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Cardioprotection effects of diosgenin from *Dioscorea bulbifera* against isoproterenol-induced myocardial infarction

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Abstract

This research plan is to examine the cardioprotective effects of bioactive diosgenin molecules isolated from Dioscorea plants on Isoproterenol (ISO)-induced myocardial infarction. Male albino Wistar rats were treated with D.bullifera and Diosgenin (20 mg/kg) daily for four weeks. After the experiment, the animals were injected with ISO (85 mg/kg) subcutaneously at 24 hour intervals for two days. The results describe the myocardial injury induced by ISO, which is manifested as an increase in serum cardiac marker enzymes CK-MB, C.K.P., G.O.T., and G.P.T. And L.D.H. and essential oxidative stress markers, such as LPO and G.S.H. In addition, significant increases in serum total cholesterol, triglycerides, and low-density lipoprotein cholesterol levels were also observed, while serum high-density lipoprotein cholesterol levels were reduced. Histopathological studies are also related to the above parameters. In addition to cardiac markers, lipid peroxidation levels in plasma and heart are also elevated. However, antioxidant enzymes such as glutathione peroxidase (G.P.X.), catalase, S.O.D. and reduced glutathione (G.S.T.) are significantly reduced in the heart after ISO-induced myocardial infarction. These findings summarized the cardioprotective effects of diosgenin and dioscorea on cardiac markers and antioxidant activity.

Keywords: *Myocardial infarction; Isoproterenol; Lipid profile; Cardiac markers; Diosgenin.*

Abbreviations*:*

G.O.T., glutamic oxaloacetic transaminase, G.P.T., glutamic pyruvic transaminase, G.S.H., reduced glutathione, G.P.X., glutathione peroxidase, G.S.T., glutathione s-transferase, G.S.H., reduced glutathione, L.D.H., lactate dehydrogenase, ISO, Isoproterenol, LPO, lipid peroxidation, S.O.D., superoxide dismutase, HDL, high-density lipoprotein, LDL, low-density lipoprotein.

Introduction

Worldwide, myocardial infarction (MI) is the leading cause of death in humans. Myocardial infarction is a major health problem and a cause of death in developed and developing countries. According to the World Health Organization survey report, by 2020, heart disease and stroke will become the leading causes of death worldwide. Animal models of isoproterenol-induced myocardial infarction have been used to evaluate cardiac marker enzymes (Farvinet al. 2006 and Wexler BC., 1978). Isoproterenol (ISO) is a synthetic catecholamine and β-adrenergic agonist that can cause severe oxidative stress in the heart muscle, leading to an infarction similar to myocardial necrosis. It also knows how to stimulate lipid

peroxidation and generate free radicals to prevent uncompetitive necrosis of the heart membrane. The new lead molecule is guaranteed to cure ischemic heart disease. (Kasik and Prince 2006). "Although many conventional drugs can be used to prevent coronary artery disease, their effects are usually limited due to their side effects" (Subasini et al. 2009). In addition, the presence of alkaloids, tannins, plant sterols, flavonoids, and saponins have been exposed to reduce LDLC and increase HDL levels. Regular physical exercise can lower low-density lipoprotein cholesterol and increase high-density lipoprotein cholesterol, but it has been shown to improve cardiovascular health through other mechanisms and is the cornerstone of weight loss. Generally speaking, through weight loss, regular exercise, moderate alcohol consumption and lower dietary cholesterol, such as eating red meat, LDLC can be reduced by about 10-15% (Kumar, 2015).

D. bulbifera L. (D.B.), belonging to the family of Dioscoreaceae. D.B. generally called air yam or air potato or bitter yam. It is a climber plant with tuberous roots. D.B. has been commonly used in the Chinese system of medicine as an appreciated herb in the process of reconstruction and upholding kidney stone related problems. This herb was also initiated to have a valuable effect in treating diseases of the lungs and spleen and many types of dysentery, diarrhoea, fruitful digestion and metabolism. In Asia, this herb has been highly suggested for treating metabolic disorders (Ahmed et al. 2009). It has been conventionally used to lower the hyperglycemic index, providing a more continuous form of energy and better defence against obesity, dyslipidemia and diabetes. However, this property has not yet been systematically proven (Saravanan et al. 2011). Some literature reports are accessible on D. bulbifera use in diabetes mellitus and other related metabolic disorders, but not technically authenticated study has been carried out to justify its potential in experimental cardioprotection from ischemic heart diseases. There are several studies on tuber medicinal plants, the antioxidant activity of tubers, and the stems and leaves used in traditional medicine used in the evaluation of tuber animal models with cardioprotective effects. However, this study plans to examine the cardioprotective effects of ISO-induced diosgenin using cardiac markers in serum and heart tissue and antioxidant activities in rats.

Methods and Materials

Chemicals

Isoproterenol purchased from the Sigma Chemical Company, St. Louis, MO, U.S.A. All the chemicals and other reagents used analytical grade.

Plant materials

D. bulbifera (tubers) are identified by Rabinat Herbarium, St. Joseph College, Trichy, Palayamkottai and Botanical survey CCRAS Unit, Chennai, Tamil Nadu, India. The plant herbarium are deposited in the department of CARISM herbarium, and it was kept as DB-0062-2009. Hydroalcoholic extract of *D. bulbifera* screened for medicinally active constituents through the different qualitative and quantitative chemical methods. Hydroalcoholic extract of *D.bulbifera* gave positive test for terpenes, steroids, isoflavonoids, saponins and glycosides. Thin-layer chromatography of the hydroalcoholic extract of *D. bublifera* exposed purple, red, orange and green spots by Liebermann's test for steroids are present in the hydroalcoholic extract. *D.bulbifera* tubers were collected and authenticated by the pharmacognostic observations (Subasini et al., 2009) Fig 1 (A & B) and were selected for the new lead molecules for the management of coronary artery disease.

Extraction

The tubers of *D. bulbifera* were harvested and dried in the shade for 15 days. They are roughly course

powdered with a mixer blender. The dried tubers were extracted with water and alcohol by the cold osmosis method. The extract was then dried under a vacuum and stored in the refrigerator. The preparation of diosgenin was carried out according to the method of (Ghosh 2013). Narula et al. (2007), high content of diosgenin was found in *D. bulbifera* L., which is a potentially active biomolecule of diosgenin. Zhang et al. (2006) reported that diosgenin has powerful hypoglycemic and cholesterol-lowering activities.

Experimental animals

Thirty-six male albino Wistar rats weighing about 150-200 g were randomly selected. They are placed under standard laboratory conditions, room temperature (21ºC ± 2ºC) and 55-60 % relative humidity. They are fed a standard diet, including pellets and ad libitum drinking water. The study was conducted in accordance with CPCSEA guidelines and was approved by the Institutional Animal Ethics Committee (IAEC) of the SwamyVivekandha School of Pharmacy (Proposal No.: SVCP / IAEC / Pharm / 07/2012).

Experimental protocol for Isoproterenol (ISO) induced myocardial infarction

Male Wistar rats (weight 150-200 g) were randomly divided into six groups, each group containing six animals. The experimental myocardial infarction consisted of two subcutaneous injections of 85 mg/kg of Isoproterenol dissolved in physiological saline at 24-hour intervals. At the end of the 30th day, take the D.B. extract and diosgenin 30 minutes later. On day 31st day (24 hours after the first dose of ISO), the second dose of ISO was given.

Group 1: Received vehicle 5% Tween 80 in saline p.o., - normal control

Group 2: Received 85 mg/kg of Isoproterenol in sc. - disease control.

Group 3: Received 100 mg/kg of D.B. extract in oral (two times a day every 12 hrs)

Group 4: Received 200 mg/kg of D.B. extract by the oral route

Group 5: Received 10 mg/kg of diosgenin by the oral route via feeding tube.

Group 6: Received diosgenin 20 mg/kg + ISO treated; injected with Isoproterenol at 1 mg/kg body weight and two times a day for the period of one month. Diosgenin was administered to the 5th and 6th group of animals three days before to Isoproterenol.

After administration of 2nd dose of ISO, animals are fasted for 24 hours and at the end of 24thhour (i.e. 48 hours after the administration of 1stdose of ISO). The experiment was completed after one month, and then after overnight fasting, the animals are killed by cervical decapitation.

Biochemical parameters

Blood was collected in a test tube, the homogenate was centrifuged at 3000 rpm for 15 minutes, and the supernatant was used for various biochemical parameters. For all biochemical evaluations in the heart, a homogenate of myocardial tissue was prepared in 10 % phosphate-buffered saline. Take 10 % of the cardiac tissue in a tissue homogenizer and prepare it in a 0.1 M Tris HCl buffer solution (pH 7.4). Estimate levels of lipid peroxidation (L.P.O) (Okhawa et al. 1979), lactate dehydrogenase (L.D.H.) (King, 1965a), glutamate oxaloacetate aminotransferase (G.O.T) and glutamate pyruvate aminotransferase (G.P.T) (Mohun and Cook, 1957), glutathione peroxidase (GPx) (Rotruck et al. 1973), reduced glutathione (G.S.H.) (Ellman, 1959) and glutathione (G.S.T.) (Moron, 1979). Catalase activity was estimated by (Sinha 1972) superoxide dismutase (S.O.D.) based on (Kakkar et al. 1984), using standard Spinreact kits to analyze CK-MB estimates and creatine phosphokinase estimates (C.P.K., EC2. 7.3 .2)

(Okinaka et al. 1961).

Estimation of serum cholesterol

Serum cholesterol and triglycerides were predictable using standard cholesterol estimation Randox kit (Catalogue no. CH 201, TR 1697- Zlatkis et al. 1953). Serum HDL-cholesterol was projected from Randox (Catalogue no. CH 204).

Statistical Analysis

The results were statistically presented here as the means ± means by one-way Analysis of Variance (ANOVA), and groups were compared by Duncan Multiple Range Test (DMRT) used by SPSS software version 12.0. A value of $p<0.05$ was considered statistically significant.

Result and Discussion

Cardio protective potential of D. bulbifera

Myocardial infarction is commonly induced by different small animals such as mice, rats and rabbits of invasive methods. In this research work, the myocardial muscles of the heart induced by the administration of Isoproterenol, this chemical compound used for arterial and bronchial smooth muscles dilatation. It also affects the heart by increasing contractility and stroke volume. Evaluating the cardioprotective effect of D. bulbifera using different groups of rats were treated with D.bulbifera (D.B.) and diosgenin for one month.

Fig. 1: *D.bulbifera* and its tubers. Fig (A) Exomorphic features of bulbs
Fig (B) Twining perennial herb

Effect of D.B. & diosgenin on cardiac markers

Effect of D.B and diosgenin on cardiac enzymes like CK-MB, C.P.K., L.D.H., G.O.T., G.P.T. activities in Isoproterenol induced Myocardial infarction rats. Therefore, examining the D.B and diosgenin could reorganize the ISO induced myocardial damage. Myocardium muscles of the heart having an elevated concentration of proteins and enzymes, which are released into the extracellular fluid. In this research, D.B. and diosgenin have inhibited the release of cardiac marker enzymes in the serum compared with ISO treated rats.

The activity of CK-MB, C.P.K., L.D.H., G.O.T., G.P.T. and alkaline phosphatase (A.L.P.) is in the heart tissue of the diseased rats in the second group was significantly reduced, and the serum level was increased ($p<0.05$, Table 1 and Table 2). Sathish et al. (2003) also tested these diagnostic markers in ISO-treated rats. Ghosh et al. (2013) stated that the level of enzymes in the serum is directly proportional to the number of necrotic cells in the damaged heart tissue. Heart muscle contains many proteins and enzymes that, once damaged, are released into the extracellular fluid and measured as potential marker enzymes. These enzymes, mainly CK-MB, C.P.K., L.D.H., G.O.T., G.P.T., are released into the circulation due to myocardial degeneration and myocardial cell necrosis (Wexler and Greenberg, 1978). ISO causes a series of metabolic events in myocardial tissue, beginning with anaerobic glycolysis, inhibiting the ATP-dependent transport process in the cell membrane, electrolyte transfer, cell oedema, and finally loss of membrane integrity. cellular (Saravanan et al., 2011). This leads to the release of these specific marker enzymes from the heart.-

In the present study, D.B. and diosgenin have prevented the acute release of diagnostic marker enzymes in the serum as compared with ISO treated group of rats. The important boost of investigative markers in ISO treated rats has also been reported by Sathish et al. (2003). The activity of CK-MB, C.P.K., L.D.H., G.O.T., G.P.T. and alkaline phosphatase (A.L.P.) is observed to be decreased significantly in heart tissue and increased in serum of diseased group II of animals compared to the normal group of animals ($p<0.05$, Table 1 & 2). The quantity of necrotic cells in the injured cardiac tissue is directly proportional to the number of enzymes present in the serum (Ghosh et al., 2013). In comparison to the diseased group II of animals, pretreatment with diosgenin reduced the activity of these enzymes in serum while increasing it in heart tissue ($p<0.05$, Table 1).

A useful cardiac marker for myocardial infarction is serum CK-MB (Suchalatha et al., 2004**).** Table 1 shows that the serum CK-MB level increases ($p<0.05$) in diseased conditions. Similar results have been observed by Vimal & Devaki (2004). In treatment animals with diosgenin, it is found to decrease significantly ($p<0.05$) at both doses. The percentage of decrease is calculated as 45 % at 10 mg/kg b.wt and 48.4 % at 20 mg/kg b.wt., respectively. But, treating animals with diosgenin serum CK-MB level is found to decrease and seem to reach the normal level at a lower dose of 10 mg/kg b.wt. itself. The same value also maintains in the dose of 20 mg/kg b.wt. of diosgenin.

Table 1. Effect of D.B. &diosgenin on cardiac markers in ISO induced MI in rats

Groups	CK-MB	CK Serum	CK Heart	LDH Serum	LDH Heart
Normal	54.9 ± 3.1^{a}	1.53 ± 0.14^{a}	300.9 ± 7.2^{a}	3.6 ± 0.6^{a}	44.6 ± 4.3^{b}
ISO	308.4 ± 91.3^{b}	3.8 ± 1.14^{b}	170.3 ± 14.8^{b}	7.0 ± 1.3^{b}	24.1 ± 1.9^{a}
DB(100)+ISO	172.0 ± 55.9^{ab}	2.08 ± 0.45^{a}	295.2 ± 28.4^{a}	5.1 ± 0.02^{ab}	32.3 ± 4.9^{ab}
DB(200)+ISO	159.0 ± 6.92^{ab}	1.90 ± 0.48^{a}	276.7 ± 15.8^{a}	4.2 ± 0.9^{ab}	37.9 ± 6.9^{ab}
DG 10 (mg/kg)	51.6 ± 3.6^{a}	2.16 ± 0.56^{a}	272.9 ± 24.0^{a}	5.2 ± 0.2^{ab}	35.3 ± 7.1^{ab}
DG 20 (mg/kg)	53.1 ± 35.0^{a}	1.6 ± 0.6^{a}	266.8 ± 36.2^{a}	4.3 ± 0.2^{ab}	39.6 ± 6.2^{ab}

Values are mean ± S.D. (n=6). A significant difference was observed between different groups using One Way ANOVA followed by DMRT. Values with different letters like a,b, ab of the same column are differed significantly (p<0.05).Note: Activity of creatine kinase(C.K.), L.D.H., are represented as nM of phosphate liberated/min/mg of protein, nM of pyruvate liberated /min/mg of protein, µM of pyruvate formed/min/mg of protein, µM of pyruvate formed/min/mg of protein.

Table 2. Effect of D.B. &diosgenin on cardiac enzymes in ISO induced MI in rats

Groups	SGOT Serum	SGOT Heart	SGPT Serum	SGPT Heart	ALP Serum	ALP Heart
Normal	5.7 ± 1.1^{a}	132.1 ± 3.1^{b}	7.1 ± 0.9^{a}	175.9 ± 10.4^{b}	225.2 ± 10.5^{a}	10.6 ± 1.5^{b}
ISO	8.5 ± 0.7^{b}	105.8 ± 4.5^{a}	10.5 ± 0.2^{c}	137.1 ± 5.0^{a}	181.1 ± 7.6^{c}	19.7 ± 2.3^{a}
DB(100)+ISO	6.9 ± 0.4^{ab}	114.0 ± 9.5^{ab}	9.5 ± 0.5^{bc}	148.9 ± 9.2^{ab}	186.9 ± 10.7^{bc}	18.6 ± 1.9^{a}
DB(200)+ISO	6.7 ± 0.7^{ab}	119.2 ± 8.4^{ab}	8.6 ± 1.1^{abc}	150.4 ± 9.3^{ab}	200.1 ± 10.7^{bc}	17.2 ± 0.6^{ab}
DG 10 mg/kg	7.9 ± 0.5^{ab}	110.2 ± 8.4^{ab}	9.3 ± 0.6^{bc}	141.8 ± 11.3^{a}	183.7 ± 4.9^{abc}	15.5 ± 0.8^{a}
DG 20 mg/kg	6.4 ± 0.7^{ab}	121.4 ± 9.7^{ab}	7.7 ± 0.2^{ab}	151.2 ± 12.5^{ab}	193.2 ± 3.5^{ab}	14.1 ± 1.3^{ab}

Values are mean ± S.D. (n=6). A significant difference was observed between different groups using One Way ANOVA followed by DMRT. Values with different letters like a,b,c, ab of the same column are differed significantly (p<0.05).Note: Activity of SGOT, SGPT and *alkaline phosphatase*(A.L.P.) are represented as nM of phosphate liberated/min/mg of protein, nM of pyruvate liberated /min/mg of protein, µM of pyruvate formed/min/mg of protein, µM of pyruvate formed/min/mg of protein.

Effect of D.B. & diosgenin on glutathione and lipid peroxidation

When administering D.B and diosgenin, the serum increased the levels of oxidative stress markers, such as L.P.O, G.S..H and G.P.X compared to control animals. It was observed in the heart and serum of rats of disease group II (Table 3. p <0.05). It was observed that the rats previously treated with ISO and Diosgenin (groups III, IV and V) significantly reduced the levels of these markers (p <0.05, Table 3) compared to the diseased rats of group II. Subasini et al. (2014) explained that "ISO undergoes oxidation that leads to the formation of superoxide anions, and the propagation of the chain reaction leads to R.O.S." In this study, the activity of enzymatic antioxidants such as S.O.D., catalase, GPx, and G.S.T. (p <0.05, Table 4) Compared to normal rats in serum and cardiac tissue in the diseased heart of rats. Regarding the treatment of D.B. animals and diosgenin, the activity of antioxidant enzymes and other non-enzymatic antioxidants was observed to increase in a dose-dependent manner in animals of groups III, IV, and V against diseased animals of group II. Note that the evidence that ISO induces increased oxidative stress markers in rats may be due to the production of free radicals.

Table 3. Effect of D.B. &diosgenin on glutathione and lipid peroxidation in ISO induced MI in rats

Groups	LPO	LPO	G.S.H.	G.S.H.	GPx	GPx

	Serum	Heart	Serum	Heart	Serum	Heart
Normal	0.56± 0.08^{a}	0.92±0.1^{a}	29.6±8.9^{ab}	0.62±0.11^{ab}	0.19±0.04^{a}	2.7± 0.13^{ab}
ISO	0.76±0.05^{b}	1.38±0.1^{b}	24.7±2.5^{a}	0.53±0.09^{a}	0.13±0.009^{b}	2.2± 0.09^{a}
DB(100)+ISO	0.69±0.05^{ab}	1.16±0.13^{ab}	25.6±2.1^{a}	0.97±0.21^{b}	0.21±0.01^{ab}	2.4± 0.17^{ab}
DB(200)+ISO	0.64±0.03^{ab}	1.14±0.02^{ab}	37.5±7.5^{b}	0.89±0.26^{ab}	0.35±0.09^{ab}	2.5± 0.19^{b}
DG 10 mg/kg	0.72±0.03^{ab}	1.24±0.09^{ab}	28.0±5.9^{ab}	0.48±0.05^{a}	0.24±0.01^{ab}	2.3± 0.17^{ab}
DG 20 mg/kg	0.57± 0.05^{a}	1.14±0.13^{ab}	40.9±13.9^{b}	0.6±0.05^{ab}	0.22±0.02^{ab}	2.5± 0.17^{ab}

Values are mean ± S.D. (n=6). A significant difference was observed between different groups using One Way ANOVA followed by DMRT. Values with different letters like a,b, ab,c of the same column are differed significantly (p<0.05).

Table 4. Effect of D.B. & diosgenin on enzymatic antioxidant in ISO induced MI in rats

Groups	Catalase	SOD	GPX	GST
Normal	61.2 ± 1.7^{b}	1.0 ± 0.37^{b}	1.18±0.1^{b}	132.1±15.3^{b}
ISO	16.0 ± 3.2^{a}	0.57 ± 0.08^{a}	0.88 ± 0.1^{a}	72.8 ±7.8^{a}
DB(100)+ISO	32.7± 10.1^{ab}	0.89 ± 0.08^{ab}	1.22±0.21^{b}	107.8±24.6^{ab}
DB(200)+ISO	60.9 ± 9.8^{b}	1.07 ± 0.35^{b}	1.22±0.21^{b}	135.1± 13.2^{b}
DG 10 (mg/kg)	59.6 ± 9.4^{b}	1.02 ± 0.38^{b}	1.21±0.09^{b}	132.6± 16.3^{b}
DG 20 (mg/kg)	60.4 ± 11.6^{b}	0.94 ± 0.25^{ab}	1.18±0.12^{b}	132.4± 15.8^{b}

Values are mean ± S.D. (n=6). A significant difference was observed between different groups using One Way ANOVA followed by DMRT. Values with different letters like a,b, ab of the same column are differed significantly (p<0.05).Note: Activity of catalase, S.O.D., GPx and G.S.T. are represented as nM of H_2O_2consumed/min/mg of protein, Units/mg of protein, µM of G.S.H. consumed/min/mg of protein, nM of CDNB conjugated/min/mg of protein.

Effect of D.B. on lipid profiles

Lipids play an important role in cardiovascular disease, but their accumulation can lead to atherosclerosis and lead to ischemic heart disease and peripheral vascular disease. The increased risk of atherosclerosis is always associated with high levels of total cholesterol, low-density lipoproteins, very low-density lipoproteins, and low-density lipoproteins (Panda et al., 2014). Table 5 shows the cholesterol curve. Compared to normal animals, the ISO-induced group of animals resulted in increased levels of total cholesterol (T.C.), triglycerides (T.G.L.) and LDL, while the HDL level decreased (p <0.05). (Subasini., 2019) Similar results were observed. It was observed that the pretreatment animal group significantly reversed the lipid profile to normal levels (p<0.05). Those results can be seen in Table 5.

Table 5: Effect of D.B. & diosgenin on lipid profile in ISO induced MI in rats

Groups	Cholesterol	Triacylglycerol	LDL	HDL
Normal	140.0±5.2^{ab}	125.9 ± 11.1^{ab}	107.9±0.9^{ab}	18.3± 3.1^{bc}
ISO	143.2±13.8^{b}	143.2 ± 14.7^{b}	117.1±11.6^{b}	13.9± 1.3^{a}
DB(100)+ISO	157.9±13.1^{b}	142.3 ± 30.0^{b}	117.9±4.1^{b}	16.4±3.0^{abc}
DB(200)+ISO	105.0±11.7^{a}	102.1 ± 12.1^{ab}	76.2±6.3^{a}	14.7± 2.3^{ab}
DG 10 mg/kg	139.0±10.9^{ab}	141.7 ± 10.5^{b}	111.8±7.1^{b}	14.2± 2.1^{a}
DG 20 mg/kg	127.2±8.9^{ab}	85.2 ± 12.9^{a}	88.2±3.5^{a}	19.4± 2.8^{c}

Values are mean ± S.D. (n=6). A significant difference was observed between different groups using One Way ANOVA followed by DMRT. Values with different letters like a,b,ab,c,d of same column are differ significantly (p<0.05).

Conclusion

In conclusion, this study demonstrated that D.B and diosgenin significantly reduced isoprenaline-induced myocardial infarction injury in rats. This effect is related to a reduction in cardiac markers, cholesterol profile, and antioxidant research. However, more research is needed to understand the exact mechanism by which diosgenin inhibits oxidative stress in heart tissue damaged by myocardial infarction. The results of this study indicate that the cholesterol-lowering properties of diosgenin may have the potential to prevent and control cardiovascular disease.

Conflict of interest

The authors declare that there are no conflicts of interest

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