

## Cardioprotective effect of coconut kernel protein in isoproterenol administered rats

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Male albino rats were given subcutaneous injection of isoproterenol (10 mg/100g body wt) twice at an interval of 24 hr to induce myocardial infarction. The rats showed massive myocardial necrosis and increased activities of creatinine phosphokinase (CPK), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), in serum, while a decrease in nitric oxide synthase activity and lower levels of palmitate oxidation into CO<sub>2</sub> and ATP were observed in the heart. Rats pre-treated with coconut protein or L-arginine showed significantly decreased CPK, GOT and GPT activities in the serum. There was significantly higher nitric oxide synthase activity and higher rate of palmitate oxidation into CO<sub>2</sub> and increased levels of ATP in the heart in these groups. These observations indicate the cardioprotective effect of coconut protein, which may be attributed to the high content of L-arginine present in it.

Supplementation of animal protein with vegetable protein has been reported to reduce lipid levels in experimental animals and humans<sup>1</sup>. Several plant proteins including black gram protein<sup>2</sup> and cashew kernel protein<sup>3</sup> were studied in this laboratory to investigate their effect on cholesterol metabolism. Both of them showed significant cholesterol-lowering activity. Coconut kernel, a regular dietary item of the population in Kerala, contains 5–6 % proteins; the major fraction being the globulins<sup>4</sup>, which showed significant cholesterol-lowering activity in rats fed cholesterol-free and cholesterol containing diet. Analysis of the coconut kernel globulin<sup>4</sup> gave 2.13% lysine and 24.5% arginine, giving a low lysine/arginine ratio of 0.086. Previous studies have shown that lysine/arginine ratio of the protein has a significant influence on the metabolism of cholesterol<sup>5,6</sup>. Inclusion of arginine to a casein diet results in significant lowering of cholesterol levels<sup>7</sup>, while addition of lysine has the opposite effect<sup>8</sup>. Recent studies in experimental animals and in patients with coronary vascular disease suggest the potential use of orally administered L-arginine as a therapeutic agent to reduce myocardial infarction<sup>9-11</sup>. In addition, it has been reported that oral administration of L-arginine at 2% level has significant cardioprotective effect in isoproterenol-induced myocardial infarction in male albino rats<sup>12</sup>.

In view of the very high content of arginine (~24%) present in coconut kernel protein, the present study was carried out to investigate the effect of administration of coconut protein on various biochemical functions in isoproterenol administered rats. The results were also compared with rats administered L-arginine.

### Materials and Methods

Male albino rats (sprague-dawley strain, weight 200 ± 5g) were divided into three groups with 20 rats in each group. The diet for group I contained 16% casein, group II 8% casein and 8% coconut protein and group III 16% casein and L-arginine at 2% level in drinking water. Water was available *ad libitum*.

Myocardial infarction was produced in half the number of rats in each group by subcutaneous injection of isoproterenol (10 mg/100 g body wt.) twice at an interval of 24 hr. Rats administered isoproterenol exhibited signs of shock, tachycardia, dyspnea, rapid respiration, anuria and prostration. The animals surviving the second injection were sacrificed at 36 hr after the first injection. Animals in each group were deprived of food overnight, stunned by a blow at the back of the neck and sacrificed by decapitation. Blood and tissues were removed to ice cold containers for various estimations. Activities of creatine phosphokinase (EC.2.7.3.2)<sup>13</sup>, glutamate oxaloacetate transaminase (EC.2.6.1.1)<sup>14</sup> and glutamate pyruvate transaminase (EC.2.6.1.2)<sup>14</sup> in the serum were determined. Concentration of ATP<sup>15</sup> and radio-

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labelled studies of  $\beta$ -oxidation<sup>16</sup> and nitric oxide synthase<sup>17</sup> activity in the heart were also studied. Activity of CPK, GOT oxaloacetate and GPT is expressed as  $\mu$ moles of creatinine, oxaloacetate pyruvate liberated/min/l, respectively and nitric oxide synthase expressed as units/mg protein.

#### Statistical analysis

Results are expressed as mean  $\pm$  SD of six rats. The significance of the differences between mean values was determined using student's 't' test and considered significant if  $p < 0.05$ .

#### Results

Administration of isoproterenol resulted in increased glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activity in the serum, when compared to normal rats. In coconut protein fed rats, isoproterenol administration resulted in decreased activities of GOT and GPT, when compared to those fed casein alone. Creatine phosphokinase (CPK) activity in the serum was higher in all isoproterenol treated groups, when compared to normal rats. In coconut protein and L-arginine fed rats, isoproterenol administration resulted in decreased CPK activity; the activity of CPK was

significantly lower in rats fed coconut protein than those fed L-arginine (Table 1).

Nitric oxide synthase activity in the heart decreased significantly in isoproterenol treated groups, when compared to control, while feeding coconut protein and L-arginine resulted in significant increase in nitric oxide synthase activity; the activity of the enzyme was significantly lower in rats fed L-arginine than those fed coconut protein (Table 2).

Concentration of both CO<sub>2</sub> and ATP was decreased significantly in isoproterenol treated groups, when compared to normal rats. On the other hand in coconut protein and L-arginine fed rats, isoproterenol administration resulted in significant increase in the concentration of CO<sub>2</sub> and ATP, when compared to casein fed alone. The concentration of CO<sub>2</sub> was higher in isoproterenol administered rats fed coconut protein than those fed L-arginine. No significant difference was observed in concentration of ATP in rats fed coconut protein and L-arginine treated with isoproterenol (Table 2).

#### Discussion

Isoproterenol treated rats showed increased activity of CPK, GOT and GPT in the serum as compared to control (Table 1) indicating that induction of

Table 1—Activities of CPK, GOT and GPT in the serum  
[Average of the values from 6 rats in each group  $\pm$  SD]

Animal group	CPK ( $\mu$ moles of creatinine min/l)	GOT ( $\mu$ moles of oxaloacetate min/l)	GPT ( $\mu$ moles of pyruvate min/l)
Casein	1446.7 $\pm$ 33.3	125.9 $\pm$ 2.8	54.1 $\pm$ 1.2
Casein + isoproterenol	2892.4 $\pm$ 75.2 <sup>a</sup>	160.8 $\pm$ 3.7 <sup>a</sup>	65.3 $\pm$ 1.5 <sup>a</sup>
Casein + coconut protein	1560.7 $\pm$ 29.7	117.7 $\pm$ 2.8	46.4 $\pm$ 1.2
Casein + coconut protein + isoproterenol	2032.1 $\pm$ 48.8 <sup>a</sup>	139.9 $\pm$ 3.2 <sup>a</sup>	60.7 $\pm$ 1.5 <sup>a</sup>
Casein + L-arginine	1608.2 $\pm$ 37.0	105.1 $\pm$ 2.2	9.9 $\pm$ 1.1
Casein + L-arginine + isoproterenol	2456.3 $\pm$ 66.3 <sup>a</sup>	137.6 $\pm$ 2.8 <sup>a</sup>	61.1 $\pm$ 1.2 <sup>a</sup>

a,  $p < 0.01$

Table 2—Nitric oxide synthase activity, [<sup>14</sup>C]-palmitate oxidation to CO<sub>2</sub> and ATP concentration in the heart  
[Average of the values from 6 rats in each group  $\pm$  SD]

Animal group	Nitric oxide synthase (units /mg protein)	[ <sup>14</sup> C]-palmitate oxidation to CO <sub>2</sub> ( $\mu$ mole/g/min)	Concentration of ATP (n mole/ mg protein)
Casein	0.903 $\pm$ 0.022	18.3 $\pm$ 0.38	22.2 $\pm$ 0.47
Casein + isoproterenol	0.694 $\pm$ 0.015 <sup>a</sup>	12.6 $\pm$ 0.30 <sup>a</sup>	13.2 $\pm$ 0.26 <sup>a</sup>
Casein + coconut protein	1.21 $\pm$ 0.024	26.1 $\pm$ 0.52	27.5 $\pm$ 0.52
Casein + coconut protein + isoproterenol	1.05 $\pm$ 0.023 <sup>a</sup>	21.9 $\pm$ 0.39 <sup>a</sup>	19.3 $\pm$ 0.44 <sup>a</sup>
Casein + L-arginine	0.98 $\pm$ 0.02	23.6 $\pm$ 0.45	25.5 $\pm$ 0.64
Casein + L-arginine + isoproterenol	0.77 $\pm$ 0.017 <sup>a</sup>	16.4 $\pm$ 0.43 <sup>a</sup>	18.7 $\pm$ 0.52 <sup>a</sup>

a,  $p < 0.01$

myocardial infarction results in myocardial damage, which causes secretion of enzymes from the myocardium to the blood plasma.

Lower levels of uptake of palmitic acid and its oxidation to CO<sub>2</sub> were observed by Whitemer *et al.*<sup>16</sup> in ischemic and hypoxic rats. It has been reported that the oxidation was rate limiting step in fatty acid metabolism, in ischemic and hypoxic heart. Shung *et al.*<sup>18</sup> observed decrease in the concentration of ATP and creatine phosphate in the heart in myocardial infarction. Similar results were observed in the present study in isoproterenol-treated rats, suggesting that available oxygen may be more in the coconut protein administered group which may contribute to the improvement in the functioning of cardiac muscle cells. The higher levels of ATP and the ability of the heart to oxidise the labelled fatty acids at a high rate observed in the present study in isoproterenol-treated rats fed coconut protein correlates with this possibility. Judging from the serum CPK, GOT and GPT values (Table 1) it was clear that the rate of recovery from myocardial infarction was higher in the isoproterenol rats administered coconut protein.

The results of this study indicate the cardioprotective effect of coconut protein. In this connection, the observation that L-arginine administration (at 2% level) affords protection against myocardial infarction induced by isoproterenol<sup>12</sup> is relevant. In the present study, we have also observed significant cardioprotective effect on administration of L-arginine. However, the effect was lower as compared to coconut protein. In this connection reports suggest that both low and high levels of arginine administration have cytotoxic effect<sup>19,20</sup>. Low doses of arginine leads to decreased synthesis of nitric oxide which induces myocardial infarction, while high doses of arginine cause increased release of nitric oxide and continuous exposure of cardiac muscle cells to high levels of nitric oxide may reduce the cardioprotective effect.

These results indicate that the major factor responsible for the cardioprotective effect, is higher arginine content. It has been reported that cardioprotective effect of L-arginine was mediated by L-arginine-nitric oxide pathway<sup>21</sup>. Nitric oxide is synthesized in vascular endothelial cells from L-arginine by the enzyme nitric oxide synthase. Increased nitric oxide synthase activity in the heart improves cardiac function, enhances myocardial blood flow and may be beneficial in the treatment of acute myocardial infarction. Reports suggest that

nitric oxide deficiency induces myocardial infarction in hypocholesterolemic rats<sup>22,23</sup>. In the present study, isoproterenol-treated rats fed coconut protein and those administered L-arginine showed increased nitric oxide synthase activity in the heart, which may improve cardiac function.

Nitric oxide functions in the regulation of blood pressure, platelet adhesion, neutrophil aggregation as well as synaptic function in the brain. Its chemical and biological properties are well suited for its non-physiological and pathophysiological functions. It also helps in the dilation of blood vessel correcting the inadequate blood supply to the heart muscle. From these effects, it is clear that the L-arginine-nitric oxide pathway plays a protective role in cardiac function against the deleterious effects caused by the isoproterenol administration.

Thus, the results indicate that consumption of coconut protein may cause less incidence of myocardial infarction and the major factor responsible for the cardioprotective effect is its high L-arginine content.

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