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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 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 *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.

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

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.

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

(Roche Diagnostics, Switzerland). 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 cardiopreventive 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 isorhamnitin (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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References

Acikel, M., Buyukokuroglu, M., Erdogan,F., Aksoy, H., Bozkurt, E., Senocak, H., 2005. Protective effects of dantrolene against myocardial injury induced by isoproterenol in rats: biochemical and histological findings. Int. J. Cardiol. 98, 389-94.

Al Shukor, N., Van Camp, J., Gonzales, G.B., Staljanssens, D., Struijs, K., Zotti, M.J., Raes, K., Smagghe, G., 2013. Angiotensin-converting enzyme inhibitory effects by plant phenolic compounds: a study of structure activity relationships. J. Agric. Food Chem. 61 (48), 11832-9.

Alam, M.A., Sernia, C., Brown, L., 2013. Ferulic acid improves cardiovascular and kidney structure and function in hypertensive rats. J. Cardiovasc. Pharmacol. 61 (3) 240-9.

Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., Shahidi, F., 2005. Comparison of antioxidant activity, antho-cyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. J. Agric Food Chem. 53, 7592-7599.

Annapurna, A., Reddy, C.S., Akondi, R.B., Rao, S.R., 2009. Cardioprotective actions of two bioflavonoids, quercetin and rutin, in experimental myocardial infarction in both normal and streptozotocin-induced type I diabetic rats. J. Pharm. Pharmacol. 61(10), 1365-74.

Batta, A.K., Xu, G., Honda, A., Miyazaki, T., Salen, G., 2006. Stigmasterol reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption in the rat. Metabolism 55 (3), 292-299.

Borghi, C., Bacchelli, S., Esposti, D.D., Ambrosioni, E., 2006. Effects of early angiotensin-converting enzyme inhibitionin patients with non–ST-elevation acute anterior myocardial infarction. Am. Heart J. 152, 470-477.

Bhardwaj, P., Khanna, D., Balakumar, P., 2014. Catechin averts experimental diabetes mellitus-induced vascular endothelial structural and functional abnormalities. Cardiovasc. Toxicol. 14 (1), 41-51.

Boersma, E., Mercado, N., Poldermans, D., Gardien, M., Vos, J., Simoons, M., 2003. Actue myocardial infarction. Lancet. 361, 847-58.

Bian, C., Xu, T., Zhu, H., Pan, D., Liu, Y., Luo, Y., Wu, P., Li, D., 2015. Luteolin inhibits ischemia/reperfusion-induced myocardial injury in rats via down regulation of microRNA-208b-3p. Plos One 10 (12), e0144877.

Daoud, A., Drira, M., Bakari, S., Hfaieth, N., Manfgui, K., Kadri, A., Gharsallah, N., 2015. Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of date palm pollen (DPP) from two Tunisian cultivars. Arabian J. Chem. (in press). doi: 10.1016/j.arabjc.2015.07.014.

De Bono, D.P., Boon, N.A., 1992. Diseases of the cardiovascular system, in: C.R.W. Edwards, I.A. Boucheir, (Eds.), Davidson's Principles and Practice of Medicine, Churchill Livingstone, Hong Kong, pp. 249-340.

Dean, S.A., Tan, J., O'Brien, E.R., Leenen, F.H., 2005. 17beta-estradiol down regulates tissue angiotensin-converting enzyme and ANG II type 1 receptor in female rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288 (3), R759-66.

Du, H., Hao, J., Liu, F., Lu, J., Yang, X., 2015. Apigenin attenuates acute myocardial infarction of rats via the inhibitions of matrix metalloprotease-9 and inflammatory reactions. Int. J. Clin. Exp. Med. 8 (6), 8854-9.

Elberry, A., Mufti, S., Al-Maghrabi, J., Abdel-Sattar, E., Ashour, O., Ghareib, S., et al. 2011. Anti-inflammatory and antiproliferative activities of date palm pollen (*Phoenix dactylifera*) on experimentally-induced atypical prostatic hyperplasia in rats. J Inflam. 8: 40-53.

Eraslan, G., Kanbur, M., Silici, S., Liman, B., Altinordulu, S., Karabacak, M., 2008. Evaluation of protective effect of bee pollen against propoxur toxicity in rat. Ecotoxicol. Environ. Saf. 72, 931-937.

Evran, B., Karpuzoglu, H., Develi, S., Kalaz, E.B., Soluk-Tekkes,M., Olgac, V., Uysal, M., 2014. Effects of carnosine onprooxidant–antioxidant status in heart tissue, plasma and erythrocytesof rats with isoproterenol-induced myocardial infarction. Pharmacol. Rep. 66, 81-86.

Ganapathy, P., Rajadurai, M., Ashokumar, 2014. Effect of β-Sitosterol on Cardiac Troponins, Marker Enzymes and Biochemical Parameters in Isoproterenol-Induced Myocardial Infarction. J.A.I.R. 3 (5), 209 -214.

Geng, F., He, Y., Yang, L., Wang, Z., 2010. A rapid assay for angiotensin-converting enzyme activity using ultra-performance liquid chromatography-mass spectrometry. Biomed Chromatogr. 24 (3), 312-7.

Guerrero, L., Castillo M., Quiñones, J., Garcia-Vallvé, S., Arola, L., Pujadas, G., et al. 2012. Inhibition of Angiotensin-Converting Enzyme Activity by Flavonoids: Structure-Activity Relationship Studies. Plos one 7(11), e49493.

Häckl, L.P., Cuttle, G., Dovichi, S.S., Lima-Landman, M.T., Nicolau, M., 2002. Inhibition of angiotesin-converting enzyme by quercetin alters the vascular response to brandy kinin and angiotensin I. Pharmacol. 65(4), 182-6.

Harada, K., Sugaya, T., Murakami, K., Yazaki, Y., Komuro, I., 1999. Angiotensin II Type 1A Receptor Knockout Mice Display Less Left Ventricular Remodeling and Improved Survival after Myocardial Infarction. J. Am. Heart Assoc.100, 2093-2099.

Hertog, M.G., Hollman, P.C., Katan, M.B., Kromhout, D., 1993. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr. Cancer 20, 21-29.

Hung, C.H., Chan, S.H., Chu, P.M., Tsai, K.L., 2015. Quercetin is a potent anti-atherosclerotic compound by activation of SIRT1 signaling under oxLDL stimulation. Mol. Nutr. Food Res. 59(10), 1905-17.

Ibarra, M., Pérez-Vizcaíno, F., Cogolludo, A., Duarte, J., Zaragozá-Arnáez, F., López-López, J.G., Tamargo, J., 2002. Cardiovascular effects of isorhamnetin and quercetin in isolated rat and porcine vascular smooth muscle and isolated rat atria. Planta. Med. 68 (4), 307-10.

Ittagi, S., Merugumolu, V., Siddamsetty, R., 2014. Cardioprotective effect of hydroalcoholic extract of Tecoma stans flowers against isoproterenol induced myocardial infarction in rats. Asian Pac. J. Trop. Dis. 4, 378-384.

Jing, L., Wang, Y., Zhao, X., Zhao, B., Han, J., Qin, S., Sun, X., 2014. Cardioprotective effect of hydrogen-rich saline on isoproterenol-induced Myocardial Infarction in Rats. Heart Lung Circ. 1728, 1-9.

Jyoti Roy, A., Stanely Mainzen Prince, P., 2013. Preventive effects of p-coumaric acid on lysosomal dysfunction and myocardial infarct size in experimentally induced myocardial infarction. Eur. J. Pharmacol. 699 (1-3), 33-9.

Kannan, M.M., Quine, S.D., 2011. Ellagic acid ameliorates isoproterenol induced oxidative stress: Evidence from electrocardiological, biochemical and histological study. Eur J. Pharmacol. 659, 45-52.

Kannan, M.M., Quine, S.D., 2013. Ellagic acid inhibits cardiac arrhythmias, hypertrophy and hyperlipidemia during myocardial infarction in rats. Metabolism. 62 (1), 52-61.

Lagranha, C.J., Deschamps, A., Aponte, A., et al., 2010. Sex differences in the phosphorylation of mitochondrial proteins result in reduced production of reactive oxygen species and cardioprotection in females. Circ. Res. 106, 1681-1691.

Lei, L., Wang, X., Huang, W., Liu, Y., Zheng, F., Ma, K.Y., Li, Y.M., Wang, L., Man, S.W., Zhang, C., Chen, Z.Y., 2015. Cholesterol side chain analogs but not its ether analogs possess cholesterol-lowering activity. Food Funct. 6 (2), 630-4.

Li, C., Liu, Y., Lu, Q., Luo, S., 2015. Stigmasterol protects against Ang II-induced proliferation of the A7r5 aortic smooth muscle cell-line. Food Funct. 6 (7), 2266-72.

Lin, J., Steenbergen, C., Murphy, E., Sun, J., 2009. Estrogen receptor-beta activation results in S-nitrosylation of proteins involved in cardioprotection. Circulation 120, 245–254.

Lin, M.C., Yin, M.C., 2013. Preventive effects of ellagic acid against doxorubicin-induced cardio-toxicity in mice. Cardiovasc. Toxicol. 13 (3), 185-193.

Malaguti, M., Angeloni,C., Hrelia, S., 2015. Nutraceutical bioactive compounds promote healthspan Counteracting Cardiovascular Diseases. J. Am. Coll. Nutr. 34 Suppl l, 22-7.

Mehdizadeh, R., Parizadeh, M., Khooei, A., Mehri, S., Hosseinzadeh, H., 2013. Cardioprotective effect of saffron extract and safranal in isoproterenol-induced myocardial infarction in Wistar rats. Iran J. Basic Med. Sci.16, 56-63.

Metwaly, M., Dkhil, M., Al-Quraishy, S., 2014. Anti-coccidial and anti-apoptotic activities of palm pollen grains on Eimeria papillata-induced infection in mice. Biologia. 69: 254-259.

Mnafgui, K., Hajji,R., Derbali, F., Khlif, I., Kraiem, F., Ellefi, H., Elfeki, A., Allouche, N., Gharsallah, N., 2016. Protective effect of hydroxytyrosol against cardiac remodeling after isoproterenol-induced myocardial infarction in rat. Cardiovasc. Toxicol. (in press). doi: 10.1007/s12012-015-9323-1.

Mnafgui, K., Hajji, R., Derbali, F., Gammoudi, A., Khabbebi, G., Ellefi, H., Allouche, N., Kadri, A., Gharsallah, N., 2016. Anti-inflammatory, Antithrombotic and Cardiac Remodeling Preventive Effects of Eugenol in Isoproterenol-Induced Myocardial Infarction in Wistar Rat. Cardiovasc. Toxicol. (in press). doi: 10.1007/s12012-015-9343-x

Nai, C., Xuan, H., Zhang, Y., Shen, M., Xu, T., Pan, D., Zhang, C., Zhang, Y., Li, D., 2015. Luteolin exerts cardioprotective effects through improving sarcoplasmic Reticulum $Ca^{(2+)-}$ ATPase activity in rats during ischemia/reperfusion *in vivo.* Evid-Based. Compl. Alt. 365-854.

Navarrete, S., Alarcón, M., Palomo, I., 2015. Aqueous extract of tomato (*Solanum lycopersicum* L.) and ferulic acid reduce the expression of TNF-α and IL-1β in LPS-Activated macrophages. Molecules. 20(8), 15319-29.

O'Brien, P.J., Smith, D.C., Knechtel, T.J., Marchak, M.A., Pruimboom-Brees, I., Brees, D.J., et al, 2006. Cardiac troponin-I is a sensitive, specific biomarker of cardiac injury in laboratory animals. Lab. Anim. 40, 153-171.

Qureshi, A.A., Khan, D.A., Mahjabeen, W., Papasian, C.J., Qureshi, N., 2012. Suppression of Nitric Oxide Production and Cardiovascular Risk Factors in Healthy Seniors and Hypercholesterolemic Subjects by a Combination of Polyphenols and Vitamins. J. Clin. Exp. Cardiolog. S5, 8.

Rahmani, A., Aly, S., Ali, H., Babiker, A., Srikar, S., Khan, A., 2014. Therapeutic effects of date fruits (*Phoenix dactylifera*) in the prevention of diseases *via* modulation of anti-inflammatory, anti-oxidant and anti-tumour activity. Int. J. Clin. Exp. Med. 7(3), 483-491.

Rajadurai, M., Stanely Mainzen Prince, P., 2007. Preventive effect of naringin on cardiac markers, electrocardiographic patterns and lysosomal hydrolases in normal and isoproterenol-induced myocardial infarction in wistar rats. J. Toxicol. 230, 178-188.

Saxena, P., Panjwani, D., 2014. Cardioprotective potential of hydro-alcoholic fruit extract of Ananas comosus against isoproterenol induced myocardial infarction in wistar albino rats. J. Acute Dis. 228-234.

Shaik, H.A., Rasool, S.N., Reedy, K.V.A., Kareem, A.M., Krushna, S.G., 2012. Cardioprotective effect of HPLC standardized ethanolic extract of terminalia pallida fruits against isoproterenol-induced myocardial infarction in albino rats. J. Ethnopharmacol. 141, 33-40.

Simaratanamongkol, A., Umehara, K., Noguchi, H., Panichayupakaranant, P., 2014. Identification of a new angiotensin-converting enzyme (ACE) inhibitor from Thai edible plants. Food Chem. 165, 92-7.

Soni, M.G., Taylor, S.L., Greenberg, N.A., Burdock, G.A., 2002. Evaluation of the health aspects of methyl paraben: a review of the published literature. Food Chem. Toxicol. 40 (10) 1335-73.

Stanely Mainzen Prince, P., Roy, A.J., 2013. p-Coumaric acid attenuates apoptosis in isoproterenol-induced myocardial infarcted rats by inhibiting oxidative stress. Int. J. Cardiol. 168 (4), 3259-66.

Sun, J., Sun, G., Meng, X., Wang, H., Luo, Y., Qin, M., Ma, B., Wang, M., Cai, D., Guo, P., Sun, X., 2013. Isorhamnetin protects against doxorubicin-Induced cardiotoxicity *In vivo* and *in vitro*. Plos one 8 (5), e64526.

Tan, Q.G., Li, X.N., Chen, H., Feng, T., Cai, X.H., Luo, X.D., 2010. Sterols and Terpenoids from Melia azedarach. J. Nat. Prod. 73 (4), 693-697.

Teyssedou, A., 2007. LES IEC dans le post-infarctus: gros plan sur le zofe´nopril. Ann. Cardiol. Angeiol. 56,137-144.

Upaganlawar, A., Gandhi, H., Balaraman, R., 2011. Isoproterenol induced myocardial infarction: Protective role of natural products,J. Pharmacol. Toxicol. 6, 1-17.

Uzbekova, D., Makarova, V., Khvoynitskaya, L., Slepnev, A., 2003. Evaluation of bee collected pollen influence on lipid peroxidation, antioxidant system and liver function in old animals. Hepatology. 38, 203-208.

Wang, M.X., Jiao, J.H., Li, Z.Y., Liu, R.R., Shi, Q., Ma, L., 2013. Lutein supplementation reduces plasma lipid peroxidation and C-reactive protein in healthy non smokers. Atherosclerosis. 7 (2), 380-5.

Wong, W.Y., Ward, L.C., Fong, C.W., Yap, W.N., Brown, L., 2015. Anti-inflammatory γ- and δ-tocotrienols improve cardiovascular, liver and metabolic function in diet-induced obese rats. Eur. J. Nutr. (in press). doi: 10.1007/s00394-015-1064-1.

Yang, X., Yang, J., Hu, J., Li, X., Zhang, X., Li, Z., 2015. Apigenin attenuates myocardial ischemia/reperfusion injury *via* the inactivation of p38 mitogen activated protein kinase. Mol. Med. Rep. 12(5), 6873-8.

Yao, H., Shang, Z., Wang, P., Li, S., Zhang, Q., Tian, H., Ren, D., Han, X., 2015. Protection of luteolin-7-O-glucoside against doxorubicin-induced injury through PTEN/Akt and ERK pathway in H9c2 cells. Cardiovasc. Toxicol. 16(2), 101-10.

Zhang, L., Nan, C., Chen, Y., Tian, J., Jean-Charles, P.Y., Getfield, C., Wang, X., Huang, X., 2015. Calcium desensitizer catechin reverses diastolic dysfunction in mice with restrictive cardiomyopathy. Arch. Biochem. Biophys. 573, 69-76.

Zou, Z., Xu, X., Huang, Y., Xiao, X., Ma, L., Sun, T., Dong, P., Wang, X., Lin, X., 2011. High serum level of lutein may be protective against early atherosclerosis: the Beijing atherosclerosis study. Atherosclerosis. 9 (2), 789-93.

Zou, Z.Y., Xu, X.R., Lin, X.M., Zhang, H.B., Xiao, X., Ouyang, L., Huang, Y.M., Wang, X., Liu, Y.Q., 2014. Effects of lutein and lycopene on carotid intima-media thickness in Chinese subjects with subclinical atherosclerosis: a randomised, double-blind, placebo-controlled trial. Br. J. Nutr. 111 (3), 474-80.

Figure captions

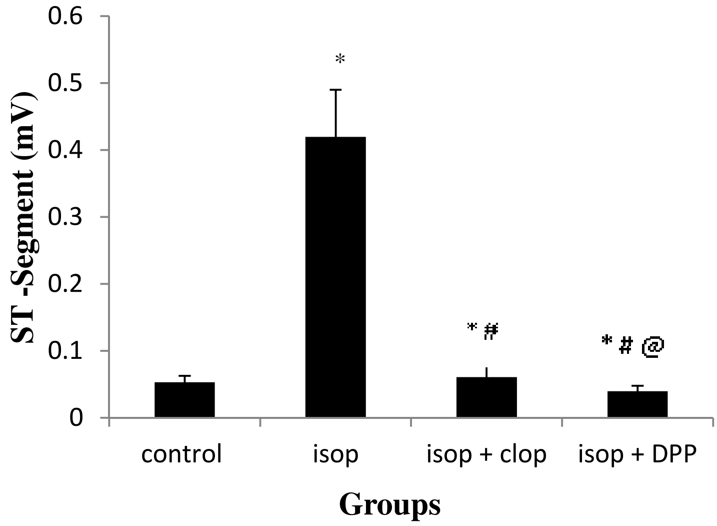
Fig. 1. Effect of DPP ethanolic extract on ST-segment elevation (mV) in the ECG (recorded from limb lead II) in normal control, isoproterenol alone injected and treated rats. Values are given as mean ± SD for group of six rats. Statistically, values are represented as follows: * $P < 0.05$ significant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


0.6
0.5
0.4
0.3
0.2
0.1
0
ST -Segment (mV)
*
* #
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control
isop
isop + clop
isop + DPP
Groups

Fig.1.

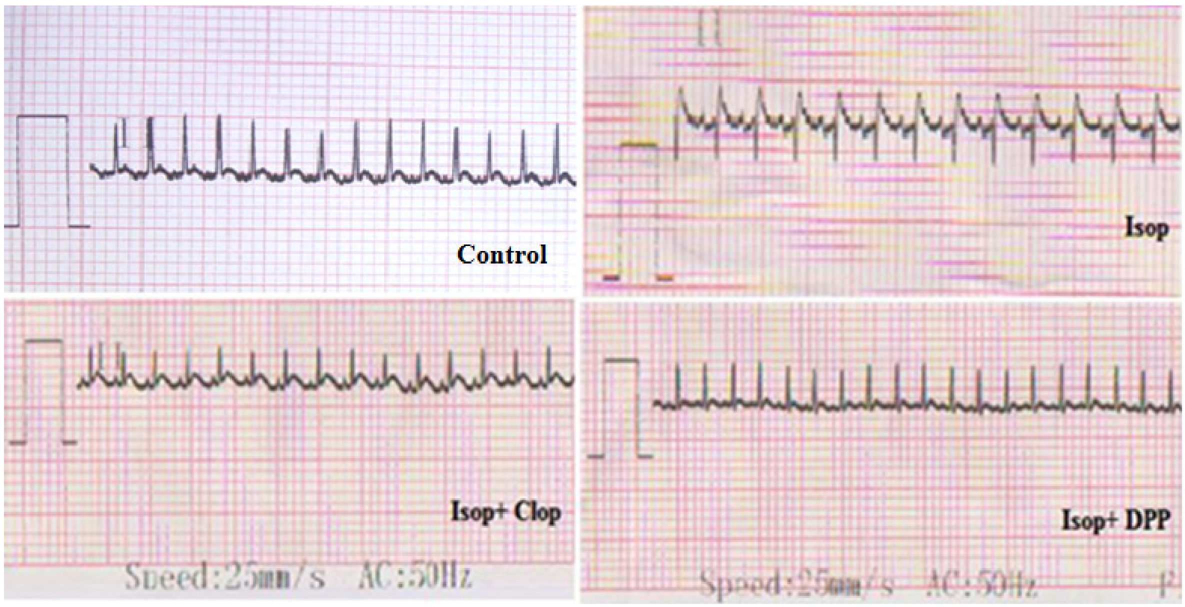
Control
Isop
Isop+ Clop
Isop+ DPP
Speed:25mm/s AC:50Hz

Fig. 2.

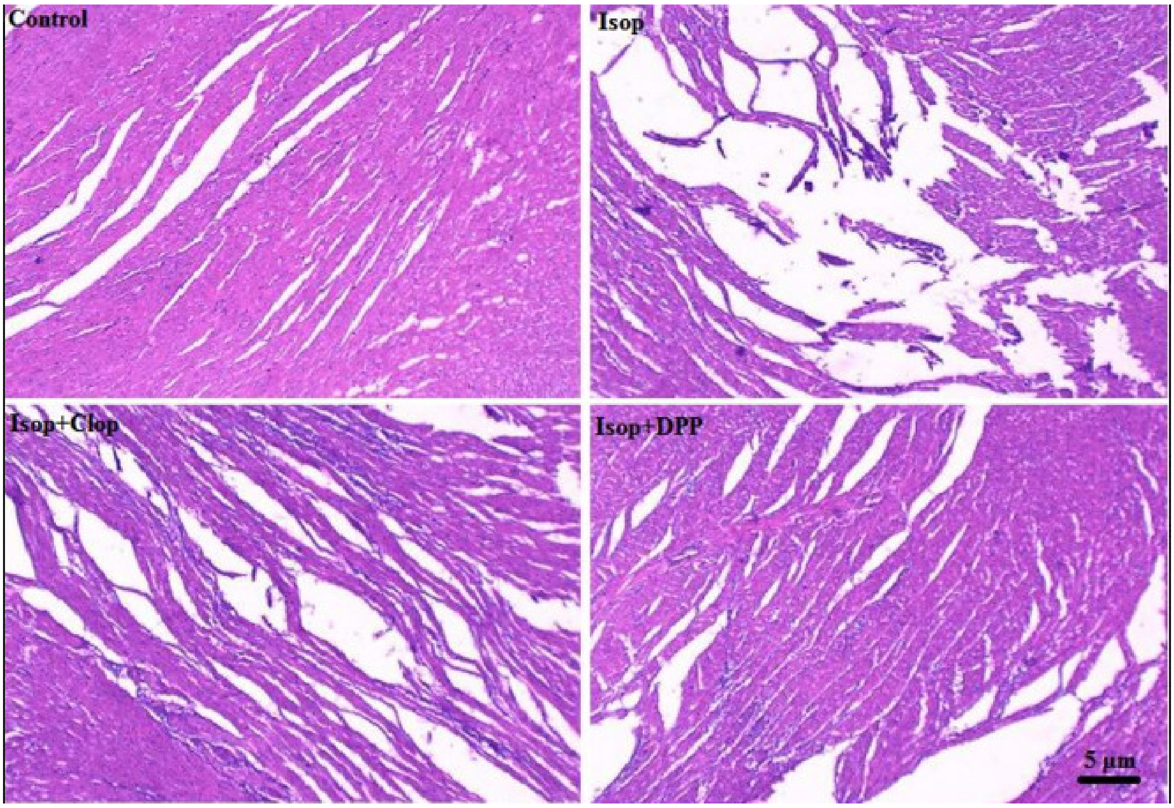
Control
Isop
Isop+Clop
Isop+DPP
5 µm

Fig. 3.

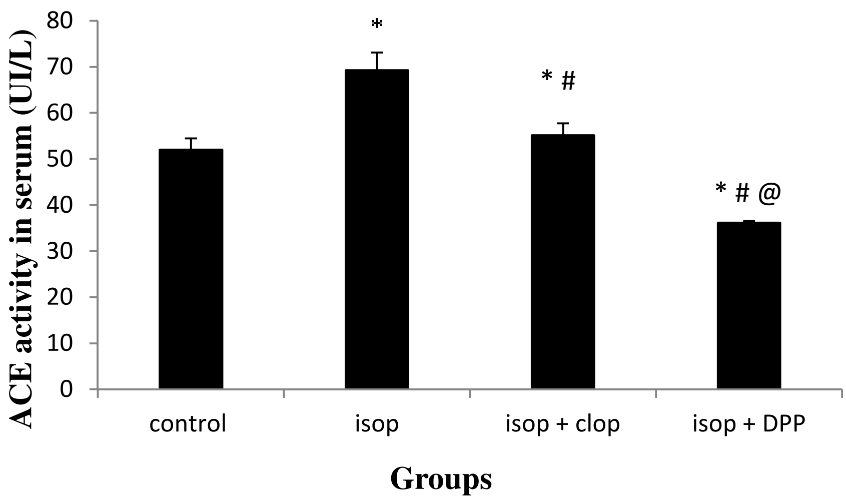
ACE activity in serum (UI/L)
80
70
60
50
40
30
20
10
0
*
* #
* # @
control
isop
isop + clop
isop + DPP
Groups

Fig. 4.

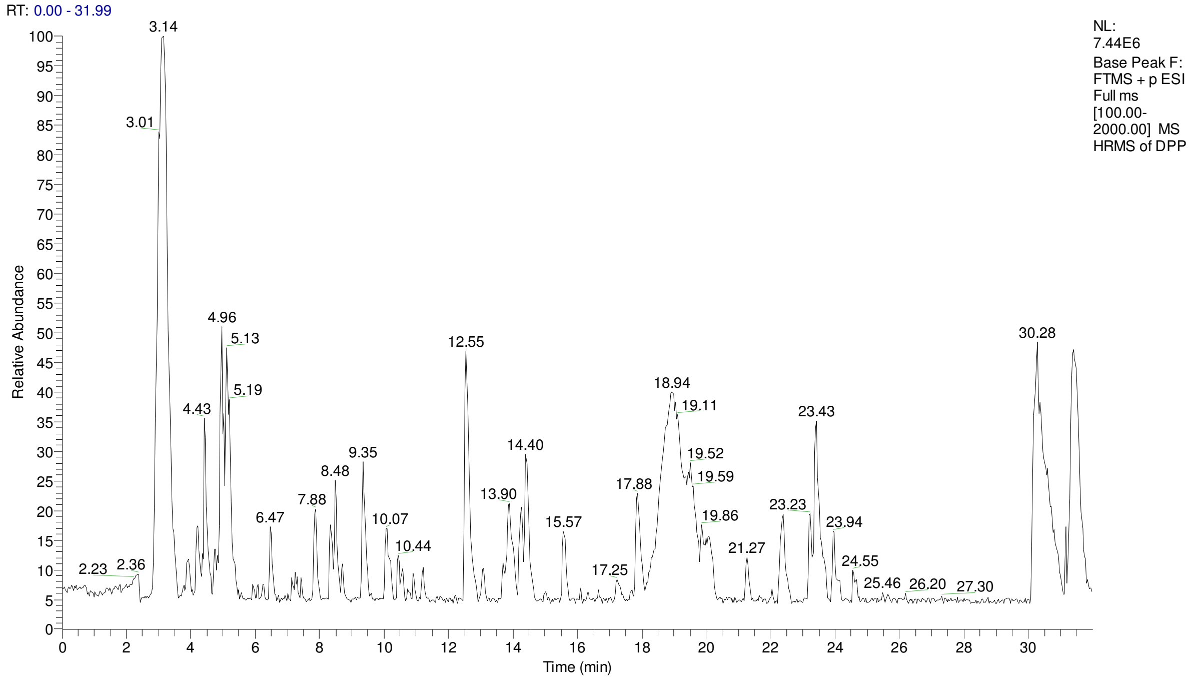
RT: 0.00 - 31.99
NL:
7.44E6
Base Peak F:
FTMS + p ESI
Full ms
[100.00-
2000.00] MS
HRMS of DPP
Relative Abundance
Time (min)
3.14
3.01
4.96
5.13
5.19
4.43
2.23
2.36
6.47
7.88
8.48
9.35
10.07
10.44
12.55
13.90
14.40
15.57
17.25
17.88
18.94
19.11
19.52
19.59
19.86
21.27
23.23
23.43
23.94
24.55
25.46
26.20
27.30
30.28

Fig. 5

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.

Parameters	Control	Isop	Isop + Clop	Isop + DPP
Body weight (g)	176.66 ± 10.44	173.66 ± 13.41	174.5 ± 13.18	199.88 ± 3.06$^{*\#@}$
Heart weigt (g)	0.82 ± 0.16	1.14 ± 0.17*	0.92 ± 0.09$^{\#}$	1.02 ± 0.14$^{*\#}$
Heart weight/body weight ratio	0.46 ± 0.08	0.65 ± 0.05*	0.54 ± 0.04$^{\#}$	0.51 ± 0.06$^{\#}$

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**.**

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

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.

Parameters	Control	Isop	Isop + Clop	Isop + DPP
Cholesterol (mmol/l)	1.57 ± 0.15	2.33 ± 0.22*	1.93 ± 0.16*#	1.85 ± 0.18*#
Triglycerides (mmol/l)	0.70 ± 0.09	0.99 ± 0.25*	0.81 ± 0.11*#	0.65 ± 0.039*#@

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.

HRESIMS [a]	Mol formula [a]	Suggested compound [b]	MS/MS fragments	Biological properties	Reference
413.3775	$C_{29}H_{48}O$	Stigmasterol (steroid)	396.3751, 353.3203, 338.2968, 257.2266	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.	Batta et al., 2006 Li et al., 2015
415.3931	$C_{29}H_{50}O$	β-Sitosterol (steroid)	398.3907, 355.3359, 340.3125, 257.2264	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.	Lei et al., 2015 Ganapathy et al., 2014
273.1844	$C_{18}H_{24}O_2$	Estradiol	256.1822,	Estrogen has been shown to increase expression of superoxide	Lagranha et al., 2010

		(steroid)	239.1794	dismutase and inhibit NADPH oxidase activity, thereby reducing oxidative stress Estrogen acting via ERβ increases protein S-nitrosylation, a common post-translational protein modification, leading to cardioprotection 17beta-estradiol downregulates tissue angiotensin-converting enzyme	Lin et al., 2009 Dean et al., 2005
457.4045	$C_{31}H_{52}O_2$	β-Sitosterol acetate (steroid)	414.3856, 398.3907	Similar effects to β-Sitosterol	Ganapathy et al., 2014 Lei et al., 2015
373.2327	$C_{21}H_{34}O_4$	2β,3β,4β-trihydroxypregn-16-one (steroid)	356.2322, 339.2295, 322.2267	No biological activity reported	Tan et al., 2010
324.9950	$C_{14}H_6O_8$	Ellagic acid (Tannin)	307.9927, 290.9905, 273.9873, 256.9845	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,	Kannan and Quine, 2013 Lin and Yin, 2013 Al Shukor et al., 2013
397.4049	$C_{26}H_{52}O_2$	Cerotic Acid (fatty acid)	380.4013	No biological activity reported.	
569.4355	$C_{40}H_{56}O_2$	Lutein (carotenoid derivative)	552.4321, 535.4301	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	Zou et al., 2011 Zou et al., 2014 Wang et al., 2013

				increasing plasma lutein concentrations and antioxidant capacity.	
397.3095	$C_{27}H_{46}O_2$	δ-tocotrienol (carotenoid derivative)	381.3152, 365.3208	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.	Wong et al., 2015 Qureshi et al., 2012
441.3209	$C_{30}H_{42}O$	8'-β-apocarotenol (carotenoid derivative)	424.3107	No biological activity reported. Precursor to Vitamin A	
583.45288	$C_{41}H_{58}O_2$	Taraxanthin (carotenoid derivative)	566.4485	No biological activity reported.	
317.1708	$C_{19}H_{24}O_4$	3, 5'-hydroxyprenyl-5-prenyl-p-coumaric acid (coumarin)	300.1720, 272.1771, 255.1743	No biological activity reported.	
217.1181	$C_{12}H_{18}O_2$	2,5-dimethoxy-p-cymene (terpenoid)	186.1015, 155.0831	No biological activity reported.	
261.1079	$C_{13}H_{18}O_4$	Trans-isomyristicin (terpenoid)	230.0917	No biological activity reported.	
413.1156	$C_{20}H_{22}O_8$	5-hydroxy-pentamethoxy-flavanone (Flavonoid)	396.1177, 365.0990, 334.0812, 303.0628	No biological activity reported.	
317.0658	$C_{16}H_{12}O_7$	Isorhamnetin (Flavonoid)	286.0472, 269.0445	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. Isorhamnetin Protects against Doxorubicin-Induced Cardiotoxicity *In Vivo* and *In Vitro*	Ibarra et al., 2002 Sun et al., 2013
291.0869	$C_{15}H_{14}O_6$	Catechin (Flavonoid)	274.0836, 258.0887	catechin is effective in reversing the impaired relaxation in restrictive cardiomyopathy myocardial cells and rescuing the restrictive	Zhang et al., 2015

				cardiomyopathy mice with diastolic dysfunction. catechin treatment prevents diabetes mellitus-induced vascular endothelial dysfunction. It also prevents of diabetic vascular endothelial dysfunction through reduction in high glucose, vascular oxidative stress, and lipid peroxidation.	Bhardwaj et al., 2014
287.0553	$C_{15}H_{10}O_6$	Luteolin (Flavonoid)	270.0520, 253.0495	Luteolin yields cardioprotective effects Luteolin pretreatment conveys anti-apoptotic effects after myocardial ischemia/reperfusion injury. Luteolin indicated potent in vitro ACE inhibitory activity with IC_{50} value of 23 μM.	Nai et al., 2015 Bian et al., 2015 Guerrero et al., 2012
301.1402	$C_{18}H_{20}O_4$	5,7,4'-trimethoxyflavane (Flavonoid)	270.1250, 239.1067, 208.0883	No biological activity reported.	
303.0499	$C_{15}H_{10}O_7$	Quercitin (Flavonoid)	286.0389	Quercitin provides cardiovascular protective effects Quercetin is a potent anti-atherosclerotic compound Quercitin indicated potent in vitro ACE inhibitory activity with IC_{50} value of 43 μM.	Malaguti et al., 2015 Hung et al., 2015 Guerrero et al., 2012
271.0601	$C_{15}H_{10}O_5$	Apigenin (Flavonoid)	254.0574	Apigenin attenuates myocardial ischemia/reperfusion Apigenin ameliorates acute myocardial infarction of rats Apigenin indicated potent in vitro ACE inhibitory activity with IC_{50} value of 196 μM.	Yang et al., 2015 Du et al., 2015 Guerrero et al., 2012
611.1608	$C_{27}H_{30}O_{16}$	Rutin (Flavonoid glycoside)	448.1009, 302.0427, 286.0472	Rutin have a cardioprotective effects in ischaemia-reperfusion injury in both normal and diabetic rats, and that protection might be in part due to the attenuation of oxidative stress and moderate increment in antioxidant reserves. Rutin indicated potent in vitro ACE inhibitory activity with IC_{50} value of 64 μM.	Annapurna et al., 2009 Guerrero et al., 2012
449.1075	$C_{21}H_{20}O_{11}$	Quercitrin (Flavonoid glycoside)	302.0421, 286.0472	Quercitrin have an inhibitory effect on the angiotensin-converting enzyme activity, similar to that of captopril.	Hackl et al., 2002
493.1345	$C_{23}H_{24}O_{12}$	Morifonoside A	331.0815	No biological activity reported.	

		(Flavonoid glycoside)			
449.1077	$C_{21}H_{20}O_{11}$	Luteolin-7-O-glucoside (Flavonoid glycoside)	286.0472	Protection of Luteolin-7-O-Glucoside Against Doxorubicin-induced cardiotoxicity. Luteolin -7-O-glucoside have an inhibitory effect on the angiotensin-converting enzyme	Yao et al., 2015 Simaratanamongkol et al., 2014
153.0543	$C_8H_8O_3$	Methyl-4-hydroxybenzoate (Phenolic derivative)	122.0362	safe food and cosmetic antibacterial and antifungal preservative.	Soni et al., 2002
179.0701	$C_{10}H_{10}O_3$	Methyl-p-hydroxycinnamate (Phenolic derivative)	148.0522	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.	Stanley Mainzen Prince and Jyoti Roy., 2013 Jyoti Roy and Stanley Mainzen Prince., 2013
195.0654	$C_{10}H_{10}O_4$	Ferulic Acid (Phenolic derivative)	178.0626, 147.0447	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.	Navarrete et al., 2015 Alam et al., 2013 Geng et al., 2010

[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).