1.

Fig. 2.
Fig. 3.

Fig. 4.
Fig. 5

NL: 7.44E6
Base Peak F:
FTMS + p ESI
Full ms
[100.00 - 2000.00] MS
HRMS of DPP
Table 1
Effect of DPP ethanolic extract on body weight, heart weight and heart weight/body weight ratio in isoproterenol induced myocardial infarction in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Isop</th>
<th>Isop + Clop</th>
<th>Isop + DPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>176.66 ± 10.44</td>
<td>173.66 ± 13.41</td>
<td>174.5 ± 13.18</td>
<td>199.88 ± 3.06*##@</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>0.82 ± 0.16</td>
<td>1.14 ± 0.17*</td>
<td>0.92 ± 0.09#</td>
<td>1.02 ± 0.14##</td>
</tr>
<tr>
<td>Heart weight/body weight ratio</td>
<td>0.46 ± 0.08</td>
<td>0.65 ± 0.05*</td>
<td>0.54 ± 0.04#</td>
<td>0.51 ± 0.06#</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of six animals each. Statistically, values are presented as follows: * P < 0.05 significant differences compared to controls. # P < 0.05 significant differences compared to isoproterenol group. @ P < 0.05 significant differences to rats treated with clopidogrel.

Table 2
Effect of DPP ethanolic extract on plasma cardiac damage.

<table>
<thead>
<tr>
<th></th>
<th>ALT (UI/L)</th>
<th>LDH (UI/L)</th>
<th>CPK (UI/L)</th>
<th>Troponin-T (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75.33 ± 5.82</td>
<td>588.5 ± 45.38</td>
<td>2811 ± 120.44</td>
<td>0.46 ± 0.1</td>
</tr>
<tr>
<td>Isop</td>
<td>124 ± 31.08*</td>
<td>1591 ± 179.98*</td>
<td>4818.33 ± 401.07*</td>
<td>1.91 ± 0.23*</td>
</tr>
<tr>
<td>Isop + Clop</td>
<td>73.83 ± 8.28*#</td>
<td>1057.16 ± 101.57*#</td>
<td>3518 ± 269.97*#</td>
<td>0.59 ± 0.04*#</td>
</tr>
<tr>
<td>Isop + DPP</td>
<td>66.66 ± 1.63##@</td>
<td>807.33 ± 2.06##@</td>
<td>2974 ± 116.66##@</td>
<td>0.46 ± 0.25##</td>
</tr>
</tbody>
</table>

Alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and serum troponin-T level of control and experimental groups of rats. Values are given as mean ± SD for group of six rats. Values are statistically presented as follows: * P < 0.05 significant differences compared to controls. # P < 0.05 significant differences compared to isoproterenol group. @ P < 0.05 significant differences to rats treated with clopidogrel.
### Table 3
Effect of DPP ethanolic extract on cholesterol and triglycerides levels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Isop</th>
<th>Isop + Clop</th>
<th>Isop + DPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>1.57 ± 0.15</td>
<td>2.33 ± 0.22*</td>
<td>1.93 ± 0.16*#</td>
<td>1.85 ± 0.18*#</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.70 ± 0.09</td>
<td>0.99 ± 0.25*</td>
<td>0.81 ± 0.11*#</td>
<td>0.65 ± 0.039*#</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of six animals each.
Statistically, values are presented as follows: * P < 0.05 significant differences compared to controls. # P < 0.05 significant differences compared to isoproterenol group @ P < 0.05 significant differences to rats treated with clopidogrel.

### Table 4
HRESIMS analysis of DPP ethanolic extract and literature review of their biological properties.

<table>
<thead>
<tr>
<th>HRESIMS mass (a)</th>
<th>Mol formula</th>
<th>Suggested compound</th>
<th>MS/MS fragments</th>
<th>Biological properties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>413.3775</td>
<td>C_{29}H_{48}O</td>
<td>Stigmasterol (steroid)</td>
<td>396.3751, 353.3203, 338.2968, 257.2266</td>
<td>stigmasterol, when fed, lowers plasma cholesterol levels, inhibits intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid synthesis in Wistar as well as WKY rats. However, plasma and hepatic incorporation of stigmasterol is low. Stigmasterol inhibits excessive proliferation of vascular smooth muscle cells, a crucial event in the pathogenesis of several cardiovascular diseases, including atherosclerosis and restenosis.</td>
<td>Batta et al., 2006; Li et al., 2015</td>
</tr>
<tr>
<td>415.3931</td>
<td>C_{29}H_{50}O</td>
<td>β-Sitosterol (steroid)</td>
<td>398.3907, 355.3359, 340.3125, 257.2264</td>
<td>β-sitosterol reduce plasma cholesterol by 18% and is poorly absorbed in the intestine. β-sitosterol possess cardioprotective role in isoproterenol -induced myocardial infarction in rats.</td>
<td>Lei et al., 2015; Ganapathy et al., 2014</td>
</tr>
<tr>
<td>273.1844</td>
<td>C_{18}H_{22}O_{2}</td>
<td>Estradiol</td>
<td>256.1822, 257.2266</td>
<td>Estragen has been shown to increase expression of superoxide</td>
<td>Lagranha et al., 2010</td>
</tr>
<tr>
<td>Molecular Formula</td>
<td>Molecular Weight</td>
<td>Description</td>
<td>Biological Activity</td>
<td>References</td>
<td></td>
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<tr>
<td>-------------------</td>
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<td></td>
</tr>
<tr>
<td>C_{31}H_{52}O_{2}</td>
<td>457.4045</td>
<td>β-Sitosterol acetate (steroid)</td>
<td>Similar effects to β-Sitosterol</td>
<td>Ganapathy et al., 2014, Lei et al., 2015</td>
<td></td>
</tr>
<tr>
<td>C_{31}H_{52}O_{2}</td>
<td>373.2327</td>
<td>2β,3β,4β-trihydroxy pregn-16-one (steroid)</td>
<td>No biological activity reported</td>
<td>Tan et al., 2010</td>
<td></td>
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<tr>
<td>C_{14}H_{34}O_{2}</td>
<td>324.9950</td>
<td>Ellagic acid (Tannin)</td>
<td>Oral pretreatment with ellagic acid was safe and effective in cardio protection against isoproterenol-induced arrhythmias, hypertrophy and myocardial necrosis. Anti-lipid peroxidation property and anti hyperlipidaemic activity through 3-hydroxy-3 methyl glutaryl CoA reductase inhibition by ellagic acid may be the reasons for the beneficial action of ellagic acid against experimentally induced myocardial infarction. Ellagic acid is a potent cardiac protective agent against doxorubicin-induced cardiac oxidative, inflammatory and apoptotic stress. ellagic acid showed some ACE inhibition at a concentration of 0.75 mM.</td>
<td>Kannan and Quine, 2013, Lin and Yin, 2013, Al Shukor et al., 2013</td>
<td></td>
</tr>
<tr>
<td>C_{26}H_{52}O_{2}</td>
<td>397.4049</td>
<td>Cerotic Acid (fatty acid)</td>
<td>No biological activity reported.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_{40}H_{50}O_{2}</td>
<td>569.4355</td>
<td>Lutein (carotenoid derivative)</td>
<td>Lutein may play a protective role in the prevention of early atherosclerosis. Lutein supplementation significantly increased the serum concentrations of lutein with a decrease in carotid artery intima-media thickness being associated with lutein concentrations. Lutein supplementation reduces biomarkers of cardiovascular diseases risk via decreased lipid peroxidation and inflammatory response by</td>
<td>Zou et al., 2011, Zou et al., 2014, Wang et al., 2013</td>
<td></td>
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increasing plasma lutein concentrations and antioxidant capacity.

<table>
<thead>
<tr>
<th>CAS</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Description</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td>397.3095</td>
<td>C_{27}H_{46}O_{2}</td>
<td>381.3152, 365.3208</td>
<td>δ-tocotrienol improved inflammation, heart structure and function as well as cardiovascular function in diet-induced obese rats. Diet supplementation with δ-tocotrienol, reduce cardiovascular risk factors in humans when used as nutritional supplements with, or without, other dietary changes.</td>
<td>Wong et al., 2015</td>
</tr>
<tr>
<td>441.3209</td>
<td>C_{16}H_{32}O</td>
<td>424.3107</td>
<td>No biological activity reported.</td>
<td></td>
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<tr>
<td>583.45288</td>
<td>C_{41}H_{58}O_{2}</td>
<td>566.4485</td>
<td>No biological activity reported.</td>
<td></td>
</tr>
<tr>
<td>317.1708</td>
<td>C_{10}H_{24}O_{8}</td>
<td>300.1720, 272.1771, 255.1743</td>
<td>No biological activity reported.</td>
<td></td>
</tr>
<tr>
<td>217.1181</td>
<td>C_{12}H_{18}O_{2}</td>
<td>186.1015, 155.0831</td>
<td>No biological activity reported.</td>
<td></td>
</tr>
<tr>
<td>261.1079</td>
<td>C_{13}H_{18}O_{4}</td>
<td>230.0917</td>
<td>No biological activity reported.</td>
<td></td>
</tr>
<tr>
<td>413.1156</td>
<td>C_{20}H_{22}O_{8}</td>
<td>396.1177, 365.0990, 334.0812, 303.0628</td>
<td>No biological activity reported.</td>
<td></td>
</tr>
<tr>
<td>317.0658</td>
<td>C_{16}H_{12}O_{7}</td>
<td>286.0472, 269.0445</td>
<td>Isorhamnetin produced endothelium-independent vasodilator effects in rat aorta, rat mesenteric arteries, rat portal vein and porcine coronary arteries. The arterial, venous and coronary vasodilator effects may contribute to the protective effects of flavonoids in ischaemic heart disease observed in epidemiological studies.</td>
<td>Ibarra et al., 2002, Sun et al., 2013</td>
</tr>
<tr>
<td>291.0869</td>
<td>C_{15}H_{14}O_{6}</td>
<td>274.0836, 258.0887</td>
<td>Catechin is effective in reversing the impaired relaxation in restrictive cardiomyopathy myocardial cells and rescuing the restrictive</td>
<td>Zhang et al., 2015</td>
</tr>
</tbody>
</table>
cardiomyopathy mice with diastolic dysfunction. Catechin treatment prevents diabetes mellitus-induced vascular endothelial dysfunction. It also prevents diabetic vascular endothelial dysfunction through reduction in high glucose, vascular oxidative stress, and lipid peroxidation.

<table>
<thead>
<tr>
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<th>Function</th>
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<tbody>
<tr>
<td>287.0553</td>
<td>C_{13}H_{10}O_{6}</td>
<td>Luteolin (Flavonoid)</td>
<td>Bhardwaj et al., 2014</td>
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<tr>
<td>301.1402</td>
<td>C_{16}H_{20}O_{4}</td>
<td>5,7,4'-trimethoxyflavane (Flavonoid)</td>
<td>No biological activity reported.</td>
</tr>
<tr>
<td>303.0499</td>
<td>C_{15}H_{10}O_{7}</td>
<td>Quercitin (Flavonoid)</td>
<td>Malaguti et al., 2015</td>
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<tr>
<td>271.0601</td>
<td>C_{15}H_{10}O_{5}</td>
<td>Apigenin (Flavonoid)</td>
<td>Yang et al., 2015</td>
</tr>
<tr>
<td>611.1608</td>
<td>C_{27}H_{30}O_{16}</td>
<td>Rutin (Flavonoid glycoside)</td>
<td>Annapurna et al., 2009</td>
</tr>
<tr>
<td>449.1075</td>
<td>C_{21}H_{21}O_{11}</td>
<td>Quercitrin (Flavonoid glycoside)</td>
<td>Hackl et al., 2002</td>
</tr>
<tr>
<td>493.1345</td>
<td>C_{23}H_{24}O_{12}</td>
<td>Morifonoside A</td>
<td>No biological activity reported.</td>
</tr>
<tr>
<td><strong>Molecular Formula</strong></td>
<td><strong>Molecular Mass</strong></td>
<td><strong>Description</strong></td>
<td><strong>References</strong></td>
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<tr>
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<tr>
<td>Luteolin-7-O-glucoside</td>
<td>449.1077</td>
<td>C_{21}H_{20}O_{11} Protection of Luteolin-7-O-Glucoside Against Doxorubicin-induced cardiotoxicity. Luteolin-7-O-glucoside have an inhibitory effect on the angiotensin-converting enzyme.</td>
<td>Yao et al., 2015</td>
</tr>
<tr>
<td>Methyl-4-hydroxybenzoate</td>
<td>153.0543</td>
<td>C_{8}H_{8}O_{3} safe food and cosmetic antibacterial and antifungal preservative.</td>
<td>Soni et al., 2002</td>
</tr>
<tr>
<td>Methyl-p-hydroxycinnamate</td>
<td>179.0701</td>
<td>C_{10}H_{10}O_{3} p-coumaric acid protected the myocardial infarcted rat's heart against apoptosis by inhibiting oxidative stress. p-coumaric acid have Preventive effects of on myocardial infarct size in experimentally induced myocardial infarction.</td>
<td>Stanley Mainzen Prince and Jyoti Roy., 2013</td>
</tr>
<tr>
<td>Ferulic Acid</td>
<td>195.0654</td>
<td>C_{10}H_{10}O_{4} Ferulic acid may contribute to prevention of chronic inflammatory diseases, a part of the pathophysiology of Cardiovascular Diseases. Ferulic acid improved the structure and function of the heart and blood vessels in hypertensive rats. Ferulic acid indicated potent in vitro ACE inhibitory activity with IC_{50} values of 10.898 +/- 0.430.</td>
<td>Navarrete et al., 2015</td>
</tr>
</tbody>
</table>

**Footnotes:**

*a* High Resolution Electrospray Ionization Mass Spectrometry (HRESIMS) using Xcalibur 3.0 and allowing for M + H and M + Na adducts.

*b* The suggested compound according to Dictionary of Natural Products (DNP 23.1, 2015 on DVD).