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Pharmacological evaluation of *Vitis vinifera* and *Zingiber zerumbet* on electrocardiographic, biochemical alterations and antioxidant status in isoproterenol-induced myocardial infarction in rats

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Abstract: The *Vitis vinifera* (VV) and *Zingiber zerumbet* (ZZ) are popular functional foods which are used for the treatment of cardiovascular ailments. These possess antiproliferative, antiplatelet and antioxidant effects. The current study has been designed to ascertain their effectiveness against Isoproterenol (ISO)-induced myocardial infarction (MI). Chronic administration of VV and ZZ was accessed for its cardio-protective effect in ISO-induced MI rats. Male albino rats were treated with VV (250 mg/kg, p.o.), ZZ (200 mg/kg, p.o.) and its combination (*Vitis vinifera* + *Zingiber zerumbet)* VZ for 30 days prior to ISO administration (85 mg/kg, S/C). Electrocardiography (ECG) and Blood Pressure (BP) were measured using PowerLab data acquisition system. Biochemical serum markers, tissue histopathology and HPLC finger printing were performed. The VV, ZZ and its combination VZ showed significant protective effects on ST segment elevation, cardiac biomarkers; Troponin I (Trop I), creatine kinase-MB (CK-MB), alanine transaminase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), enhanced the cardiac antioxidant defense system, restored the hematological (WBCs, RBCs, Platelets) & coagulation parameters and improved the lipid profile and histopathological alterations such as tissue necrosis, infiltration and edema which were observed only in ISO administered rats. These results indicate that *V. vinifera* and *Z. zerumbet* possess cardio protective effects possibly mediated through maintenance of endogenous antioxidant levels, cardiac biomarkers and lipid parameters.

Keywords: Isoproterenol, myocardial infarction, antioxidant, cardio-protective, necrosis.

INTRODUCTION

Cardiovascular diseases (CVDs) are one of the prevailing contributors of death throughout the world. About 23.6 million population will be affected with CVDs at the end of 2030 according to WHO statistics. Among complications of CVDs, MI is major alarming one occurring due to variations among oxygen demand of myocardial tissue and blood supply to cardiac tissue. The major risk factors for myocardial infarction are hypertension, smoking, obesity, hypercholesterolemia and diabetes mellitus (Touloumi *et al*., 2020).

Isoproterenol (ISO), a selective beta-adrenergic agonist, is one of the main precursor of myocardial oxidative stress which causes necrosis along with multiple complications such as hyperlipidemia, elevated cardiac markers and hyperglycemia. Auto-oxidation of ISO initiates the free radical production. Therapeutic approaches involving antioxidants might be useful against oxidative stress in multiple CVDs including MI. In current era, a considerable focus is being done on "health-promoting antioxidants" which are natural in origin and regularly used in our daily nutrition. Natural and herbal supplements provide economical and harmless substitutes for therapeutic use as well as for prophylactic measures (Liperoti *et al.,* 2017).

Among natural plants, the grapevine (*Vitis vinifera*), along with the common name black grape, belongs to the family *Vitaceae* and is native to Western Asia and Southern Europe. Traditionally, dried fruits of VV were used to treat stomachic, cough, hoarseness and act as laxative & demulcent. It possesses a wide range of anthocyanins, resveratrol, phenolic acids, catechins and procyanidins which demonstrated cardioprotective effects and reduced CVS mortality. Flavonoids, potent phytochemical and vital nutraceutical possess antioxidant, anti-inflammatory (Magrone *et al*., 2019), anticancer (Meo *et al*., 2019) and antibacterial properties. A study has shown the beneficial effects of purple grapes on CVS by inhibiting thrombus formation (Oscar *et al*., 2021). The skin of black and red grapes contains high amount of resveratrol, one of the most potent natural antioxidant was found to improve myocardial reperfusion injury by decreasing necrotic and apoptotic cell death (Kimble *et al.,* 2019).

Zingiber zerumbet (Linnaeus) Smith, commonly known as bitter ginger, belong to family Zingiberaceae,

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indigenous to the Malaysia, tropics of Asia and South-East Asia and is used as an edible ginger. Fresh rhizomes were used traditionally as an anti-inflammatory, anti-rheumatic, antipyretic and to cure edema in Mediterranean regions. This flavoring food is also helpful to relieve headache and treat high cholesterol levels. Phytochemical screening revealed the presence of several active constituents, like zerumbone, kaempferol and quercetin as plant constituents (Kalantari *et al.,* 2017). The most potent constituent, zerumbone possesses diverse pharmacological activity including antibacterial, anti-inflammatory (Somchit *et al.,* 2012), antiplatelet (Jantan *et al.,* 2005), antioxidants (Hamdi *et al.*, 2015), anti-atherogenic (Hemn *et al*., 2015) and anti-proliferative activities (Al-Zubairi, 2018).

Antioxidants impart a variety of beneficial metabolic functions in appropriate combinations such as free radical scavengers and defensive actions. The hydrophilic and lipophilic combination of antioxidants showed synergistic effects in previous studies (Huwait, 2019). Considering the antioxidant potential of VV and ZZ, it was supposed that these plants could elicit cardioprotective actions and reduce myocardial damage induced by ROS and free radicals. Because of popular utility and valuable pharmacological profile, we designed this study to investigate the potential of Black grapes and Bitter ginger as cardio protective agents in animal model of isoproterenol (ISO)-induced myocardial infarction (MI) and evaluate their combined effects on hemodynamic, electrocardiographic and biochemical parameters.

MATERIALS AND METHODS

Preparation of plant extracts

The *Vitis vinifera* and *Zingiber zerumbet* were purchased from local market and authenticated by a Taxonomist "Dr. Mansoor Hameed" of Department of Botany, University of Agriculture Faisalabad and samples were deposited in herbarium against voucher no. 224-21-06 and 8421-01.

Preparation of plant extracts were made by macerating 1500 g of grape berries and 1200 g of dried ginger powder in methanol and water (70:30) for 5 days with occasional shaking. After filtration, extract was concentrated through rotary evaporator under reduced pressure at 40C°. The weight of final extracts of grapes and ginger were 250 g and 210 g respectively. The percentage yield was calculated. The extraction yield of grapes (*Vitis vinifera*) and ginger (*Zingiber zerumbet*) were 16.66% and 17.5%, respectively.

In-vitro determination of TPC, TFC and DPPH activity

The measurement of total phenolic contents (TPC), total flavonoid contents (TFC) and DPPH radical scavenging activity was assessed as previously described in literature (Fuad *et al.,* 2020).

HPLC analysis of methanolic extracts of Vitis vinifera and Zingiber zerumbet

High performance liquid chromatography (HPLC) was performed to segregate the flavonoids and phenolic acids along with vitamins from samples. The sample was run through HPLC equipped with UV-Vis detector (SPD-10.AV, Shimadzu, Japan) and pump (LC-10AT, Shimadzu, Japan). A C18 phase column of width 250 × 4.6 mm2, film thickness 5 µm, was employed for quantification and analysis. The composition of mobile phase for flavonoids consists of Methanol: H2O (50:50) + 2 ml acetic acid while mobile phase for ZZ comprise of solution A (H_2O: Acetic acid-94:6) and solution B (Acetonitrile 100%) with a flow rate of 1ml/min. the peaks were measured at 280 nm through a detector and Peaks and retention times of sample were compared with standard to identify analytes.

Animal procurement and maintenance

The study was carried out on male albino Wistar rats of 200-300 g. The rats were restrained in plastic cages at controlled conditions of temperature and humidity, 25-28˚C and 50%, respectively with 12 h light and dark cycles. During study, rats were fed standard pellet diet along with water *ad libitum*. Ethical approval for study was obtained from Institutional Review Board of GCUF having reference No. GCUF/IRB/759.

Experimental design and protocol

Isoproterenol (85 mg/kg, s.c. at 24 h interval) dissolved in normal saline for two successive days was utilized to induce myocardial infarction (Goyal *et al*., 2010). Forty two rats were distributed randomly into six groups which comprise of seven animals in each group.

Group 1: termed as Normal control group (NC), received standard pellet diet and water for 30 days , Group 2 (ISO group): animals were treated with standard pellet diet and water for 30 days, along with ISO (85 mg/kg, sc) on 29th and 30th day at an interval of 24hr .Group 3, 4, 5 & 6 designated as standard group (STD group), Test group 1 (TG1), Test group 2 (TG2), Test group 3 (TG3), receiving Metoprolol (10 mg/kg/day, po), VV extract (250 mg/kg/day, p.o.), ZZ extract (200 mg/kg/day, p.o.) and VZ extract (VV, 250 mg/kg/day + ZZ, 200 mg/kg/day p.o.) respectively, for 30 days prior to ISO administration (85 mg/kg, sc, at 24 hr interval) on 29th and 30th day.

Electrocardiography (ECG) & Blood Pressure (B.P.) measurement

The recording of ECG was measured at 24 hr after second injection of ISO on 30th day by PowerLab Data Acquisition System (AD Instruments, Australia) connected to a computer enabled with LabChart professional software version 8 (AD Instruments, Australia). The rats were anaesthetized under mild anesthesia. After 15 min of anesthesia, electrodes were implanted under skin of all animals for standard limb lead

II recording. Heart rate, ST segment, P wave, QT and R-R intervals were calculated from ECG recordings.

At start of study, rats were trained a week before monitoring of blood pressure. Systolic diastolic and mean blood pressure were measured at the end of study under minimal stress and strain by using tail cuff equipped with PowerLab by AD instrument attached to Lab Chart computer software (Li *et al.,* 2020).

Biochemical investigation

The animals were sacrificed after measurement of B.P. & ECG and collection of blood samples from each group was done. The serum was separated after centrifugation which was employed for further analysis.

Heart to Body weight ratio calculation

Heart weight to body weight ratio was calculated according to formula (Goyal *et al.,* 2010).

$$Heart\ to\ Body\ weight\ ratio = \frac{Heart\ weight\ (g)}{Body\ weight} \times 100$$

Assessment of cardiac biomarkers & anti-oxidants

Cardiac biomarkers such as Trop I, CK-MB, LDH, ALT and AST were estimated from collected serum using BECKMAN COULTER USA and Routine Chemistry Analyzer (DXC- 700 and AU-480) (Lippi et al., 2018). Serum level of enzymes CAT, SOD and GPx were measured according to method described in literature (Katerji *et al.,* 2019).

Assessment of coagulation parameters

The prothrombin time (PT) and activated partial thromboplastin time (APTT) were analyzed from plasma by using Coagulation analyzer Humaclot Pro Analyzer (HUMAN, Germany) (Araya *et al.,* 2021)

Assessment of serum biochemical parameters

The serum lipid profile including total cholesterol (TC), triglycerides (TG), HDL and LDL cholesterol were analyzed using BECHMAN COULTER (DXC-700AU) chemistry analyzer (Yong and Young, 2017).

Hematological parameters such as White Blood cells (WBCs), Red Blood Cells (RBCs), and Platelets were estimated by hematology analyzers (Sysmex KX-21, Japan and Abacus 380 Diatron) (Burr *et al.,* 1992). Blood glucose level was determined using diagnosis reagent kit (DiaSys Diagnostic Systems GmbH, Germany) (Husni *et al*., 2016).

Histopathological evaluation

The rat heart was immediately dissected out after sacrifice and fixed in 10% buffered formalin after washing. The fixed cardiac tissues were prepared for paraffin embedding and almost 5 µm thick sections were separated after cutting and staining with hematoxylin and eosin (H&E). The tissue sections were mounted and examined at 40X under light microscope (Sultana *et al*., 2019).

STATISTICAL ANALYSIS

All parameters were measured in triplicate and values are presented as mean ± SEM. The Statistical analysis was performed using Minitab 21 and GraphPad Prism (Version 7.0. San Diego, USA). Data was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's post-test. The significance level was expressed as $p < 0.05$.

RESULTS

Quantitative phytochemical analysis

Results showed that *Vitis vinifera* possessed more phenolic contents (96.8 mg GAE/g) than *Zingiber zerumbet* (37.8 mg GAE/g), while flavonoid contents of *Zingiber zerumbet* (71.71 55 mg QE/g) were in excess than *Vitis vinifera* (68.55 mg QE/g). The *Vitis vinifera* showed significantly higher (87.69%) DPPH activity than *Zingiber zerumbet* (40.80%).

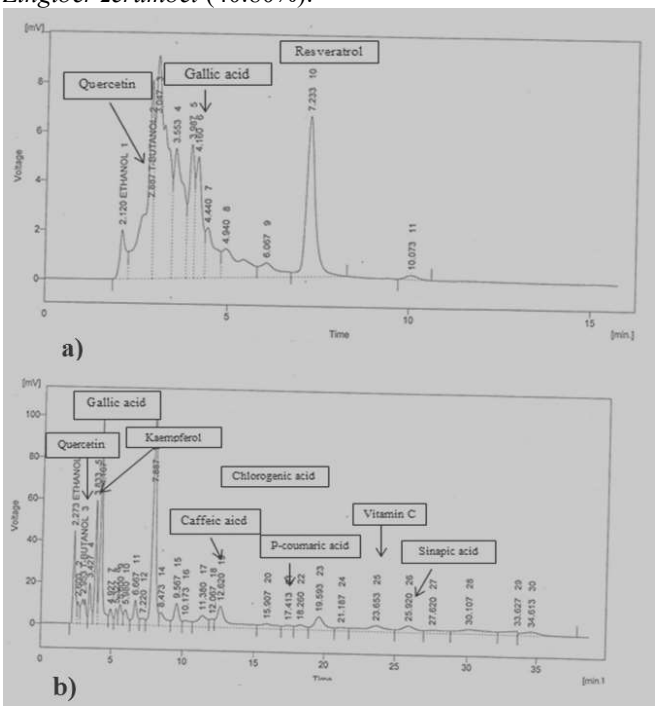
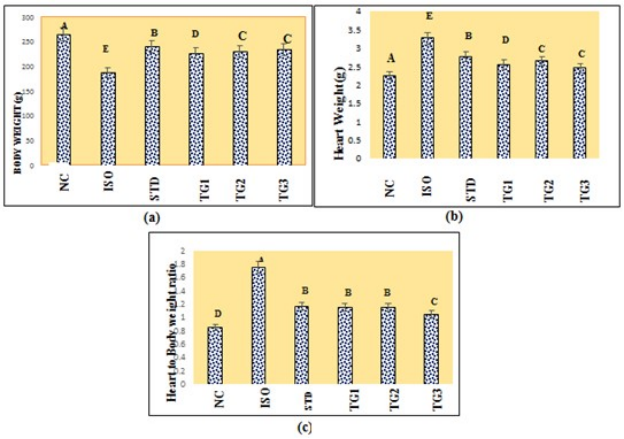


Fig. 1: HPLC analysis of a) *V. vinifera;* b) *Z. zerumbet*

HPLC-based quantification

The HPLC analysis revealed that *Vitis vinifera* possessed important bioactive compounds like quercetin (15.53 ppm), gallic acid (41.55 ppm), resveratrol (4.89 ppm) while *Zingiber Zerumbet* contain kaempferol (132.31 ppm), Vitamin C (17.94 ppm), Quercetin (15.53ppm), Gallic acid (41. 55 ppm), Caffeic acid (25.30 ppm), Chlorogenic acid (14.51 ppm), P-coumaric acid (1.14 ppm) and Sinapic acid (3.11 ppm) (fig. 1, table 1).



Varying alphabets in different bars show that data is significantly different (P<0.05) when compared with disease control whereas NC: Normal control; ISO: isoproterenol treated; STD: Metoprolol; TGI: VV+ISO; TG2: ZZ+ISO; TG3: VV+ZZ+ISO

Fig. 2: Effect of VV and ZZ on a) Heart weight b) Body Weight c) Heart to Body weight Ratio

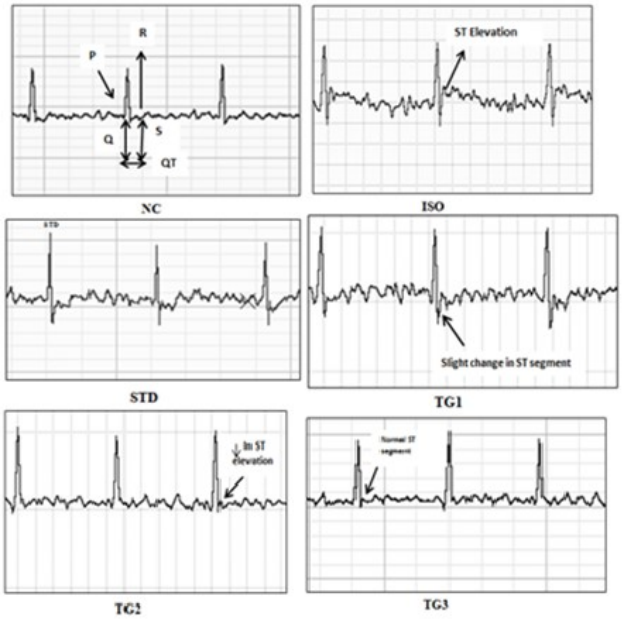
Effects on Heart to Body weight ratio

Fig. 2 presents the effect of ISO administration and treatment groups on heart weight, body weight and heart weight (wt) to body weight (wt) ratio. The heart weight and the ratio between heart to body weight was significantly enhanced (*P*< 0.05) in ISO administered rats as compared to normal rats. The STD group significantly increased body weight and decreased heart to body weight ratio as compared to ISO treated group. No significant difference (*P*>0.05) was observed in rats pretreated with VV and ZZ alone, but the co treatment of both plants significantly enhance heart to body weight ratio in comparison to control group.

Effects on ECG changes and Heart rate

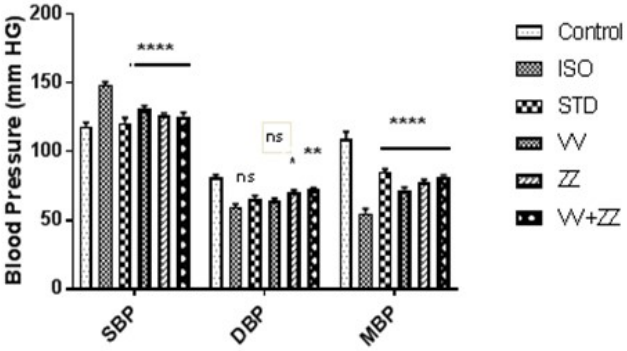
Electrocardiographic pattern of normal and ISO treated animal is presented in fig. 3 and table 2. The NC and STD group revealed normal ECG pattern, whereas animals in ISO administered group exhibited a significant (P<0.05) ST elevation and reduction in R-R interval in comparison to NC, which is an indication of infarcted myocardium. A significant (P<0.05) reduction in P wave, QRS complex along with significant rise in heart rate was observed in ISO-administered rats in comparison to NC rats.

Vitis vinifera or *Zingiber zerumbet* treatment in ISO treated rats (VV + ISO or ZZ + ISO) produced a significant (*P*<0.05) reduction in ST segment and QT interval along with subsequent rise in QRS complex and RR interval in comparison to ISO treated rats. The VV and ZZ co-administration in ISO treated rats (VV + ZZ + ISO) decreased ST segment significantly (*P*<0.05) and enhanced P wave, QRS complex and RR interval significantly (*P*<0.05) in comparison to ISO, VV + ISO or ZZ + ISO injected rats. Heart rate did not alter significantly (*P*>0.05) in NC and ISO injected rats.



Whereas NC: Normal control; ISO: isoproterenol treated; STD: Metoprolol; TG1: VV + ISO; TG2: ZZ + ISO; TG3: VV + ZZ + ISO

Fig. 3: Effects of VV and ZZ on ISO-induced electrocardiographic changes.



Comparison is made between normal control, Isoproterenol group (ISO) and treatment groups (i.e. TG1: VV (250 mg/kg/day, p.o) + ISO: ZZ (200 mg/kg/day, p.o) + ISO; TG3 VV (250mg/kg/day, +ZZ (200 mg/kg/day,p.o) + ISO. Results are presented as mean ± SEM (n=6) and analyzed by one-way ANOVA followed by Bonferroni's post-test. Ns, non-significant; ***p<0.001, **p<0.01 and *p<0.05 as compared to the disease control.

Fig. 4: Effect of VV and ZZ on Blood Pressure

Effects on systolic, diastolic and mean blood pressure (SBP, DBP and MBP)

The Diastolic blood pressure and Mean blood pressure were reduced significantly (*P*<0.05) in ISO administered rats when compared with NC rats. The VV+ISO, ZZ+ISO and VV+ZZ+ISO treatment slightly improved the DBP in comparison to only ISO injected rats. However, a significant improvement (*P*<0.05) in MBP has been observed in combination (VZ) group in comparison to ISO administered rats (fig. 4).

Table 1: HPLC analysis of VV and ZZ methanolic extracts

	Compound name	Retention time (min)	Area (mV.s)	Area [%]	Ppm
Vitis Vinifera	Quercetin	2.887	112.58	17.0	15.53
	Gallic acid	4.160	62.018	9.4	41.55
	Resveratrol	7.233	112.707	17.0	4.89
Zingiber Zerumbet	Compound name	Retention time (min)	Area (mV.s)	Area [%]	Ppm
	Quercetin	2.953	293.34	3.3	15.53
	kaempferol	3.833	735.104	31.114	132.31
	Gallic acid	4.167	1154.80	13.0	41.55
	Caffeic acid	12.620	550.396	6.2	25.30
	Chlorogenic acid	15.907	186.210	2.1	14.51
	P-coumaric acid	17.413	87.354	1.0	1.14
	Vitamin C	23.653	338.365	3.8	17.94
	Sinapic acid	25.920	239.75	2.7	3.11

Table 2: Effects of VV and *ZZ* on ECG parameters, Cardiac biomarkers and antioxidants

Parameters	Group 1 Control	Group 2 ISO	Group 3 STD	Group 4 TG1	Group 5 TG2	Group 6 TG3
ECG changes and heart rate						
ST (mv)	0.073 ± 0.482^{D}	0.303 ± 0.872^{A}	0.302 ± 0.042^{A}	0.181 ± 0.983^{B}	0.142 ± 1.032^{C}	0.133 ± 1.322^{C}
QRS Complex (sec)	0.042 ± 0.532^{A}	0.026 ± 0.872^{D}	0.029 ± 0.672^{D}	0.032 ± 1.032^{C}	0.037 ± 1.045^{C}	0.040 ± 0.893^{B}
P wave (sec)	0.030 ± 0.943^{A}	0.018 ± 0.832^{D}	0.017 ± 0.742^{D}	0.020 ± 0.643^{C}	0.022 ± 0.743^{C}	0.025 ± 0.829^{B}
R-R interval (sec)	0.230 ± 0.872^{B}	0.141 ± 0.642^{E}	0.256 ± 0.482^{A}	0.160 ± 1.492^{D}	0.177 ± 0.733^{D}	0.190 ± 0.393^{C}
Heart Rate (bpm)	241.5 ± 0.643^{F}	370.6 ± 0.743^{A}	263.8 ± 0.742	280.1 ± 0.853^{C}	320.7 ± 0.643^{B}	275.6 ± 1.022^{D}
Cardiac biomarkers and antioxidants						
cTnI (ng/ml)	7.2 ± 0.583^{D}	16.6 ± 1.492^{A}	10.9 ± 0.643^{C}	13.0 ± 0.643^{B}	12.1 ± 0.722^{B}	11.8 ± 1.032^{C}
CK-MB (IU/L)	790.9 ± 1.042^{F}	1171.2 ± 1.103^{A}	803.0 ± 0.933^{E}	849.8 ± 0.943^{B}	815.6 ± 0.744^{D}	830.04 ± 0.892^{C}
LDH (IU/L)	294.5 ± 0.933^{F}	564.7 ± 0.853^{A}	298.6 ± 0.984^{E}	376.9 ± 0.643^{B}	351.8 ± 0.492^{C}	333.6 ± 0.853^{D}
Catalase (Pmol/mg of protein)	80.18 ± 0.643^{A}	33.00 ± 0.743^{E}	57.20 ± 0.742^{D}	63.53 ± 0.853^{C}	66.91 ± 0.643^{C}	71.73 ± 1.022^{B}
SOD (U/mg of protein)	31.30 ± 0.853^{A}	10.71 ± 0.643^{D}	23.90 ± 1.022^{B}	16.89 ± 0.754^{C}	18.34 ± 0.854^{C}	21.89 ± 0.492^{B}
Gpx (nmol/min/mg of protein)	29.1 ± 0.583^{A}	9.0 ± 0.393^{E}	25.0 ± 1.032^{B}	21.8 ± 0.829^{C}	19.6 ± 0.782^{D}	23.9 ± 0.893^{C}

Values are expressed as mean ± SE (n = 6);. Varying alphabets in different columns shows that data is significantly different ($p < 0.05$) when compared with disease control

Effects on cardiac biomarkers and antioxidants

Table 2 indicates the cardiac enzymes (Trop I, CK-MB, LDH) activities were significantly ($P<0.05$) increased in ISO treated group in comparison to control rats. The VV, ZZ and VZ pretreatment in ISO treated animals significantly ($P<0.05$) declined the Trop I, CK-MB and LDH level. There was a significant ($P<0.05$) decrease in antioxidant enzymes (SOD, CAT, GPx) in ISO administered animals. The Pre co-treatment of VV, ZZ and VZ for 30 days restored the values of antioxidants in comparison to NC. The STD group normalizes the ISO induced changes in animals.

Effects on lipid profile

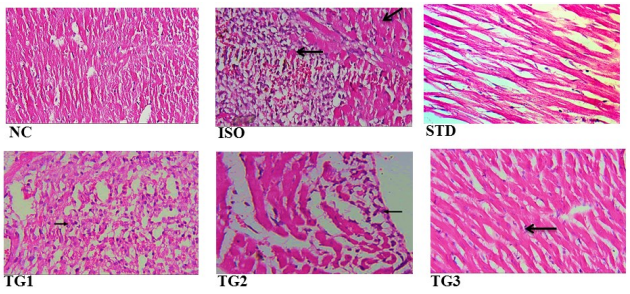
A significant increase in TC, TG, LDL and decrease in HDL level was observed in ISO injected rats as shown in table 3. The prior administration of *VV* and *ZZ* for 30 days along with ISO administration on 29th and 30th day caused a marked ($P<0.05$) reduction in TG, TC and LDL level with a significant increase in HDL. No significant change has been observed on lipid profile in STD group.

Effects on Hematological parameters

Table 3 represents the effect of ISO, VV and ZZ on WBCs, RBCs, Hb and Platelets, in which all hematological parameters were, increased significantly ($P < 0.05$) in ISO injected animals in comparison to NC. The treatment group restored the ISO induced changes in hematological parameters. A significant ($P<0.05$) reduction in WBCs and Platelets has been observed after the co-administration of VV and ZZ for 30 days along with ISO administration on 29th and 30th day. The combination VZ also restored the values of RBCs and Hb. The STD did not have significant effect on RBCs and Hb.

Effects on Plasma coagulation parameters, Renal Function Tests (RFTs)* and *Liver Function Tests (LFTs)
Isoproterenol significantly (p<0.05) decreased Plasma PT and APTT and increased LFTs and RFTs as compared to NC (table 3). Pretreatment with VV, ZZ, its combination and metoprolol significantly (p<0.05) prolonged the PT and APTT, and preserved blood glucose, LFTs and RFTs in comparison to ISO treated group (table 3). The prolonged effect of combined treatment with VV and ZZ was more significant (p<0.05) than the separate treatments.

Effects on Histopathology
Histopathological study showed that ISO induces myocardial necrosis, infiltration and edema in cardiac tissues. However, VV, ZZ and VZ pretreatment significantly reduction (P<0.05) in the incidence of cardiac infiltration, necrosis and edema and restored the pathological changes (fig. 5).



NS) Normal cardiomyocytes ISO) Confluent necrosis and degenerative changes along with cellular infiltration and swollen myoctes in isoproterenol treated group; STD) Insignificant confluent necrosis and edema are seen in metoprolol (10 mg kg-1) treated group; TG1) Partial suppression of diffuse confluent necrosis and edema are seen in TG1 TG2) Treatment group showing relatively lesser degree of confluent necrosis and decreased infiltration of inflammatory cells; TG3) Treatment group showing well preserved cardiac muscle fiber without any evidence of focal necrosis.

Fig. 5: Effects of *V. vinifera* and *Z. zerumbet* (alone and in combination) on isoproterenol-induced histo-pathological (H & E stained, at 40×) changes in cardiac tissues

DISCUSSION

The presence of polyphenols was quantified through HPLC analysis of methanolic extract of both *Vitis vinifera* and *Zinigiber zerumbet* which confirmed the existence of gallic acid, sinapic acid, cholorgenic acid, quercetin and vitamin C. Phenols possess anti-inflammatory effects. Quercetin inhibits the production of inflammatory mediators such as TNF-α and Nitric oxide. Gallic acid and sinapic acid also produce anti-inflammatory effects while chlorogenic acid acts immunoprotective and antibacterial. The findings of HPLC analysis current study are in agreement with previous HPLC results (Nag *et al.,* 2013).

The beneficial effects of several CVS drugs including antioxidants have been evaluated through a standardized model of isoproterenol-induced myocardial infarction. ISO, a non-selective beta blocker and synthetic catecholamine is used to produce MI through ROS production which ultimately leads to oxidative stress (Patel *et al*., 2010).

The heart weight increased significantly after ISO administration which may lead towards increase in heart to body weight ratio. Multiple factors contribute to increase in heart weight such as presence of inflammatory cells in necrotic areas and fluid accumulation in intramuscular space. Previous literature supports these results (Abbas, 2016). Pretreatment with VV, ZZ and VZ significantly declined the heart weight and heart to body weight ratio. Combined treatment with VZ was more effective than pretreatment with either VV or ZZ.

The ECG is valuable tool to investigate heart functioning in normal and diseased states. The cardiac necrosis produced by ISO causes elevation of ST segment and decreased R wave amplitude, P wave intensity and QRS complex. The abnormal ECG parameters might be due to successive cell membrane loss in injured cardiac cell. The ST segment elevation reveals the difference in electric potential between ischemic and non-ischemic zones while decreased R wave amplitude might be linked with onset of cardiac edema. The changes in ECG pattern by ISO were in line as previously described by Patel *et al*. (2010). Administration of VV or ZZ reduced the rise in ST segment and increase R wave amplitude induced by ISO. The combined effect was more pronounced due to an additive action (VV+ZZ).

The isoproterenol increases heart rate which might be due to insufficient blood supply to heart and positive chronotropic effects. The increased heart rate demands for higher oxygen consumption which may lead towards ischemia. Pretreatment with VZ bring down the heart rate in ISO treated rats which is supported by previous studies (Abbas, 2016).

In current study, ISO administered rats exhibited MI as evident by significant decrease in DBP and MBP. Activation of sympathetic nervous system and systemic vasodilation bring about these hemodynamic changes. The decrease in BP is in accordance with previous studies (Goyal *et al.,* 2010). Supplementation of VV and ZZ alone and its combination, VZ in ISO treated rats attenuated significantly these hemodynamic alterations and improved SBP, DBP and MAP.

Cardiac troponin I (cTnI) is a highly selective and sensitive diagnostic biomarker of MI. Increased levels of cTnI can predict the cardiac ischemia and subsequent cardiac death (Abbas, 2016). The ISO treated rats showed

Table 3: Effects of VV and *ZZ* on hematological and biochemical variables

Physical Parameters	Group 1 Control	Group 2 ISO	Group 3 STD	Group 4 TG1	Group 5 TG2	Group 6 TG3
Lipid Profile (mg/dL)						
TG (mg/dl)	77.9 ± 0.722^{E}	158.5 ± 0.983^{A}	140.2 ± 0.843^{B}	107.7 ± 1.032^{C}	104.5 ± 1.492^{C}	96.2 ± 0.854^{D}
TC (mg/dl)	60.42 ± 0.583^{E}	133.69 ± 1.492^{A}	127.03 ± 0.643^{B}	88.20 ± 0.643^{D}	92.90 ± 0.722^{C}	80.57 ± 0.853^{D}
HDL (mg/dl)	50.3 ± 0.722^{A}	20.0 ± 0.983^{E}	28.1 ± 0.843^{D}	39.1 ± 1.032^{C}	41.7 ± 1.492^{C}	45.2 ± 0.854^{B}
LDL (mg/dl)	47.6 ± 0.873^{F}	98.1 ± 0.872^{A}	75.0 ± 1.832^{B}	62.6 ± 0.103^{D}	68.8 ± 1.422^{C}	57.1 ± 1.053^{E}
Hematological Parameters						
WBCs ($10^3/\mu L$)	9.1 ± 0.482^{E}	15.5 ± 0.642^{A}	9.9 ± 0.048^{E}	12.4 ± 0.832^{C}	13.5 ± 0.853^{B}	10.9 ± 0.872^{D}
RBC ($10^6/\mu L$)	7.9 ± 0.583^{E}	12.2 ± 1.492^{B}	13.6 ± 0.643^{A}	11.0 ± 0.643^{C}	11.9 ± 0.722^{C}	10.9 ± 1.032^{D}
Hb (g/dL)	14.8 ± 0.873^{C}	18.1 ± 0.872^{A}	17.0 ± 1.832^{B}	12.3 ± 0.103^{E}	11.6 ± 1.422^{F}	13.1 ± 1.053^{D}
Platelets ($10^3/\mu L$)	684.6 ± 0.583^{F}	773.8 ± 0.393^{A}	709.9 ± 1.032^{D}	748.8 ± 0.829^{B}	723.8 ± 0.782^{C}	707.7 ± 0.893^{E}
Plasma Coagulation Parameters						
PT (sec)	12.91 ± 1.492^{A}	8.78 ± 0.733^{C}	12.68 ± 1.322^{A}	11.63 ± 0.854^{B}	11.10 ± 0.593^{B}	12.30 ± 0.744^{A}
APTT (sec)	16.78 ± 0.984^{A}	10.40 ± 1.053^{D}	13.90 ± 0.953^{B}	12.80 ± 0.854^{C}	11.61 ± 0.893^{C}	13.43 ± 0.833^{B}
RFTs						
Urea (mg/dl)	16.31 ± 0.532^{E}	55.86 ± 0.872^{A}	45.28 ± 0.672^{B}	28.85 ± 1.032^{D}	32.95 ± 1.045^{C}	24.88 ± 0.893^{D}
Glucose (mg/dl)	79.33 ± 0.832^{F}	278.5 ± 0.103^{A}	275.3 ± 0.833^{B}	256.8 ± 1.032^{C}	231.6 ± 1.302^{D}	219.16 ± 1.021^{E}
LFTs						
ALT (U/L)	29.46 ± 0.733^{E}	91.13 ± 1.322^{A}	81.22 ± 0.743^{B}	69.16 ± 0.742^{C}	64.55 ± 1.045^{D}	60.32 ± 1.022^{D}
AST (U/L)	102.1 ± 0.872^{F}	178.2 ± 0.642^{A}	167.7 ± 0.482^{B}	153.6 ± 1.492^{C}	149.5 ± 0.733^{D}	140.9 ± 0.393^{E}
Bilirubin (mg/dl)	0.05 ± 0.943^{D}	0.50 ± 0.832^{A}	0.48 ± 0.742^{B}	0.06 ± 0.643^{C}	0.05 ± 0.743^{D}	0.06 ± 0.829^{C}

Values are expressed as mean ± SE (n = 6); Varying alphabets in different columns and rows show that data is significantly different ($p < 0.05$) when compared with disease control

increase level of cTn1. These findings are supported by previous evidences (Subbaiah *et al*., 2017). The pre-co treatment with VZ reduced serum cTn1 in comparison to ISO treated rats.

Several diagnostic markers are present in heart which are indicators of MI, when it undergo degradation, it will releases cardiac contents into extracellular fluid (Ansari *et al.*, 2019). The serum CK-MB is another important diagnostic indicator of MI. Cytosolic enzymes such as LDH, ALT, AST & CK-MB, discharge from necrotic tissue to blood after membrane permeability is disrupted (Ansari *et al.,* 2019). ISO significantly enhanced the concentrations of these enzymes (LDH, ALT, AST & CK-MB) within serum which were supported by previous reports and gives a clear indication of cardiac damage and leakiness of cell membrane. Pre-treatment with VZ significantly reduced the level of serum markers, suggesting that combined effect was more effective than alone VV and ZZ in maintaining the membrane integrity and preventing enzyme discharge.

Free radical scavengers like SOD, CAT and GPx act as first line antioxidant enzymes of cellular defense against oxidative damage and reduce ROS. ISO administration produced a marked reduction in antioxidant enzymes (SOD, CAT, GPx) in current investigation. Pretreatment with VV and ZZ restored the levels of antioxidant enzymes (SOD, CAT. GPx). It might be due to direct radical scavenging effect of resveratrol and zerumbone. Indirect effect might be due to its ability to enhance the antioxidant role which was supported by previous studies (Adam *et al.,* 2016; Nazeih *et al*., 2020).

A marked elevation in TC and TG has been detected in ISO administered rats that might be due to increased flux of fatty acids and lipid biosynthesis from cardiac cAMP, reduced VLDL removal from plasma and decreased lipoprotein lipase activity. ISO administered rats showed decrease in HDL along with increase in LDL, TC & TG. This is in line with previous studies in which grapes lower TC, TG and LDL in hypercholesterimic wistar rats (Devi, 2017)

In ISO treated rats, there is increase in WBCs, RBCs, Platelets and Hb values. These findings are consistent with previous literature as ISO causes a significant increase in RBC, hemoglobin and hematocrit values as compared to control and defined that ISO produces a significant increase in platelet count. VV and ZZ treatment reversed the alteration in hematological parameters induced by ISO which was supported by previous study (Hasona *et al*., 2019). The ZZ has less effect on hematological parameters as compared to VV. The previous report also support these findings in which ZZ rhizomes did not show significant effect on hematological parameters in acute and sub-acute toxicity studies (Chang *et al*., 2012).

The human plasma coagulation parameters Prothrombin time (PT) and APTT have been reduced in ISO treated rats which is in accordance with previous studies in which clotting and bleeding time were significantly reduced after ISO treatment that might be due to activation of beta adrenergic receptors (Kristensen *et al.,* 1988). The VV and ZZ co-treatment significantly enhance the PT and

APTT and provide synergistic effect. The previous literature demonstrated that grapes and ginger significantly prolonged the PT and APTT in comparison to control group (Marx *et al.,* 2015; Bijak *et al*., 2019).

Isoproterenol treated animals exhibited increase in level of urea. The co treatment of VV, ZZ and VZ suppressed the ISO induced alterations in renal profile as supported by previous studies and provide synergistic effect. ISO enhances serum glucose level by stimulation of glycogenolysis and cause insulin resistance (Hoff and Koh, 2018). In current investigation, ISO increase blood glucose level but co treatment with VV, ZZ and VZ restored the blood glucose level within normal range supported by previous studies (Tzeng *et al.,* 2013; Bao *et al.,* 2015).

CONCLUSION

Current investigation demonstrates that *Vitis vinifera* and *Zingiber zerumbet* possess cardio protective effects and preserve the infarcted myocardium possibly through increase of endogenous antioxidant levels (Catalase peroxidase, Superoxide dismutase, Glutathione peroxidase), decrease of cardiac biomarkers (Troponin I, Creatinine Kinase-MB, Lactase dehydrogenase) and lipid parameters (TG, TC, LDL along with increase in high density lipoproteins. The results of current study suggest that *Vitis vinifera* and *Zingiber zerubmet* can be used as an adjunct therapy for MI prophylaxis.

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REFERENCES

Abbas AM (2016). Cardioprotective effect of resveratrol analogue isorhapontigenin versus omega-3 fatty acids in isoproterenol-induced myocardial infarction in rats. *J. Physiol. Biochem*., **72**(3): 469-84.

Adam SH, Giribabu N, Kassim N, Kumar KE, Brahmayya M, Arya A and Salleh N (2016). Protective effect of aqueous seed extract of *Vitis vinifera* against oxidative stress, inflammation and apoptosis in the pancreas of adult male rats with diabetes mellitus. *Biomed. Pharmacother.,* **81**(5): 439-452.

Al-Zubairi A (2018). Anti-proliferative activity of zerumbone against tumour cell lines. *Online J. Biol. Sci.* **18**(2): 123-129.

Ansari MA, Iqubal A, Ekbbal R and Haque SE (2019). Effects of nimodipine, vinpocetine and their combination on isoproterenol-induced myocardial infarction in rats. *Biomed Pharmacother*. **109**(1): 1372-1380.

Araya S, Mamo MA, Tsegay YG, Atlaw A, Aytenew A, Hordofa A, Negeso AE, Wordofa M, Niguse T, Cheru M and Tamir Z (2021). Blood coagulation parameter abnormalities in hospitalized patients with confirmed COVID-19 in Ethiopia. *PLoS One,* **16**(6): e0252939.

Bao L, Zhang Z, Dai X, Ding Y, Jiang Y and Li Y (2015). Effects of grape seed proanthocyanidin extract on renal injury in type 2 diabetic rats. *Mol Med. Rep*., **11**(1): 645-52.

Bijak M, Sut A, Kosiorek A, Saluk-Bijak J and Golanski J (2019). Dual anticoagulant/antiplatelet activity of polyphenolic grape seeds extract. *Nutrients*, **11**(1): 93.

Burr ML, Holliday RM., Fehily AM and Whitehead PJ (1992). Hematological prognostic indices after myocardial infarction: Evidence from the diet and reinfarction trial (DART). *The Eur. Heart J.* **13**(2): 166-170.

Chang CJ, Tzeng TF, Liou SS, Chang YS and Liu IM (2012). Acute and 28-day subchronic oral toxicity of an ethanol extract of *Zingiber zerumbet* (L.) Smith in rodents. *Evid. Based Complement. Alternat. Med.,* 2012: 608284.

Devi S and Singh R (2017). Antioxidant and anti-hypercholesterolemic potential of *Vitis vinifera* leaves. *Phamracogn. J.,* **9**(4): 565-572.

Fuad AFA, Alwi A, Zulkifly NAH and Mohamed NA (2020). Total phenolic, total flavonoids content and antioxidant activity of Mangifera sp. leaf extracts. *J. Agrobiotechnology*, **11**(1S).

Goyal SN, Arora S, Sharma AK, Joshi S, Ray R, Bhatia J, Kumari S and Arya DS (2010). Preventive effect of crocin of *Crocus sativus* on hemodynamic, biochemical, histopathological and ultrastuctural alterations in isoproterenol-induced cardiotoxicity in rats. *Phytomedicine,* **17**(4): 227-232.

Hamdi OAA, Ye LJ and Alfarizal MN (2015). Neuro-protective and antioxidant constituents from *Curcuma zedoaria* rhizomes. *Rec. Natl. Prod.,* **9**(3): 349-355.

Hasona N and Morsi A (2019). Grape seed extract alleviates dexamethasone-induced hyperlipidemia, lipid peroxidation, and hematological alteration in rats. *Indian J. Clin. Biochem.,* **34**(2): 213-218.

Hemn HO, Noordin MM, Rahman HS, Hazilawati H, Zuki A and Chartrand MS (2015). Anti-hypercholesterolemic and antioxidant efficacies of zerumbone on the formation, development, and establishment of atherosclerosis in cholesterol-fed rabbits. *Drug Desi. Devel. Ther.,* **9**(1): 4173-4208.

Hoff R and Koh CK (2018). Isoproterenol induced insulin resistance leading to diabetic ketoacidosis in type 1 diabetes mellitus. *Case Rep. Endocrinol.,* 2018: 4328954.

Husni A, Purwanti D and Ustadi (2016). Blood glucose level and lipid profile of streptozotocin-induced diabetes rats treated with sodium alginate from *Sargassum crassifolium*. *J. Biol. Sci*., **16**(3): 58-56.

Huwait EA (2019). Combination of vitamin E and L-carnitine is superior in protection against isoproterenol-induced cardiac injury: histopathological evidence. *Folia Morphol (Warsz)*, **78**(2): 274-282.

Jantan I, Rafi IAA, and Jalil J (2005). Platelet-activating factor (PAF) receptor-binding antagonist activity of Malaysian medicinal plants, *Phytomedicine,* **12**(1-2): 88-92.

Kalantari K, Moniri M, Boroumand Moghaddam A, Abdul Rahim R, Bin Ariff A, Izadiyan Z and Mohamad R (2017). A review of the biomedical applications of zerumbone and the techniques for its extraction from ginger rhizomes. *Molecules*, **22**(10): 1645.

Katerji M, Filippova M and Duerksen-Hughes P (2019). Approaches and methods to measure oxidative stress in clinical samples: Research applications in the cancer field. *Oxid. Med. Cell. Longev.,* pp.1-29.

Kimble R, Keane KM, Lodge JK and Howatson G (2019). Dietary intake of anthocyanins and risk of cardiovascular disease: A systematic review and meta-analysis of prospective cohort studies. *Crit. Rev. Food Sci. Nutr.,* **59**(18): 3032-3043.

Kristensen SD, Milner PC and Martin JF (1988). Bleeding time and platelet volume in acute myocardial infarction: A 2 year follow up study. *Thromb. Haemostasis.*, **59**(3): 353-356.

Li M, Li X and Yang L (2020). Cardioprotective effects of garcinol following myocardial infarction in rats with isoproterenol-induced heart failure. *AMB Expr.,* **10**(1): 137.

Liperoti R, Vetrano DL, Bernabei R and Onder G (2017). Herbal Medications in Cardiovascular Medicine. *J. Am. Coll. Cardiol.,* **69**(9): 1188-1199.

Lippi G, Ferrari A, Gandini G, Gelati M, Lo Cascio C and Salvagno G (2018). Analytical evaluation of the new Beckman Coulter access high sensitivity cardiac troponin I immunoassay. *Clin. Chem. Lab. Med*., **56**(1): 157-161.

Magrone T, Magrone M, Russo MA, and Jirillo E (2019). Recent advances on the anti-inflammatory and antioxidant properties of red grape polyphenols: *in vitro* and *in vivo* studies. *Antioxidants (Basel)*, **9**(1): 35.

Marx W, McKavanagh D, McCarthy AL, Bird R, Ried K, Chan A and Isenring L (2015). The Effect of Ginger (*Zingiber officinale*) on platelet aggregation: a systematic literature review. *PLoS One,* **10**(10): e0141119.

Meo F Di, Aversano R, Diretto G, Demurtas OC, Villano C, Cozzolin S, Filosa S, Carputo D and Crispi S (2019). Anti-cancer activity of grape seed semi-polar extracts in human mesothelioma cell lines. *J. Funct. Foods*., **61**(1): 1-10.

Nag A, Bandyopadhyay M and Mukherjee A (2013). Antioxidant activities and cytotoxicity of *Zingiber zerumbet* (L.) Smith Rhizome. *J. Pharmacogn. Phytochem.,* **2**(3): 102-108.

Nazeih A, Asmah H, Wahida FI and Nurul F (2020). Protective effects of *Zingiber zerumbet* rhizome extract on monosodium urate crystal-induced gout rat model. *J. Appl. Pharm. Sci*., **10**(4): 69-074.

Óscar A Muñoz-Bernal, Alma J Coria-Oliveros, Laura A de la Rosa, Joaquín Rodrigo-García, Nina del Rocío Martínez-Ruiz, Sonia G Sayago-Ayerdi and Emilio Alvarez-Parrilla (2021). Cardioprotective effect of red wine and grape pomace. *Int. Food Res. J.,* **140**: 110069.

Patel V, Upaganlawar A, Zalawadia R and Balaraman R (2010). Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: A biochemical, electrocardiographic and histo-architectural evaluation. *Eur. J. Pharmacol*., **644**(1-3): 160-8.

Somchit M, Mak J, Ahmad Bustamam A, Zuraini A, Arifah A, Adam Y and Zakaria Z (2012). Zerumbone isolated from *Zingiber zerumbet* inhibits inflammation and pain in rats. *J. Med. Plants Res.,* **6**(2): 177-180.

Subbaiah GV, Mallikarjuna K, Shanmugam B, Ravi S, Taj PU and Reddy KS (2017). Ginger treatment ameliorates alcohol-induced myocardial damage by suppression of hyperlipidemia and cardiac biomarkers in rats. *Pharmacogn. Mag*., **13**(Suppl 1): S69-S75.

Sultana F, Ahmad N, Saeed F, Jalal F and Hafeez Z (2019). Hydrolysate of extruded soy proteins concentrate attenuates post myocardial infarction remodeling and improves heart health. *Int. J. Biosci.*, **15**(1): 175-184.

Touloumi G, Karakosta A, Kalpourtzi N, Gavana M, Vantarakis A, Kantzanou M, Hajichristodoulou C, Chlouverakis G, Trypsianis G, Voulgari PV, Alamanos Y, Makrilakis K, Liatis S, Chatzipanagiotou S and Stergiou G (2020). High prevalence of cardiovascular risk factors in adults living in Greece: The EMENO National Health Examination Survey. *BMC Public Health*, **20**(1): 1665.

Tzeng TF, Liou SS, Chang CJ and Liu IM (2013). The ethanol extract of *Zingiber zerumbet* attenuates streptozotocin-induced diabetic nephropathy in rats. *Evid. Based Complement. Alternat. Med.,* 340645.

Yong KL and Young JC (2017). Proposal of modified hil-indices for determining hemolysis, icterus and lipemia interference on the beckman coulter au5800 automated platform. *Lab. Med. Online*. **7**(2): 6.