



RESEARCH ARTICLE

Phytochemical Screening and Evaluation of Cardioprotective Activity of Ethanolic Extract of *Trigonella foenum - graecum* against Isoproterenol Induced Myocardial Infarction Rats

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ABSTRACT

The objectives of the present study were phytochemical screening and evaluate the effects of ethanol extract of *Trigonella foenum-graecum* on cardiac functions and histopathological changes in isoproterenol-induced myocardial infarction (MI). The whole plant was extracted with ethanol by soxlet apparatus and subjected to analysis the phytochemical and evaluates the cardioprotective activity through wistar rats. They were assigned to 5 groups of normal control, isoproterenol, pre-treatment with ethanol extract of *Trigonella foenum-graecum* 200 and 400 mg/kg of body weight the extract one in a day concurrent with myocardial infarction rats. And verapamil standard drug in the dose of 5µmol/kg body weight. All groups except normal groups received isoproterenol (85 mg/kg) for 2 consecutive days was used to induce myocardial infarction. Phytochemical screening indicated the presence of important principal active compound in ethanol extract of *Trigonella foenum-graecum*. The results of the study demonstrate that ethanol extract of *Trigonella foenum-graecum* strongly protected the myocardium against isoproterenol-induced infarction and proved that the cardio protective effects.

KEYWORDS

Myocardial infarction, *Trigonella foenum-graecum*, Isoproterenol

INTRODUCTION

Nature has been a source of medicinal plants and plant derived products are used as medicines, recently there is a greater global interest in non-synthetic natural drugs derived from plant/ herbal sources due to safe alternative, better tolerance, lesser cost and minimal adverse drug reactions. The plant based drugs continue to play an important role in the primary health care of about 80-85% of the world's population.¹ Cardiovascular disease (CVD) is a major important cause of morbidity

and mortality in developing counters due to increased high prevalence of risk factors and also aging of their populations.² According to WHO 17.3 million people died from CVDs in 2008, over 80% of CVD death take place in low and middle income countries.³ An estimated that by 2030 more than 23 million people in world 2.6 million people in India's will die annually from CVDs. There are different way of preventing and treating cardiovascular disease. Besides drug therapy and life style changing, dietary modification and supplementation play an increasingly important role in the conservative treatment of CVDs. Current interest has focused on plant based natural drug treatments. Many plants species and their

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constituents are used in indigenous system of medicine for the treatment of myocardial infarction. Recently there renewed interested in medicinal products and food products derived from medicinal plants that have been found to have certain preventive actions in the treatment of coronary heart disease.

Fenugreek (*Trigonella foenum-graecum* L. *Leguminosae*) is one of the oldest medicinal plants, originating in India and Northern Africa. An annual plant, fenugreek grows to an average height of two feet. The leaves consisting of three small obovate to oblong leaflets. It is cultivated worldwide as a semi-arid crop, and its seeds are a common ingredient in dishes from the Indian Subcontinent. The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal use. Applications of fenugreek were documented in ancient Egypt, where it was used in incense and to embalm mummies. In modern Egypt, fenugreek is still used as a supplement in wheat and maize flour for bread-making. In ancient Rome, fenugreek was purportedly used to aid labour and delivery. In traditional Chinese medicine, fenugreek seeds are used as a tonic, as well as a treatment for weakness and edema of the legs.⁴ In India, fenugreek is commonly consumed as a condiment and used medicinally as a lactation stimulant. There are numerous other folkloric uses of fenugreek, including the treatment of indigestion and baldness. The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol absorption and thought to help lower sugar levels.⁵ Therefore, fenugreek seeds are used as a traditional remedy for the treatment of diabetes and hypercholesterolemia in Indian and Chinese medicines.⁶ It is reported to have restorative and nutritive properties and to stimulate digestive processes, useful in healing of different ulcers in digestive tract.



Figure 1: *Trigonella foenum-graecum* medicinal plant and seeds

The seed includes strong mucilage which makes it a beneficial treatment for intestinal inflammation and ulcers. The ability of *Trigonella foenum-graecum* seed to adjust many enzymes, including those related to glucose and lipid metabolism.^{7,8}

Trigonella foenum-graecum reduces the amounts of calcium oxalate in the kidneys which often contributes to kidney stones. In animal studies, fenugreek appeared to lesser the chance of developing colon cancer by blocking the action of certain enzymes.^{9,10} Fenugreek is currently used as a source of steroid synthesis, one of its active constituents from which other steroids can be synthesized¹¹, so the present research has been designed the whole plant is used to investigation of phytochemical screening and evaluate the cardioprotective property of ethanol extract of *Trigonella foenum-graecum* in isoproterenol induced myocardial injured experimental rats.

MATERIALS AND METHOD

Collection of Plant material

The medicinal plant was collected from Adhiparasakthi Agricultural College Medicinal garden, Kalavai, Vellore district, Tamil nadu, India. The plant authenticated by Dr. P. Jayaraman, Professor, Institute of Herbal Botany Plant Anatomy Research Center, Chennai. A voucher specimen no: PARC/2013/2027 was deposited in center for further analysis.

Preparation of Plant Extract

After the collection of *Trigonella foenum-graecum*, the medicinal plant they were placed in clean tray and allowed for shade drying. The whole plant was subjected to surface sterilization using ethanol and then dried in shade. Then dried plant was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve (20 mesh).

The medicinal plant *Trigonella foenum-graecum* powdered (100 g) were defatted by treating with pet-ether and then extracted with ethanol solvent by using soxlet apparatus. The solvent was removed under vacuum to get the solid mass. The residue was weighed and stored in air and water proof containers, kept in refrigerator at 4°C. From this stock, fresh preparation was made whenever required.

Phytochemical Analysis

Phytochemical screening of *Trigonella foenum-graecum* medicinal plant ethanol extract was done for the presence of various phytoconstituents by using standard procedures.¹²

Experimental Animals

Healthy adult wistar albino rats (weighing 160 - 210g) were used in the experiments.

Animals were housed in polypropylene cages at 22±2°C with relative humidity of 45- 55% under 12 hour's light and dark cycle. They were feed with standard laboratory animal feed (Hindustan Lever Ltd., India) and water *ad libitum*.

Approval of Experimental Protocol

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical committee (IAEC) of Adhiparasakthi College of Arts and Science, Kalavai, constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 282/ac/09/ CPCSEA). Ethical guidelines were strictly followed during all the experiments.

Oral Acute Toxicity Study

Acute toxicity Study was performed according to Organisation for Economic Co-operative and Development guidelines (OECD) No. 423. Albino rats of either sex were divided into six groups with six animals in each. *Trigonella foenum-graecum* ethanol extract was administered orally as single doses to rats at different dose levels of 50, 250, 500, 1000, 1500, and 2000 mg/kg b.w. Animals were observed individually for behavioural changes for the first 30 minutes & mortality up to 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total 14 days.¹³

Induction of Myocardial Infarction

At the end of the treatment period, all the animals, except the normal untreated rats that served as the control group, were administered isoproterenol (ISO) 85 mg/kg, intera vein injection for two consecutive days on the 31 and 32 day at an interval of 24 h. to induce myocardial injury.¹⁴

Experimental Design

The rats were divided into five groups with six rats in each group.

Group I: Normal rats received 3ml Saline through oral.

Group II: Serve as Isoproterenol induced myocardial infarcted rats. Rats were administration Isoproterenol (85mg/kg body weight i.v.) on the 31st and 32nd day at an interval of 24 h.

Group III: Serve *Trigonella foenum-graecum* ethanol extract treated group. Rats pre-treat with *Trigonella foenum-graecum* methanol extract (200mg/kg body weight) given orally up to 32 days and followed by Isoproterenol.

Group IV: Serve *Trigonella foenum-graecum* ethanol extract treated group. Rats pre-treat with *Trigonella foenum-graecum* methanol extract (400mg/kg body weight) given orally up to 32 days and followed by Isoproterenol.

Group V: Serve as Standard drug group. Animals are administration of Verapamil in the dose of 5µmol/kg body weight (i.v) on 14th day and 30th day, followed by Isoproterenol (85mg/kg body weight, i.v.) after 30min.

Collection of Blood and Heart Tissues

At the end of 32th day, after treatments, all the animals were sacrificed by decapitation by mild anesthesia and the fasting blood samples of each group were collected separately into sterilized dry centrifuge tubes, and allowed to coagulate for 30 min. at 37°C.

The clear serum obtained after centrifugation was used for the estimation for blood glucose, total protein, bilirubin, lipid profiles including total cholesterol, TG, HDL, LDL and cardiac enzymes like lactate dehydrogenase (LDH), creatine phosphokinase total, creatine phosphokinase MB (CPK) and serum aspartate amino transferase (AST or SGOT) using respective kits. The Heart tissues were excised immediately and immersed in Physiological saline.

It was suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) and cut into small pieces. The required amount was weighed and homogenized using a Teflon homogenizer (Inco, India). The clear supernatant was used for the estimation of cardiac enzymes using respective kits.

Histological Assessment

The heart was excised and washed immediately with ice-cold saline, then fixed in 10% buffered formalin 10% stored buffered formalin were embedded in paraffin; 5µm thick sections were cut and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histological changes.

Statistical Analysis

The statistical analysis was performed by ANOVA under one way classification followed by Bonferroni multiple comparison test, changes were considered significant at the P-value of < 0.05 and < 0.01 level of significance. The values were expressed as mean ± SD.

RESULTS AND DISCUSSION

Phytochemical Investigation

Phytochemical investigation of *Trigonella foenum-graecum* methanol extract revealed the presence of flavonoids, terpenoids, alkaloids, saponins and phytosterols are important active constituents.

Oral Acute Toxicity Study

In acute toxicity study, it was found that the animal were safe up to a maximum dose of 2000 mg/kg b.w. There were no changes in the normal behavioural pattern and no signs and symptoms of toxicity and mortality were observed.

Bio-chemical Parameters Analysis

Table 1 show that the blood glucose, total protein, bilirubin, total cholesterol, triglyceride, HDL and LDL levels in normal and experimental animals.

The blood glucose, bilirubin, total cholesterol, triglyceride, and LDL levels are significantly (P<0.01) increased and total protein and HDL levels are decreased in ISO induced myocardial infarction rats when compared to normal group. Pre-treatment with daily oral administration of *Trigonella foenum-graecum* ethanol extract significantly (P<0.01) decreased in blood glucose, bilirubin, total cholesterol, triglyceride and LDL level and the HDL level was return back to normal when compared to ISO treated group (Table 1). When compared to the 200mg/kg of body weight, the dose 400 mg/kg of b.w. have the greater ability to prevention of cardiac damage.

Myocardial and Serum Marker Enzymes

In Table 2 shows the myocardial markers enzymes of LDH, CPK total, CPK (MB) and AST levels in normal and experimental animals.

In Table 2 represent, the exposure to ISO (Group - II) significantly (P<0.01) decreased the myocardial LDH, CK total and CK (MB) levels and there were no significant changes in the level of myocardium AST when compared to normal group. After the Pre-treatment with daily

Table 1: Effect of pretreatment with *Trigonella foenum-graecum* ethanol extracts on general and lipid profile marker compounds in control and experimental animals

Parameters	Normal (Saline 10ml/kg b.w)	Isoproterenol (85mg/kg.b.w)	TFGEE (200mg/kg b.w)	TFGEE (400mg/kg b.w)	Verapamil (5µmol/kg b.w)
Blood glucose (mg/dl)	72.02±1.1	184.10±0.1***	94.21±0.1***	82.11±1.0***	81.22±1.1** *
Total protein(g/dl)	7.1.0±1.2	4.5.4±1.5***	6.2.1±2.1***	6.8.3±1.1***	6.9± 1.6**
Bilirubin (mg/dl)	0.8± 3.1	2.1±1.1***	1.1±21***	0.8±1.1***	0.9±1.1**
Total Cholesterol (mg/dl)	137.3±1.2	198.1±0.1***	136.1±2.1***	126.2±3.2***	108.2±1.2** *
Triglycerides (mg/dl)	76.11±2.3	130.1±1.2***	96.1±4.3***	84.4±6.2***	95.1±1.3***
HDL (mg/dl)	36.11±1.3	18.32±2.3***	31.65±3.1***	33.41±5.1***	35.11±2.3** *
LDL (mg/dl)	20.02±1.2	42.46±3.2***	27.52±1.4***	21.20±6.2***	22.02±0.2**

All value expressed as mean±SD; One way analysis of variance followed by Bonferroni multiple comparison test, *** P<0.01, ** P<0.05. HDL - High density lipoprotein; LDL- low density lipoprotein. *Trigonella foenum-graecum* ethanol extracts (TFGEE).

Table 2: Effect of pretreatment with *Trigonella foenum-graecum* ethanol extracts on myocardial marker enzymes in control and experimental animals.

Parameters	Normal (Saline 10ml/kg b.w)	Isoproterenol (85mg/kg.b.w)	TFGEE (200mg/kg b.w)	TFGEE (400mg/kg b.w)	Verapamil (5µmol/kg b.w)
LDH (IU/l)	76.21±3.1	26.1±0.1***	52.11±1.0***	65.25±0.0***	68.22±1.1***
CK total (IU/l)	146.11±4.1	66.0±1.5***	127.13±0.1***	130.19±1.1***	13.8± 2.6**
CK (MB) (IU/l)	23.22±1.1	10.1±1.1***	20.4±1.1***	22.1±21***	18.22±1.5**
AST(IU/l)	68.10± 21	63.64±0.1***	72.21±11***	66.11±00***	67.21 ±11***

All value expressed as mean±SD; One way analysis of variance followed by Bonferroni multiple comparison test,*** P<0.01, ** P<0.05. *Trigonella foenum-graecum* ethanol extracts (TFGEE). LDH- Lactate dehydrogenase, Creatine Kinase CK Total – CK (MB) – Creatine Kinase Myocardial Band, AST – Aspartate transaminase.

oral administration of *Trigonella foenum-graecum* ethanol extract significantly ($P < 0.01$) increased in myocardial LDH, CK total and CK (MB) levels. When compared to 200mg the 400mg/kg.b.w. have the highly significantly retain the normal levels.

In TABLE 3 shows the serum markers enzymes of LDH, CPK total, CPK (MB) and AST levels in normal and experimental animals. The exposure to ISO (Group - II) significantly ($P < 0.01$) increased the serum LDH, CK total, CK (MB) and AST levels when compared to normal group. After the Pre-treatment with *Trigonella foenum-graecum* ethanol extract significantly ($P < 0.01$) decreased in serum LDH, CK total, CK (MB) and AST levels were compared to 200mg the 400mg/kg.b.w. have the highly significantly prevent the cardiac damage.

Histopathological Examination

Histopathological examination of the myocardium of normal rat showed clear integrity of myocardial cell membrane (Fig.2).

Endocardium and pericardium were within normal limits. No inflammatory cell infiltration was observed. The group of ISO-treated rats showed moderate to marked myocytic necrosis with moderate infiltration of lymphocytes and macrophages (Fig.3). The changes were more prominent along the endocardium and in papillary muscles.

Minimal-to-mild multifocal myocytic necrosis with removal of sarcoplasm and mild diffuse inflammatory cell infiltration along the endocardium was observed in the *Trigonella foenum-graecum* ethanol extracts 200mg/kg.bw (Fig.4). And 400mg/kg.b.w treated rat showed clear integrity of myocardial cell membrane (Fig 5). Endocardium and pericardium were within normal limits. No inflammatory cell infiltration was observed.

Minimal-to-mild focal myocytic necrosis and minimal diffuse lymphocytic infiltration along the endocardium was seen in the heart sections of the standard drug verapamil treated group (Fig. 6).

Table 3: Effect of pretreatment with *Trigonella foenum-graecum* ethanol extracts on serum marker enzymes in control and experimental animals.

Parameters	Normal (Saline 10ml/kg b.w)	Isoproterenol (85mg/kg.b.w)	TFGEE (200mg/kg b.w)	TFGEE (400mg/kg b.w)	Verapamil (5µmol/kg b.w)
LDH (IU/l)	35.21±3.1	136.1±0.1***	46.11±1.0***	42.25±0.0***	40.22±1.1***
CK total (IU/l)	56.11±4.1	166.0±1.5***	73.13±0.1***	64.19±1.1***	62.8± 2.6**
CK (MB) (IU/l)	25.22±1.1	150.1±1.1***	39.4±1.1***	30.1±21***	28.22±1.5**
AST(IU/l)	28.10± 21	89.64±0.1***	43.21±11***	31.11±00***	35.21 ±11***

All value expressed as mean±SD; One way analysis of variance followed by Bonferroni multiple comparison test,*** $P < 0.01$, ** $P < 0.05$. *Trigonella foenum-graecum* ethanol extracts (TFGEE). LDH- Lactate dehydrogenase, Creatine Kinase CK Total – CK (MB) – Creatine Kinase Myocardial Band, AST – Aspartate transaminase.

DISCUSSION

Isoproterenol induced myocardial infarction is widely used as a model of evaluating cardioprotective drugs.¹⁵ Isoproterenol, a potent synthetic catecholamine, induces sub endocardial myocardial ischemia, hypoxia, and finally fibroblastic hyperplasia with decreased myocardial compliance which closely resembles local myocardial infarction-like pathological changes seen in human myocardial infarction.¹⁶ The high dose of isoproterenol is ability to destroy myocardial cells. As a result of this, cytosolic enzymes such LDH, CK, and AST were released into the blood stream and serve as the diagnostic markers of myocardial tissue damage. The amount of these cellular enzymes present in heart reflects the alteration in plasma membrane integrity and/of permeability.¹⁷ Changes in the level of myocardial markers LDH, CPK total, CPK (MB), and AST in both heart homogenate and serum in ISO-treated rats (Table 2 & 3) conforms the onset of myocardial necrosis. Oral administration of *Trigonella foenum-graecum* ethanol extracts (400mg/kg b.w) caused significant changes in the level of cardiac markers of LDH, CPK total, CPK (MB), and AST. When compared to the 400mg/kg, the dose 200 mg/kg of body weight has the highly significant effect of prevention of heart damage compared to normal animals.

Lipids play an important role in cardiovascular diseases, not only by way of hyperlipidemia and the development of atherosclerosis, but also by modifying the composition, structure and stability of the myocardium. High levels of circulating cholesterol along with TG and their accumulation in the heart tissue is usually accompanied by cardiovascular damage.¹⁸ In the present study, ISO evidenced its hyperlipidemic effect by increasing serum TC, TG and LDL levels and decreased levels of HDL in comparisons with normal controls. High levels of LDL show positive correlation with MI, while increased levels of HDL have a negative correlation. Our earlier studies reported hyperlipidemia in ISO induced myocardial necrosis.¹⁹ An increase in LDL and along with a

decrease in HDL was observed in ISO induced rats. LDL is capable of carrying the highest concentration of cholesterol is evidence to increased serum TC.²⁰

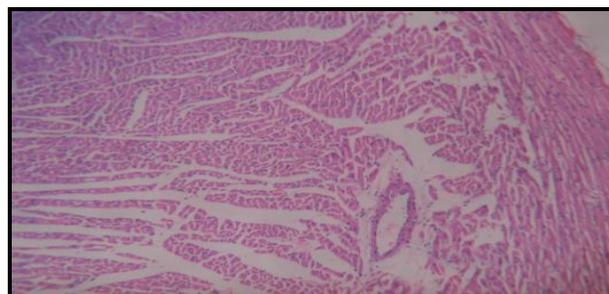


Figure 2: Hematoxylin and Eosin Staining of Heart Normal Rats

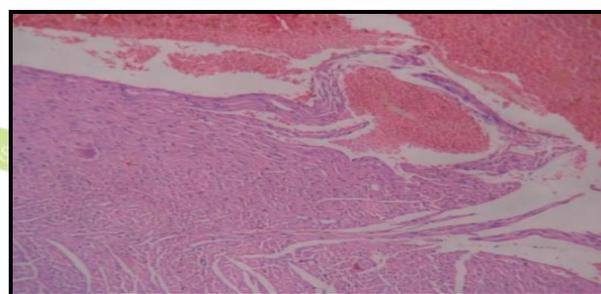


Figure 3: Hematoxylin and Eosin Staining of Heart ISO Treated Rat

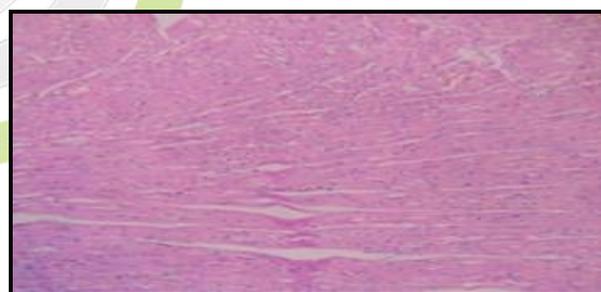


Figure 4: Hematoxylin and Eosin Staining of Heart Rats Treated with TFG (200 mg/kg) and ISO

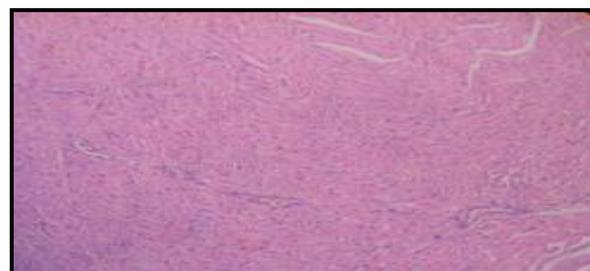


Figure 5: Hematoxylin and Eosin Staining of Heart Rats Treated with TFG (400mg/kg) and ISO

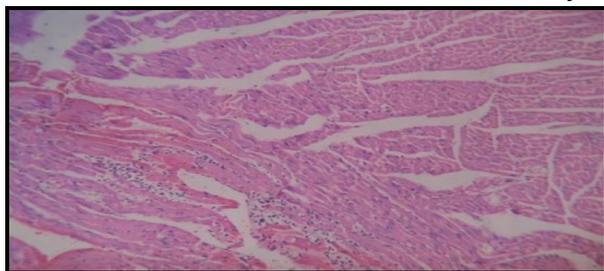


Figure 6: Hematoxylin and Eosin Staining of Heart in Rats Treated with verapamil (5 μ mol/kg) and ISO Histopathological Studies of Experimental Rats

Pre-treatment with *Trigonella foenum-graecum* ethanol extracts (200 & 400mg/kg b.w) significantly decreased the increased TC, TG and LDL levels and increased the levels of HDL (Table 1). These alterations in lipid profile might be due to the presence of major active constituents of Medicinal plant.

In conclusion from the biochemical and histopathological evidence that the *Trigonella foenum-graecum* ethanol extracts on 400 & 200mg/kg body weight, both produced significant cardioprotection in isoproterenol induced myocardial infarction animals.

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