CARDIOPROTECTIVE EFFECT OF NIGELLA SATIVA SEED AND OIL ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

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INTRODUCTION

Cardiovascular diseases have become the number one killer disease in many parts of the world. An increased risk of coronary heart disease is associated with a high serum cholesterol concentration and low density lipoprotein (LDL) and decreased high density lipoprotein (HDL) (Sheela Sasikumar and Shyamala Devi, 2001). Myocardial infarction is commonly known as a heart attack. This is most commonly due to occlusion (blockage) of a coronary artery following the rupture of a vulnerable atherosclerotic plaque, which is an unstable collection of lipids (fatty acids) and white blood cells (especially macrophage) in the wall of an artery (Mallinson, 2010).

Although modern drugs are effective in preventing the disorders their use in often limited because of their side effects and adverse reactions. A wide assay of plants an alternative therapy for ischemic heart disease. Moreover, the plant kingdom represents a largely unexplored reservoir of biologically active compounds.

Nigella sativa commonly known as black seed have been used for nutritional and medicinal purposes in many Middle Eastern countries, and other parts of the world (El-Dakhakhani et al., 2000; Ghosheh et al., 1990). They have been traditionally used in the treatment of a number of ailments including respiratory health, stomach and intestinal health, kidney hypertension, bladder and liver function, circulatory and immune system supported and for general overall well being (Baser et al., 1986; Handa, 1998; Deliorman et al., 2002; Malhotra, 2006) N.sativa has been shown to produce multi-systemic beneficial actions (Ali and Blunden, 2003), including hypoglycemic (Bamosa et al., 1997), hypocholestermic (Bamosa et al., 2002) and antioxidant effects.

In the present investigation on cardio protective activity was conducted on isoproterenol induced myocardial infarcted rats were given pretreatment with or without crude and ethanolic extract of Nigella sativa effect and protection was studied mainly on the enzymes and lipid metabolism.

MATERIALS AND METHODS

Plant material

The Nigella sativa is collected from Adhiparasakthi Agricultural college medicinal park, in kalavai- 632506, Tamil Nadu-India. The Nigella sativa was dried and crushed to fine powder formed.

Animals

Healthy adult male Swiss albino rats (weighing 160-250g) were used throughout the experiment. Animals were maintained at 22 ± 2°C with 45-55% relative humidity, 12h light and dark cycle. They were housed in well-ventilated polyurethane cages and have free access of tap water and laboratory pellet feed.

Preparation of crude extract

100g of N.sativa seed were grinded and mixed with water

KEY WORDS

Nigella sativa
Isoproterenol
LDH
Myocardial infarction

ABSTRACT

The cardio protective effect of black cumin crude and ethanolic extract was studied on some biochemical parameters in male albino rats. The values were compared with known cardio protective agent verapamil. The rats were divided into five groups. Group I serve as a healthy control, group II was induced with Isoproterenol (2mg/kg/body wt) intravenously after 48h the rats were sacrificed, group III was treated with standard Verapamil (3μmol/kg/body wt) for 2 days, followed by Isoproterenol after 2nd day of administration intravenously. After 48h the rats were sacrificed. The Nigella sativa seed extract was given in the dosage of (1000mg/kg/body wt) and oil extract was (2ml/ kg/body wt) given orally for 21 days daily to the rats of group IV and V followed by isoproterenol as in group II. The observation clearly indicates the level of LDH, CPK, AST, ALT, lipid profile, etc. were altered in myocardial infarction condition when compare to normal. The altered levels of all the parameters were maintained at near normal on pretreatment with Nigella sativa extracts. The above study clearly indicates the benefits of raw black cumin.

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and given to the rats. The crude powder extract was also given at the dose of 1000 mg/kg body weight.

**Preparation of ethanolic extract**

100g of *N. sativa* seed were grinded and extract with 200 mL of ethanol by the method of continuous hot extraction at 60ºC for 6h and evaporated. The extract yielded was viscous oil. This residual extract was dissolved in DMSO (dimethyl sulfoxide) 2 mL/kg of dose was given orally to the rats.

**Experimental design**

In the experimental a total of 30 rats were used. They were divided in to five groups in each group six rats were selected. Group I is a healthy control, group II was induced with Isoproterenol (2mg/kg/body wt) intravenously. After 48 hours the rat were sacrificed. Group III was treated with standard Verapamil (5μmol/kg/body wt) for 2 days, followed by Isoproterenol after 2nd day of administration intravenously. After 48h the rats were sacrificed. The Nigella sativa seed extract was given in the dosage of (1000mg/kg/body wt) and oil extract was (2mL/kg/body wt) given orally for 21 days daily to the rats of group IV and V followed by Isoproterenol as in group II.

**Sample collection**

After 48h of Isoproterenol administration the animals were sacrificed by decapitation. Blood sample were collected and serum is separated for all biochemical analysis. Heart tissue was dissected out and washed in ice cold saline immediately.

**Histopathological studies**

Histopathological evaluation was performed on lower portion of the heart tissue. Fresh heart tissue were excised and then fixed in 10% formalin for 24h. The fixative was removed by washing through running tap water for overnight. After dehydration through a graded series of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Section were cut into 5μm thickness and stained with hematoxylin and cosin. After repeated dehydration and cleaning, the sections were mounted and observed under light microscope with magnification of 10 xs for histological changes.

**Biochemical studies**

Lactate dehydrogenase and creatinine phosphokinase was studied by the method of king (1965) and Okinaka et al., (1961). The activities of Aspartate transaminase and alanine transaminase were estimated by the method of Reitmann and Frankel (1957). The levels of creatine and protein were estimated by the method of Reitmann and Frankel (1957). The level of transaminase were transaminase and alanine transaminase in normal and experimental rats. Injection of single high dose of Isoproterenol induced the necrotic damage to the myocardial membrane (Manjula et al., 1992).

Isoproterenol is well known cardio toxic agent due to its ability will destruct myocardial cells. As a result of these cytosolic enzymes Aspartate transaminase and alanine transaminase were released in to blood stream and serve as the diagnostic markers of myocardial damage (Gurgun et al., 2008).

The aim of the present investigation was to evaluate the protective effect of pretreatment of *Nigella sativa* medicinal plant in Isoproterenol induced rats. Our results clearly indicates that the consumption of *Nigella sativa* seed (1000mg/kg/body wt) and oil (2mL/kg/body wt) maintain the cholesterol, enzymes and the other biochemical parameters in the level of significance <0.01, <0.05 in myocardial infracted rats, when compare to normal rats.

Table 1 depicts the levels of serum enzymes creatinin phosphokinase (CPK), lactate dehydrogenase, Aspartate transaminase, alanine transaminase was released in to blood stream and serve as the diagnostic markers of myocardial damage (Jorge et al., 1998).

**RESULTS AND DISCUSSION**

The prior administration of *Nigella sativa* seed crude powder and ethanolic oil extract showed a significantly (<0.01 and <0.05) maintain the levels of serum marker enzymes. Because the reduced extent of myocardial damage and therefore restricted the leakage of these enzymes from the myocardium (Jorge et al., 1998). Table 2 depicts the levels of cholesterol, triglycerides, HDL, LDL, and VLDL in normal and experimental rats. The level of cholesterol, triglycerides, LDL, VLDL is increased and HDL is decreased in myocardial infarcted group II rats. The pretreatment on *Nigella sativa* seed oil showed significantly <0.01 and <0.05 maintain the level in group IV and V, when compare to group I normal rats.

High level of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage.

**Figure 1: Electrophoretic pattern of LDH isoenzymes in serum of Normal and experimental animals**

Lane 1 = Normal; Lane 2 = Isoproterenol induced; Lane 3 = *Nigella sativa* crude powder extract treated; Lane 4 = *Nigella sativa* oil ethanolic extract treated
Table 1: Activities of serum CPK, LDH, AST, ALT in normal, induced, standard, Nigella sativa seed and oil treated rats. Values are expressed as Mean±SD

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Normal</th>
<th>Inducer Isoproterenol</th>
<th>Verapamil + Isoproterenol</th>
<th>Nigella sativa seed + Isoproterenol</th>
<th>Nigella sativa oil + Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CPK</td>
<td>80.54±0.875</td>
<td>145.57±0.916</td>
<td>75.38±0.702</td>
<td>82.34±0.535*</td>
<td>84.116±2.874**</td>
</tr>
<tr>
<td>2.</td>
<td>LDH</td>
<td>145.79±1.167</td>
<td>219.42±0.50</td>
<td>148.69±1.022</td>
<td>148.69±1.022*</td>
<td>149.23±2.810**</td>
</tr>
<tr>
<td>3.</td>
<td>AST</td>
<td>55.53±O.561</td>
<td>95.70±0.873</td>
<td>53.601±0.510</td>
<td>60.21±2.775*</td>
<td>61.13±5.391**</td>
</tr>
<tr>
<td>4.</td>
<td>ALT</td>
<td>19.4±0.718</td>
<td>25.6±0.802</td>
<td>19.4±6.65</td>
<td>23.64±2.309*</td>
<td>22.73±2.320**</td>
</tr>
</tbody>
</table>

Level of significance p<0.01*, p<0.05**, CPK, LDH, AST- IU/L

Table 2: Activities of serum cholesterol, triglycerides, HDL, LDL and VLDL in normal, induced, standard, Nigella sativa seed and oil treated rats. Values are expressed as Mean±SD

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Normal</th>
<th>Inducer Isoproterenol</th>
<th>Verapamil + Isoproterenol</th>
<th>Nigella sativa seed + Isoproterenol</th>
<th>Nigella sativa oil + Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cholesterol</td>
<td>73.95±1.15</td>
<td>161.15±0.72</td>
<td>79.70±0.72</td>
<td>77.88±3.30*</td>
<td>79.81±10.72**</td>
</tr>
<tr>
<td>2.</td>
<td>Triglycerides</td>
<td>7.29±0.39</td>
<td>16.93±0.49</td>
<td>7.79±0.45</td>
<td>10.68±2.51*</td>
<td>10.31±2.50**</td>
</tr>
<tr>
<td>3.</td>
<td>HDL</td>
<td>45.52±0.65</td>
<td>31.51±0.907</td>
<td>49.36±7.80</td>
<td>45.94±1.90*</td>
<td>44.80±1.95**</td>
</tr>
<tr>
<td>4.</td>
<td>LDL</td>
<td>31.54±0.51</td>
<td>45.12±0.306</td>
<td>29.84±0.56</td>
<td>30.72±0.668*</td>
<td>30.94±1.37**</td>
</tr>
<tr>
<td>5.</td>
<td>VLDL</td>
<td>29.41±0.59</td>
<td>35.48±0.99</td>
<td>29.84±0.56</td>
<td>30.72±0.668*</td>
<td>30.94±1.37**</td>
</tr>
</tbody>
</table>

Level of significance p<0.01*, p<0.05**, Cholesterol, triglycerides, HDL, LDL, VLDL- mg/dl

Table 3: Activities of serum troponin T and protein in normal, standard, Nigella sativa seed and oil treated myocardial infarcted rats. Values are expressed as Mean±SD

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Normal</th>
<th>Inducer Isoproterenol</th>
<th>Verapamil + Isoproterenol</th>
<th>Nigella sativa seed + Isoproterenol</th>
<th>Nigella sativa oil + Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Troponin T</td>
<td>0.385±0.10</td>
<td>1.27±0.57</td>
<td>0.383±0.081</td>
<td>0.65±0.131*</td>
<td>0.628±0.172**</td>
</tr>
<tr>
<td>2.</td>
<td>Protein</td>
<td>6.28±0.228</td>
<td>7.91±0.68</td>
<td>6.28±0.327</td>
<td>6.79±0.356*</td>
<td>6.92±0.669**</td>
</tr>
</tbody>
</table>

Level of significance p<0.01*, p<0.05**

(Sheela Sasikumar and Shyamala Devi, 2001)

Isoproterenol administration resulted in significant increase in the free fatty acid level. Hypertriglyceridemia seen in Isoproterenol treated rats, a condition observed in ischemic heart disease, is due to decrease in the activity of lipo protein lipase in the myocardium resulting in decrease uptake of triglycerides from circulation.

The N. sativa seed and oil is contains thymoquinone may be responsible for the hypocholesteremic action in rats. The levels are maintained at near normal when compared to the normal in group IV and group V rats (Badary et al., 2000; Zaoui et al., 2002).

Administration of Isoproterenol mainly raised LDL cholesterol and decreased HDL cholesterol in group II experimental rats. High levels of LDL cholesterol have positive relation and decreased levels of HDL cholesterol have negative relation with myocardial infarction (Sheela Sasikumar and Shyamala Devi, 2001).

They have also reported HDL inhibits the uptake of LDL by arterial wall and also facilitated the transport of cholesterol from peripheral tissue to the liver where it is catabolised and excreted out of the body.

The pretreatment with Nigella Sativa seed and oil increases the HDL cholesterol. The extract mechanism of action of Nigella Sativa is not known, however it has been proved that volatile oil of Nigella Sativa has two main constituents (i.e.) Nigellone and Thymoquinone which play a key role in prevention of heart disease (Gad et al., 1963; Babayan et al., 1978; Abdel and Attia, 1993).

Tale 3 depicts the levels of protein, Troponin T in normal and experimental rats. The pretreatment on Nigella sativa seed and oil showed significantly <0.01 and <0.05 maintain the level in group IV and V, when compare to group I normal rats.

Troponin-T ng/dl
Protein g/dl

Figure 2, 3, 4 and 5: The histopathological study of different group of rats normal and experimental rats; Figure 2: A normal rat was showing normal architecture of the heart (HE× 10x); Figure 3: ISPH Myocardial infracted rat showing degenerative changes, hyalihization of muscle fibers and cellular infiltration (HE×10x); Figure 4 and 5: Nigella sativa seed and oil pretreated rat reveals less cellular infiltration, normal muscle fibers and the cardio protective effect are evident from reduced myocardial damage even after ISPH administration (HE× 10x)
Tropinin-T is a protein found in cardiac tissue. When the myocardial damage occurs by cytosolic tropinin reach the blood stream quickly resulting in a rapid peak of serum tropinin (Nigam, 2007).

In this study, significant increased level of tropinin T in serum of Isoproterenol treated rats. Increased level of tropinin was due to leakage from the damaged heart tissue into the blood stream as a result of necrosis induced by Isoproterenol in rats. Pretreatment with Nigella sativa powder and oil to Isoproterenol treated group IV and V rats restored the level of tropinin-T in serum indicates the protective action of Nigella sativa against peroxidative damage.

The increased level of protein was observed in the serum of Isoproterenol administered rat is, at least in part, due to leakage of enzymes and protein bound components from the damaged myocardium to the systemic circulation.

Thymoquinone (TQ), bioactive and the most abundant constituent of the volatile oil of the black seed, has been shown to possess an anti-inflammatory and antioxidant effects. These are also may be the protective action, of Nigella sativa seeds and oil reduces the leakage of enzymes protein bound components.

So it may be the responsible for the protective action of Nigella sativa in experimental group IV and V rats (Houghton et al., 1995 and Keuk et al., 2000).

Electrophoretic pattern of LDH isoenzymes

The Myocardial activity of LDH is high enough to make it unlikely that it could be a controlling step for lactate metabolism by the heart. In acute myocardial infarction, an estimate of the appearance and disappearance of LDH by the heart. In acute myocardial infarction, an estimate of the unlikely that it could be a controlling step for lactate metabolism of Adhiparasakthi College of Arts and Science.

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