

Research Article

Effect of Aqueous Extract of Nutmeg on Hyperglycaemia, Hyperlipidaemia and Cardiac Histology Associated with Isoproterenol-induced Myocardial Infarction in Rats

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Abstract

Purpose: The present study was designed to evaluate the hypoglycaemic and hypolipidaemic activities of the aqueous extract of nutmeg (i.e., the kernel of *Myristica fragrans*) in rat models against myocardial infarction (MI) induced by isoproterenol (ISO).

Methods: Rats were pretreated with nutmeg extract (NM) at an oral dose of 100 mg/kg/day for a period of 30 days, followed by the induction of MI by subcutaneous administration of ISO (85 mg/kg) for two consecutive days. The heart tissue was excised immediately, washed with chilled isotonic saline and used in histopathological studies. Blood was also collected from the animals and the plasma separated was subjected to biochemical analysis.

Results: In ISO-administered group, a significant ($p < 0.05$) increase in the levels of blood glucose, plasma lipids and lipoprotein lipase activity was observed along with hyalinization of muscle fibres, compared NM-pretreated ISO-administered rats. In rats treated with NM, biochemical parameters were near normal. Histological studies revealed reduced damage of heart tissue in ISO-administered rats that were pretreated with NM.

Conclusion: NM possesses protected rats against hyperglycaemia, hyperlipidaemia and cardiac tissue damage following MI. Therefore, NM should be further investigated as a prophylactic against the risk of MI.

Key words: Isoproterenol; Myocardial infarction; Glucose; Lipids; Lipoprotein lipase; Nutmeg; *Myristica fragrans*.

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INTRODUCTION

Cardiovascular diseases (CVD) remain the principal cause of death in both developed and developing countries, accounting for roughly 20% of all annual deaths worldwide. Myocardial infarction (MI) is the rapid development of myocardial necrosis caused by critical imbalance between oxygen supply and the demand of the myocardium. Oxidative stress resulting from increased production of free radicals associated with decreased levels of antioxidants in the myocardium plays a major role in CVD such as ischemic heart disease, atherosclerosis, congestive heart failure, cardiomyopathy and arrhythmias¹. Damage to the myocardial cells occurs due to the generation of toxic reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radical².

Isoproterenol (ISO), a synthetic catecholamine and β -adrenoceptor agonist, has been found to cause severe stress in the myocardium resulting in infarct-like necrosis³. Among the various mechanisms proposed to explain the ISO-induced cardiac damage, generation of highly cytotoxic free radicals through the auto-oxidation of the drug has been proposed as one of the important causative factors⁴. The consumption of diets rich in plant foods is associated with reduced risk of CVD, and this has been observed in numerous epidemiological studies⁵.

Spices have been used since ancient times not only for increasing the flavor of foods but also for their preservative and medicinal properties. *Myristica fragrans* (family: Myristicaceae) is a tropical, evergreen tree native to the Moluccas or Spice Island of Indonesia. Nutmeg is the dried seed kernel of *Myristica fragrans* and is widely used as spice and also to flavor many kinds of baked goods and vegetables. Nutmeg possesses antifungal, hepatoprotective⁶ and antioxidant properties⁷. Recent studies indicate that it is useful against damage caused by gamma radiation⁸ and also in the improvement of

mouse memory⁹. Anti-inflammatory¹⁰, antidysentric¹¹, analgesic and hypotensive¹² activities of nutmeg have also been reported, in addition to its insulin-like biological activity¹³.

The present work seeks to investigate the protective effect of the aqueous extract of nutmeg (NM) on rats in which MI was induced by ISO,

MATERIALS AND METHODS

Materials

NM was received as a gift from Chemiloids (batch no: SR/KN/CL/1/2007), manufacturers and exporters of chemicals, alkaloids and herbal extracts, based in Vijayawada, Andhra Pradesh, India.

Animals and Experimental protocol

Adult male rats of Wistar strain, weighing 120-150g, were procured from the National Centre for Animal Science, National Institute of Nutrition, Hyderabad, India. The study was approved by Animal Ethics Committee of S.K.University, Anantapur (Reg. no. 470/01/a/CPCSEA). The rats were fed with commercial pellet rat chow (M/s. Hindustan Lever Ltd., Mumbai, India) and water *ad libitum* and maintained under standard laboratory conditions with 12 :12 h light : dark cycle. The rats were divided into four groups of eight animals each. Group I rats served as positive control received 1ml of physiological saline subcutaneously (sc) for two days while group II rats were administered ISO sc (85 mg/kg body weight/day¹⁴) dissolved in physiological saline twice at an interval of 24 h for two consecutive days. Group III rats were pretreated with NM orally (100 mg/kg bw/day) for a period of 30 days while group IV rats were pretreated with NM orally (100 mg/kg bw for a period of 30 days) and then received ISO 85 mg/kg bw/ day dissolved in physiological saline sc twice at an interval of 24 h for two consecutive days.

At the end of 30 days, the animals were fasted for 12 hours to minimize dietary effects and anaesthetized with thiopentone sodium (35 mg/kg bw, ip). Blood was drawn from the external jugular vein of the rats into heparinised tubes and plasma was separated from the cells by centrifugation at 3000 rpm for 10 min and immediately used for the estimation of glucose and lipids. The heart tissue was excised immediately, washed with chilled isotonic saline and used in histopathological studies.

Plasma glucose determination

Plasma glucose was estimated by GOD-POD enzymatic method using Monozyme diagnostic kit, which is based on Trinders's method¹⁵. To 10 µl of plasma, 1.0 ml of enzyme reagent was added, mixed and incubated at 37°C for 10 min, and then 1ml of distilled water was added. A series of glucose standards ranging from 0.2 to 1.0 ml was placed in test tubes and made up to 1.0 ml with distilled water. One ml of the enzyme reagent was added to the standards and incubated at 37°C for 10 min. followed by the addition of 1.0 ml of distilled water. The red color was read spectrophotometrically at 505 nm against blank (distilled water) and the results were expressed as mg/100 ml.

Total cholesterol assessment

Plasma total cholesterol was estimated by enzymatic method using Monozyme diagnostic kit, as described by Allian et al¹⁶. To 1.0 ml of freshly reconstituted enzyme reagent, 0.01ml of plasma was added, mixed well and incubated at 37°C for 5 min. Extinction was measured spectrophotometrically at 505 nm against the blank (distilled water). The results were expressed as mg/100 ml.

Triglycerides determination

Plasma triglycerides were estimated by enzymatic method using Monozyme diagnostic kit, based on the technique of

Fossati and Principe¹⁷. To 0.01 ml of plasma, 1.0 ml of the reagent was added, mixed and incubated at 37°C for 10 min. Triglyceride standard and blank (distilled water) were also treated in a similar manner. Absorbance was read at 510nm and the values expressed as mg/dl.

Free fatty acids evaluation

Plasma free fatty acids were estimated by the method of Itaya and Ui¹⁸. To 6 ml of chloroform in a glass-stoppered tube, 1 ml of 0.5M phosphate buffer (pH 6.5) and 0.2 ml of plasma were added. The tubes were shaken for 90 sec, allowed to stand for 15 min and centrifuged. The chloroform layer was transferred to another stoppered tube and 3ml of Cu triethanolamine reagent was added. Tubes were allowed to stand for 15 min and centrifuged. The chloroform layer was filtered through Whatman No 1 filter paper. To the filtrate, 0.2 ml of 0.4% sodium diethyl dithiocarbamate was added. The intensity of the color developed was read against reagent blank at 440nm. Standard solutions of palmitic acid were run under similar conditions and the concentration of the sample calculated was expressed as mg/dl.

Phospholipids

Total plasma phospholipids were estimated by the method of Connerty¹⁹. To 1.0 ml of plasma, 16 ml of ethanol-ether mixture (3 : 1 v/v) was added dropwise with constant shaking. It was heated carefully to boiling, cooled, made up to 20 ml with more ethanol-ether mixture and filtered. 8.0 ml of the filtrate was taken and evaporated to dryness over hot water bath. To the residue, 1.0 ml of conc. sulphuric acid (H₂SO₄) was added and heated gently. When the mixture turned brown, a drop of hydrogen peroxide (H₂O₂) was added, heated further and H₂O₂ was added repeated at intervals until digestion was complete (as indicated by the change of the colour of the liquid to colourless. Few ml of water was added and boiled for a few

seconds. The liberated phosphorus was evaluated by Fiske Subbarow method.

Lipoprotein lipase

Plasma lipoprotein lipase was estimated by the method of Cherry and Crandall²⁰. The mixture, comprising of 1.0ml of enzyme source, 3ml of distilled water, 0.5ml of 0.33M phosphate buffer and 2.0ml of oil emulsion, was incubated at 37° C for 24 h and the reaction was arrested by the addition of 3ml of ethanol. Boiled enzyme source was used in the control instead of the fresh enzyme. The liberated free fatty acids were titrated with NaOH in the presence of 0.1ml phenolphthalein. One unit of activity is represented by 1ml of 0.05N NaOH required for the titration of the liberated acid from the olive oil due to hydrolysis by the lipases, and expressed as amount of glycerol formed in mg/ml.

Histological studies

Histological examination of tissue was carried out by Raghuramulu method²¹. Tissue fixation was carried out, immediately after removal from the body, with 10% neutral buffered formaldehyde solution (pH 7.0). Tissue processing was carried out as per the schedule for dehydration, clearing and paraffin infiltration. The container was filled with melted paraffin and allowed to cool to form a block of paraffin with the tissue. The block was trimmed to remove paraffin overlaying the tissue, and sections of 5µm

thickness were cut and mounted on clean glass slides which have been smeared with a drop of Mayer's egg albumin. It was then dried on a hot plate at about 50 °C for 30 min, stained with hematoxylin and eosin, and examined under a light microscope.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The results were expressed as mean ± S.E.M for rats in each group. Values of P < 0.05 were considered as significant.

RESULTS

Table 1 depicts the levels of plasma total cholesterol, triglycerides, free fatty acids (FFA) and phospholipids (PL) in normal and ISO-administered rats. Subcutaneous administration of ISO caused a significant (P < 0.05) rise in the levels of cholesterol (34.9 %), triglycerides (51.9 %), FFA (63.0 %) and PL (26.5 %) in plasma of rats, compared with positive control rats. However, in NM-pretreated rats that were given ISO, changes in the levels of cholesterol (11.0 %), triglycerides (21.7 %), FFA (53.7 %), and PL (10.6 %) were lower. NM pretreatment maintained these levels at near normal, i.e., 1.86%, 5.54%, 5.65% and 0.30% for cholesterol, triglycerides, FA and PL respectively.

Table 1: Effect of nutmeg extract (NM) and isoproterenol (ISO) on plasma lipid profile in normal and experimental rats

Group	Total cholesterol	Triglycerides	Free fatty acids	Phospholipids
Control	96.5±8.4 ^a	39.7±3.4 ^a	24.6±2.0 ^a	79.1±6.4 ^a
ISO	130.2±11.9 ^b	60.3±6.1 ^b	40.1±2.6 ^b	100.1±3.8 ^b
NM	94.7±7.8 ^a	37.5±2.1 ^a	23.2±1.2 ^a	78.9±5.7 ^a
NM+ISO	107.1±9.3 ^c	48.3±4.0 ^c	37.8±1.6 ^c	87.5±2.1 ^c

Each value is a mean ± S.E.M., n = 8. Values not sharing a common superscript (a–c) differ significantly with each other (P < 0.05).

In rats that received ISO, blood glucose level (see Figure 1) increased significantly ($P < 0.05$) by 27.1 %, while glucose level in NM-pretreated rats decreased insignificantly ($P < 0.05$) by 3.9 % compared to control. On the other hand, in NM-pretreated rats that received ISO, blood glucose increased by 8.8 %, when compared with control. Lipoprotein lipase activity data presented in Figure 2 show a significant ($P < 0.05$) decrease (35.2 %) in the activity of the enzyme in rats that received ISO when compared with controls, while the enzyme level were near normal in NM-treated (4.8 %) and NM-pretreated rats that also received ISO (4.35%).

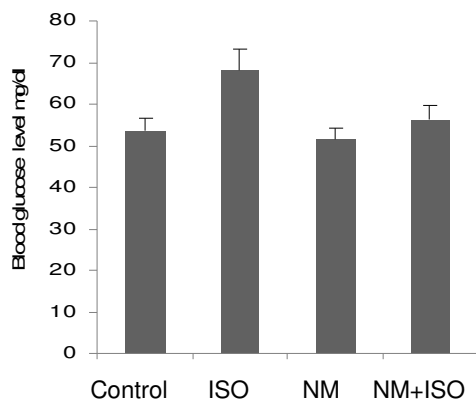


Figure 1: Blood glucose levels of control and experimental rats (Each bar is mean \pm S.E.M., $n = 8$; ISO = isoproterenol; NM = Nutmeg extract)

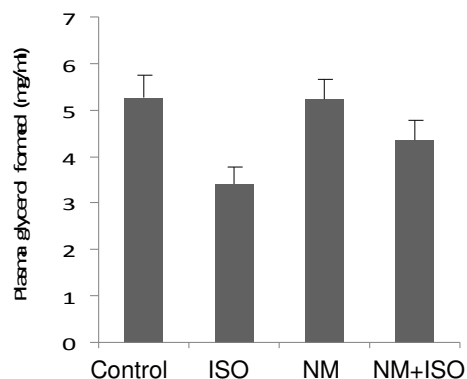


Figure 2: Changes in the activities of plasma lipoprotein lipase of control and experimental rats (Each bar is mean \pm S.E.M., $n = 8$; ISO = isoproterenol; NM = Nutmeg extract)

Photomicrographs of histopathological sections of heart tissues of the rats are shown in Figure 3, The architecture is normal in control (Figure 3(a)) and NM-treated (Figure 3(c)) groups, but increased hyalinization occurred in rats treated ISO with only (Figure 3(b)) and minimal damage in the NM-pretreated rats that also received ISO (Figure 3(d)).

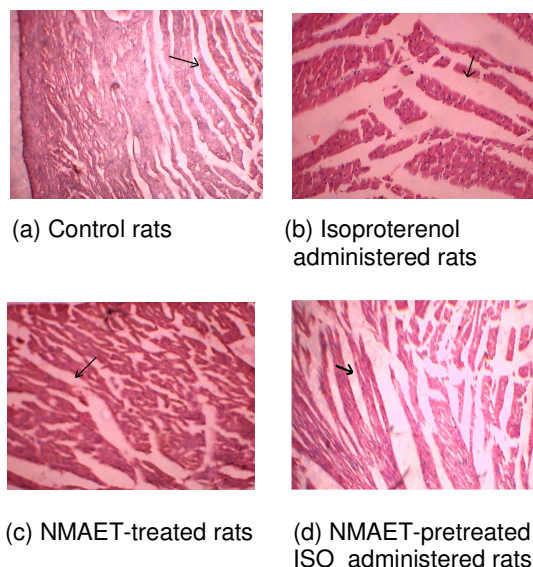


Figure 3: Photomicrograph of heart in normal and experimental animals. (**Note:** ISO = isoproterenol; NMAET = Nutmeg extract)

DISCUSSION

ISO produces relative ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension²², and induce myocardial ischemia due to cytosolic Ca^{2+} overload²³. In the group given ISO, blood glucose level increased compared to the control group. Increased levels could be due to enhanced glycogenolysis and lipolysis, through the activation of β -adrenergic receptor and phosphorylase kinase enzyme. Experimentally, ISO is capable of producing gross and microscopic myocardial necrosis and depletion of tissue enzymes in the heart. ISO-induced MI serves as a well standardized model to study the beneficial

effects of many drugs and cardiac function since it mimics the clinical conditions of myocardial infarction due to ischemia in humans²⁴. NM-pretreated rats administered ISO showed decrease (18.3%) in blood glucose when compared to rats given ISO alone, This observation is in accordance with the earlier reports of Leigh et al¹³ and this may be due to the insulin-like biological activity of NM.

Lipids play an important role in CVD, not only by way of hyperlipidaemia and the development of atherosclerosis, but also by modifying the composition, structure and stability of cellular membranes²⁵. ISO-treated rats showed an increase in the level of plasma lipids. The increased levels of plasma cholesterol and triglycerides observed in ISO-injected rats could be due to enhanced lipid biosynthesis by cardiac cyclic adenosine monophosphate²⁶. Plasma hypertriglyceridaemia, which was observed in ISO-treated rats, is due to decrease in the activity of lipoprotein lipase (Fig 2), resulting in decreased uptake of triglycerides from circulation. Hypertriglyceridaemia and increased levels of cholesterol in plasma might be responsible for altered cardiovascular functions which are often reported in ISO-induced myocardial infarction (MI)²⁷.

Increase in plasma phospholipids in rats treated with ISO was observed. Increased peroxidation of membrane causes release of phospholipids and free fatty acids via phospholipase A2²⁸. FFA is a substrate for microsomal lipid peroxidation. The higher level of plasma FFA in animals treated with ISO is due to increased lipolysis²⁹. Paritha and Devi²⁶ have also reported an increase in plasma PL in ISO-treated rats. In the group treated with NM alone the levels of cholesterol, triglycerides, FFA and PL were maintained at near normal which could be due to effective quenching of free radicals by active phytochemicals in NM, such as flavonoids, saponins and cardiac glycosides³⁰ in conjunction with its antioxidant properties³¹

thus reducing membrane lipidperoxidation, and hence decreased release of PL and FFA. The results of this study are in agreement with those of previous studies where it was reported that faecal excretion of cholesterol and PL³² and decreased activity of lipoprotein lipase³³ are responsible for decreased lipid levels in NM-pretreated rats that were given ISO.

Histopathological studies

The histopathological section of the control group (see Fig 3a) reveals normal architecture of the myocardium, with intact muscle fibres. The heart tissue of the rats given ISO (shown in Fig 3b) indicates hyalinization of muscle fibres, with focal cellular infiltration or necrosis of muscle fibres. Collection of inflammatory exudate is seen in the focal cells clearly, indicating that the heart tissue was damaged as a result of ISO administration. The heart tissue of NM-treated animals, displayed in Fig 3(c), shows no changes in cardiac structure and is similar to that of the control group. This indicates that NM did not damage the cardiac tissue. As Fig 3(d) reveals, there is minimal damage, with mild swelling of muscle cells and focal cardiac muscle fibres, in the NM-pretreated rats that received ISO. Thus NM has some protective effect on the myocardium against ISO.

CONCLUSION

This study shows that increased levels of lipids and blood glucose induced by ISO were decreased following pretreatment with NM, thus indicating that the extract has some anti-hyperlipidaemic and hypoglycemic effects in rats with MI. Furthermore, NM-pretreatment proved to be effective in reducing the extent of myocardial damage by decreasing blood lipid overload and this suggests that the extract could offer protection to the myocardium.

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