Article

Effects of Peanut (Arachis Hypogaea L.) and Its Combination with Propranolol in Isoproterenol Induced Myocardial Damage in Rats

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Abstract

Background: Ischemic heart disease is the interruption of blood supply to the heart causes several biochemical alterations which may lead to cardiac dysfunction and ultimately cell death. Medicinal plants and plant based foods such as peanut (Arachishypogaea L.) have received great attention for their salutary effects and potential to treat many aspects of ischemic heart disease due to their antioxidant property. **Objective:** To observe the effects of peanut (Arachishypogaea L.) and its combination with propranolol in isoproterenol induced myocardial damage in rats. **Method:** This experimental study was carried out in the Department of Physiology, Sir Salimullah Medical College (SSMC), Dhaka in 2012. For this purpose, 20 Wistar albino rats, age 85 to 100 days, weighing 120 to 150g (initial body weight) were included in the peanut treated group.They were sub-divided into CT-P (Cardiotoxic group with isoproterenol after peanut treatment) and CT-C (Cardiotoxic group with isoproterenol after combined treatment of peanut and propranolol). Age and weight matched 30 Wistar albino rats without any peanut supplementation were taken and sub-divided into three sub-groups, BC (Baseline control), CT (Cardiotoxic group with isoproterenol) and CT-PRO (Cardiotoxic group with isoproterenol after propranolol treatment). Each sub-group consisted of 10 rats. After taking final body weight all the rats were sacrificed on 22nd day. Blood was collected from heart & supernatant serum was preserved in deep freeze until analysis. For assessment of myocardial damage, some cardiac biomarker enzymes, like serum CK-MB & LDH were estimated by using immunoassay method. The statistical analysis was done by one way ANOVA and Bonferroni test as applicable. **Result:** In this study, percent change from initial body weight to final body weight was significantly ($p<0.01$) lower both in CT-P and CT-C as compared to that of BC. Again, the mean serum CK-MB and LDH levels were significantly ($p<0.01$) higher in CT, CT-PRO, CT-P & CT-C in comparison to that of BC. On the other hand, the serum CK-MB and LDH levels were lower in CT-PRO, CT-P and CT-C when compared to that of CT though the differences were statistically significant ($p<0.01$, $p<0.05$) in case of CT-PRO and CT-C but not significant in case of CT-P. Again, the values were also significantly lower in CT-C ($p<0.01$,$p<0.05$) as compared to that of CT-P. **Conclusion:** The present study revealed that peanut can lower serum CK-MB & LDH levels towards normal in isoproterenol induced myocardial damaged rats. However, the combined therapy of peanut with propranolol showed synergistic effect on lowering serum CK-MB & LDH levels.

Key words: Peanut, Propranolol, Isoproterenol, Cardiac biomarker enzymes

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Introduction

Ischemic heart disease is the leading cause of morbidity and mortality in industrialized countries and it is emerging as a prominent public health problem in developing countries[1].

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While Bangladesh is turning from rural based socio-economic structure towards urbanization, the ischemic heart disease in middle aged and young group is also appearing at increasing level[2].

Ischemic heart disease or myocardial infarction is the acute condition of necrosis of myocardium that occurs as a result of imbalance between coronary blood supply and myocardial oxygen demand[3]. The development of myocardial ischemia or infarction is a dynamic process with the wide spread occurrence of coronary atherosclerosis and involvement of oxidative stress in the human[4].

Isoproterenol (ISO) is a sympathomimetic non-selective â-adrenergic receptor agonist used to produce oxidative stress in experimental animals for evaluation of various cardioprotective agents[5]. High dose of isoproterenol causes free radicals generation that may attack the highly unsaturated fatty acids of the cell membrane to induce lipid peroxidation and consequently damage cell membrane[6]. Thus elevates different cardiac biomarker enzyme levels in serum such as creatine phosphokinase-MB & lactate dehydrogenase due to loss of integrity of call membrane and cell necrosis[7]. Some investigators observed that these cardio specific marker enzymes are released from heart into the blood during myocardial damage due to myofibril degeneration and myocyte damage after using high dose of isoproterenol in their study[8].

However, propranolol (PRO) is a non-selective â-adrenergic receptor antagonist, blocks the action of both $â_1$ and $â_2$ adrenergic receptors. It is widely used for the management of essential hypertension, ischemic heart disease, cardiac arrhythmias and other cardiovascular diseases[9]. Like other cardioprotective drugs long term use of propranolol also have some side effects like dyslipidemia, bradycardia, insomnia, light-headedness etc[10].

Now a day, natural plant foods are gaining popularity as it can combat various physiological changes due to their potency, less toxicity and having scientific evidences in favor of them[11]. Peanut (Arachis hypogaea L.) belongs to the family of fabaceae have gained popularity recently for their many health benefits and have been shown to lower the risk of ischemic heart diseases[12]. The potent cardioprotective activity of peanut may be due to the presence of therapeutic phytochemicals and antioxidant action[13].Multiple components of peanut including arginine, folate, tocopherol, flavonoids, resveretrol may lower the risk of ischemic heart diseases by scavenging free radicles[14]. The unsaturated fatty acid profile of nuts is thought to mediate the majority of the favorable effects on lowering cardiovascular risk, other components like fibers, vitamin-B, vitamin-E, Magnesium, copper, numerous bioactive substances may also contribute[15]. Peanut consumption is relatively safe but approximately 1% in the general population showed nut allergy[14].

Peanuts are increasingly recognized for their role in cardiovascular risk reduction as acknowledged by a Food and Drug Administration qualified heart claim in 2003[16]. Epidemiological studies estimate an approximately 35% reduction in the incidence of ischemic heart disease in the highest nut consuming groups[17]. Consumption of 142g (5oz) nuts/wk is associated with a 30-50% decrease in ischemic heart disease[18]. Different studies have evidence that myocardial damage is largely preventable by antioxidant intervention via suppression of free radical generation and augment endogenous antioxidant[19]. Leakage of cardio specific enzymes (CK-MB, LDH) from heart is the diagnostic marker of myocardial infarction[20]. Recently in an experimental study some researchers observed significant improvement in the serum cardiac biomarker enzyme (CK-MB, LDH) levels in rats treated with conventional cardioprotective drug propranolol along with nut extract in comparison to that of isoproterenol treated rats[21].

Therefore, the present study has been designed to observe the protective effect of peanut (Arachishypogaea L.) and its combination with propranolol on serum cardiac biomarker enzyme (CK-MB, LDH) levels in isoproterenol induced myocardial damaged rats.

Methods

This experimental study was conducted in the Department of Physiology, Sir Salimullah Medical College (SSMC), Mitford, Dhaka from January to December 2012. The protocol of this study was approved by Institutional Ethics Committee (IEC) of SSMC. Twenty Wistar albino rats, age 85 to100 days, weighing 120 to 150g (initial body weight) were included in the experimental group (with peanut). They were sub-divided into CT-P (Cardiotoxic group with isoproterenol after peanut treatment) and CT-C (Cardiotoxic group with isoproterenol after combined treatment of peanut and propranolol). Age and weight matched 30 Wistar albino rats without any peanut supplementation was taken as control and sub-divided into three sub-group,BC (baseline control), CT (Cardiotoxic group with isoproterenol) and CT-PRO (Cardiotoxic group with isoproterenol after propranolol treatment). Each subgroup consisted of 10 Wistar albino rats. Before grouping all the animals were acclimatized for 14 days under 12 hour dark and light cycle. During this study they had free access to food and water *ad libitum*.After acclimatization and before giving any supplementation, body weights of all the rats were measured (initial bw). However, each group consisted of 10 rats and was given basal diet for 21 consecutive days. In addition to this, animals of CT-PRO were given propranolol (10mg/kg body weight; orally) for last seven (from 15th to 21st day of study period) consecutive days, animals of CT-P were given peanut extract (500mg/kg body weight; orally) for 21 consecutive days (started from 1st day of study period), animals of CT-C were given both peanut extract (500mg/kg body weight; orally) for 21 consecutive days (started from 1st day of study period) and propranolol (10mg/kg body weight; orally) for last seven (from 15th to 21st day of study period) consecutive days. All the groups of animals except baseline control group were given isoproterenol subcutaneously (150mg/kg body weight/day) for last two (at 20th& 21st day of study period) consecutive days.After taking their final body weights (final bw), all the rats were anaesthetized with the help of chloroform (30%), and were sacrificed on 22nd day. Then their blood samples were collected from the heart. For the assessment of myocardial damage, some serum cardiac biomarker enzyme levels such as creatine phosphokinase-MB & lactate dehydrogenase were measured. The serum enzymes were measured by using immunoassay method[22]. The tests were done in the department of physiology, SSMC. Statistical analysis were done by one way ANOVA and Bonferroni test by using SPSS windows, version 16.

Results

The percent change of body weight from final to initial was significantly ($p<0.01$) lower both in CT-P and in CT-C as compared to that of baseline control (Table I).

Table I : Body weight in different groups of rats (n=50)

Parameters	Without peanut			With peanut	Combined
	BC	CT	CT-PRO	CT-P	CT-C
Initial body wt (g) (Day 1)	128.89±6.01	132.78±10.93	134.33±10.03	135.73±5.35	134.44±8.08
Final body wt (g) (Day 22)	137.11±6.43	147.83±11.06***	152.02±10.33**	142.63±5.45*$^{+}$	135.78±7.63$^{\bullet++o}$
% of change from final (F) to initial (I) body wt [(F-I)/Ix100]	6.39±1.67	6.73±1.08	6.06±0.93	-1.64±1.07^—	-3.24±4.46^^”-

Values are means ±SD. Statistical analysis was done by ANOVA test and then Bonferroni test. For final body wt (***p<0.01, **p<0.01 & *p<0.05 BC vs CT, CT-PRO & CT-P) (•p<0.01 CT-ISO vs CT-ISO-C) ($^{+}$p<0.05 &$^{++}$p<0.01 CT-PRO vs CT-P & CT-C) (^{o}p<0.01 CT-P vs CT-C). For % change of body wt (^p<0.01 & ^^p<0.01 BC vs CT-P & CT-C) (“p<0.01 CT vs CT-C) (- -p<0.01 &-p<0.01 CT-PRO vs CT-P & CT-C). BC = Baseline control CT = Cardiotoxic group with isoproterenol CT-PRO = Cardiotoxic group with isoproterenol after propranolol treatment CT-P = Cardiotoxic group with isoproterenol after peanut treatment CT-C = Cardiotoxic group with isoproterenol after combined treatment of peanut and propranolol.

Table II: Serum CK-MB and LDH levels in different groups of rats (n=50)

Cardiac biomarker		Without peanut		With peanut	Combined
enzymes (U/L)	BC	CT	CT-PRO	CT-P	CT-C
Serum	5.78±4.49	40.56±5.00	32.78±5.19	35.75±4.53	29.16±4.74
CK-MB	(1-14)	(33-48)*	(25-40)*^	(29-42)*	(23-38)*^-
Serum	209.11±32.29	739.11±124.74	684.11±124.72	732.00±101.25	601.67±94.25
LDH	(161-261)	(515-890)º	(510-870)º+	(520-855)º	(470-733)º+#

Statistical analysis was done by ANOVA test & then Bonferroni test was performed to compare between groups. Figures in parenthesis indicate ranges. For cardiac biomarker enzyme CK-MB, LDH levels (*p<0.01& ºp<0.01 BC vs CT, CT-PRO, CT-P & CT-C) (^p<0.01& +p<0.01 CT vs CT-PRO& CT-C) (-p<0.01& #p<0.05 CT-P vs CT-C). BC = Baseline control CT = Cardiotoxic group with isoproterenol CT-PRO = Cardiotoxic group with isoproterenol after propranolol treatmen CT-P = Cardiotoxic group with isoproterenol after peanut treatment CT-C = Cardiotoxic group with isoproterenol after combined treatment of peanut and propranolol.

Again, the mean serum CK-MB & LDH levels were significantly (p<0.01) higher in CT, CT-PRO, CT-P & CT-C in comparison to that of baseline control. On the other hand, the serum CK-MB and LDH levels were lower in CT-PRO, CT-P and CT-C when compared to that of CT though the differences were statistically significant (p<0.01, p<0.05) in case of CT-PRO and CT-C but not significant in case of CT-P. Again, the levels were also significantly lower in CT-C (p<0.01, (p<0.05) as compared to that of CT-P (Table II).

Discussion

In the present study, the percent changes of body weight were almost similar to the findings reported by the various investigators from different countries[21].

Again, in this study serum levels of CK-MB and LDH were significantly higher in CT (Cardiotoxic group with isoproterenol) in comparison to BC (Baseline control group). Furthermore, levels of these variables were significantly lower in CT-PRO (Cardiotoxic group with isoproterenol after propranolol treatment) and CT-C (Cardiotoxic group with isoproterenol after combined treatment of peanut and propranolol) when compared to that of CT (Cardiotoxic group with isoproterenol). Moreover, significantly lower levels of CK-MB and LDH were also found in CT-C (Cardiotoxic group with isoproterenol after combined treatment of peanut and propranolol) in comparison to that of CT-P (Cardiotoxic group with isoproterenol after peanut treatment). Almost similar findings were also observed by different researchers by using different nuts & herbal plants[9, 21].

Administration of high dose of isoproterenol subcutaneously produces oxidative stress and generate free radicals in the myocardium[6]. It is well recognized that free radicals generated in ischemic tissues causes lipid peroxidation with resultant degradation of tissue defense system, leading to myocardial damage and necrosis[5, 23]. However, rats that received isoproterenol at the dose of 150mg/kg body weight showed diffuse myocardial necrosis and leakage of myocardial enzymes[21].

Again, high dose of Isoproterenol enhances susceptibility of myocardial cell membrane to the isoproterenol mediated peroxidative damage resulting in increase release of diagnostic marker enzymes into systemic circulation[13, 24]. Isoproterenol mediated production of oxidant metabolites exert their toxic effect and causes modification of cell membrane permeability thereby cause leakage of cardiac enzymes[25]. Again, it has been suggested that isoproterenol also enhanced lipid biosynthesis in the

myocardium which in turn leads to loss of integrity of cell membrane and render the membrane more porus and permeable or may rupture thus results in enzyme leakage[7,8]. Some investigators reported that lipid peroxidation and its products play important role in ischemic heart disease and leakage of cardiac enzymes from damaged heart tissue induced by isoproterenol[6, 23].

Moreover, some investigator suggested that propranolol mediated cardioprotection is mainly by scavenging reactive oxygen species, thus decreases lipid peroxidation, maintain myocardial integrity and ultimately prevent leakage of cardiac biomarker enzymes[9].

Again, some other investigators observed that resveratrol, present in peanut inhibit lipid peroxidation by scavenging free radicals and thus blocking the lipid chain reaction[26]. Folate content of peanut lowers plasma homocysteine status which is independent risk factors for development of ischemic heart disease[27]. Peanut is a rich source of unsaturated fatty acid. It has been suggested that a simultaneous reduction in saturated fatty acid and an increase in unsaturated fatty acid in diet can lower the cardiovascular risk in individual with peanut consumption due to its antioxidant action[13, 15]. Flavonoid, the main bioactive compound in peanut, increases the cardiac glutathione content thereby maintain cell membrane integrity with concomitant decrease of enzyme leakage from cardiocytes and protect cardiac tissue from damage[26].

In the present study, myocardial damage was observed in rats treated with isoproterenol as evidenced by their elevated levels of serum CK-MB and LDH.

Again, lower levels of serum CK-MB and LDH were observed in CT-P (Cardiotoxic group with isoproterenol after peanut treatment) and CT-C (Cardiotoxic group with isoproterenol after combined treatment of peanut and propranolol) of the present study suggested the cardioprotective role of peanut against isoproterenol induced myocardial damage and subsequent leakage of cardiac enzymes. Moreover, in this study combined therapy of peanut and propranolol showed synergistic cardioprotective effect than when they were used alone. These effects are most likely due to increasing the endogenous antioxidant levels and free radical scavenging activity of peanut and propranolol.

Conclusion

The present study clearly emphasize the beneficial action of peanut as a cardioprotective plant food, proved to be effective in reducing the extent of myocardial damage. However, combined therapy of peanut with propranolol showed synergistic effect on preventing myocardial damage by scavenging free radicals.

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