Article

Protective Effect of Peanut (Arachis hypogaea L) and Its Combination with Propranolol on Dyslipidemia in Isoproterenol Induced Cardiotoxic Rats

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Abstract

Background: Cardiotoxicity can be developed due to prolonged use of higher doses of some drugs, exposure to some chemicals, toxins or infectious agents and also by some disease conditions. Natural plant food such as peanut (Arachis hypogaea L.) may have free radical scavenging and lipid lowering activity, thereby, can be used for the prevention and management of heart disease. **Objective:** To observe the protective effect of peanut (Arachis hypogaea L) and its combined action with propranolol on dyslipidemia in Isoproterenol induced cardiotoxic rats. **Methods:** This experimental study was carried out in the Department of Physiology, Sir Salimullah Medical College (SSMC), Dhaka from January to December, 2012. Twenty Wistar albino rats, age 85-100 days, weighing 120 to 150g (initial body weight) were included in the experimental group (Group B, with peanut). They were further sub-divided into group B_1 (isoproterenol treated group after peanut treatment), and group B_2 (isoproterenol treated group after combined treatment of peanut and propranolol). Age and weight matched 30 Wistar albino rats without any peanut supplementation was taken as control (group A) and divided into three sub-groups, group A_1 (baseline control), group A_2 (isoproterenol treated control) and group A_3 (isoproterenol treated control after propranolol treatment). Each subgroup consisted of 10 rats. After taking final body weight all the rats were sacrificed on 22nd day. Blood was collected from heart and supernatant serum was preserved in deep freeze until analysis. For assessment of lipid profile status, serum total cholesterol (TC), low density lipoprotein cholesterol (LDL- C) and high density lipoprotein cholesterol (HDL-C) were estimated by standard method. The statistical analysis was done by one way ANOVA and Bonferroni test as applicable. **Results:** In this study, percent change from initial body weight to final body weight was significantly ($p<0.01$) lower both in isoproterenol treated group after peanut treatment and in isoproterenol treated group after combined treatment of peanut and propranolol as compared to that of baseline control. Again, this value was almost similar and the difference was not statistically significant between isoproterenol treated group after peanut treatment and isoproterenol treated group after combined treatment of peanut and propranolol. Again, the mean serum TC ($p<0.01$) and LDL-C ($p<0.05$) were significantly lower in isoproterenol treated group after peanut treatment and isoproterenol treated group after combined treatment of peanut and propranolol in comparison to those of isoproterenol treated control group. Moreover, the mean serum HDL-C was significantly ($p<0.01$) higher in isoproterenol treated group after combined treatment of peanut and propranolol, in comparison to that of isoproterenol treated control group. Furthermore, the mean serum TC and LDL-C were non significantly higher and serum HDL-C was significantly ($p<0.01$) higher in isoproterenol treated group after combined treatment of peanut and propranolol when compared to those of isoproterenol treated group after peanut treatment. **Conclusion:** The present study revealed that peanut alone can maintain blood lipid level by decreasing TC and LDL-C levels and by increasing HDL-C level in isoproterenol induced cardiotoxic rats. However, the combined therapy of peanut with propranolol showed synergistic effect on preventing dyslipidemia.

Key words: Peanut, Propranolol, Isoproterenol, Dyslipidemia

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Introduction

Cardiotoxicity is the electrophysiological dysfunction of heart and myocardial damage.[1] It may be caused by chemotherapy treatment with cytotoxic drugs such as doxorubicin, epirubicin, cisplatin etc, adverse effects of heavy metals intake like lead, cadmium etc, and an incorrectly administered drug such as high dose of isoproterenol and also by some cardiotoxins.[2]

Coronary 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.[3] While Bangladesh is turning from rural based socio-economic structure towards urbanization, the coronary heart disease in middle aged and young group is also appearing at increasing level.[4] Urbanization is characterized by a marked increase in the intake of energy dense foods, a decrease in physical activity and an increased level of psychosocial stress, all of which promote the development of diabetes, hypertension and dyslipidemia.[5] All of these are the important risk factors for the development of atherosclerosis and ischemic heart disease.[6]

Isoproterenol is a sympathomimetic non-selective â-adrenergic receptor agonist used to produce myocardial injury in experimental animals for evaluation of various cardioprotective agents.[7] High dose of isoproterenol causes severe oxidative stress in the myocardium resulting in infarction, it also generate free radicals and stimulate lipid peroxidation.[8]

However, propranolol is a non-selective β-blocker[17] used in patients with hypertension, ischemic heart disease, cardiac arrhythmias and other cardiovascular diseases.[9] But long term use of propranolol may produce dyslipidemia, bradycardia, insomnia, light-headedness etc.[10]

Arachis hypogaea L. known as peanut belongs to the family of fabaceae have been valued for their high nutritional content throughout the world for many years.[11] Peanuts are energy dense foods that are particularly rich in fat, mostly unsaturated fatty acids and this unsaturated fatty acid of nuts through its lipid lowering effect may be responsible for their protective effects against ischemic heart disease.[12] Peanuts are also a rich source of vitamin-B, vitamin-E, magnesium, copper, phosphorus, plant protein, arginine, dietary fiber and numerous bioactive substances like flavonoids, resveratrol and plant sterols.[13] Peanut consumption is relatively safe but approximately 1% in the general population showed nut allergy.[14]

Some researchers observed that consuming about an ounce of peanuts every day can reduce the risk of heart diseases by up to half.[15] Moreover some other researchers found that consumption of peanut 5 times per week (about 155g of nuts/week) reduced the risk of death from coronary heart diseases by 35%.[16] Recently in a study some researchers observed that peanut oil significantly reduces LDL-cholesterol level.[17] Some other researchers showed that peanut consumption may improve blood HDL-cholesterol levels.[18] It is well established that, dyslipidemia is one of the risk factor for the development of coronary heart disease.[6]

Therefore, the present study has been designed to observe the protective effect of peanut (Arachis hypogaea L) and its combined action with propranolol on dyslipidemia in Isoproterenol induced cardiotoxic rats. It is also expected that the result of this study would make peanut acceptable among the people as a rich source of nutrients with medicinal value for the prevention of coronary heart disease.

Methods

This experimental study was conducted between January and December 2012 in the Department of Physiology, Sir Salimullah Medical College (SSMC), Mitford, Dhaka. Ethical permission was taken from Institutional Ethics Committee (IEC) of SSMC. Twenty wistar albino rats, age 85-100 days, weighing 120 to 150g (initial body weight)

were included in the experimental group (Group B, with peanut). They were further sub-divided into group B_1 (isoproterenol treated group after peanut treatment), and group B_2 (isoproterenol treated group after combined treatment of peanut and propranolol). Age and weight matched 30 Wistar albino rats without any peanut supplementation was taken as control (group A) and divided into three sub-groups, group A_1 (baseline control), group A_2 (isoproterenol treated control) and group A_3 (isoproterenol treated control after propranolol treatment). Each subgroup consisted of 10 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*. Each subgroup consisted of 10 rats and was given basal diet for 21 consecutive days. In addition to this, animals of propranolol treated control group were given propranolol (10mg/kg body weight; orally) for last seven (from 15th to 21st day of study period) consecutive days, animals of peanut treated group were given peanut extract (500mg/kg body weight; orally) for 21 consecutive days (started from 1st day of study period), animals of combined treated group 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) consecutive days. All the groups of animals except baseline control group were given isoproterenol subcutaneously (150mg/kg body weight/day) for the last two (20th & 21st day) consecutive days. After acclimatization and before giving any supplementation, body weights of all the rats were measured (initial bw). After giving isoproterenol, propranolol and peanut including the baseline control rats, were anaesthetized with the help of chloroform (30%) and sacrificed on 22nd day. Blood was collected from heart and supernatant serum was preserved in deep freeze until analysis. Before anaesthetized the rats their body weights (final bw) were taken. For the assessment of lipid profile status, serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were measured by enzymatic method[19]. All the above 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.

Preparation of peanut (Arachis hypogaea L.) extract[20]

Peanuts were purchased from Mohammadpur town hall market, Dhaka, dried in sunlight for 2 days and grinded in an electrical grinder. The grinded peanut (1000g) was subjected to cold extraction with the use of chloroform:methanol for 7 days with frequent shaking every day. Then the extract was filtered, evaporated by using rotatory evaporator under reduced pressure. The yield of the peanut oil was 500ml and was fed to the experimental animals.

Results

The percent change of body weight from final to initial was significantly ($p<0.01$) lower both in isoproterenol treated group after peanut treatment and in isoproterenol treated group after combined treatment of peanut and propranolol as compared to that of baseline control, and was almost similar and the difference was not statistically significant between isoproterenol treated group after peanut treatment and isoproterenol treated group after combined treatment of peanut(Table I).

Again, the mean serum TC ($p<0.01$) and LDL-C ($p<0.05$) were significantly lower in isoproterenol treated group after peanut treatment and isoproterenol treated group after combined treatment of peanut and propranolol in comparison to those of isoproterenol treated control group. Moreover, the mean serum HDL-C was significantly ($p<0.01$) higher in isoproterenol treated group after combined treatment of peanut and propranolol, in comparison to that of isoproterenol treated control group. Furthermore, the mean serum TC and LDL-C were non significantly higher and serum HDL-C was significantly ($p<0.01$) higher in isoproterenol treated group after combined treatment of peanut and propranolol when compared to those of isoproterenol treated group after peanut treatment (Table II).

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

Groups	Body weight (g)		% of change from final (F) to initial (I) body weight [(F-I)/I ×100]
	Initial (Day 1)	Final (Day 22)	
A_1(n=10)	128.89 ± 6.01	137.11 ± 6.43	6.39 ± 1.67
A_2(n=10)	132.78 ± 10.93	147.83 ± 11.06	6.73 ± 1.08
A_3(n=10)	134.33 ± 10.03	152.02 ± 10.33	6.06 ± 0.93
B_1(n=10)	135.73 ± 5.35	142.63 ± 5.45	-1.64 ± 1.07
B_2(n=10)	134.44 ± 8.08	134.78 ± 7.63	-3.24 ± 4.46

Statistical analysis:

	p value		
A_1vs A_2	0.06^{ns}	0.00^{**}	0.31^{ns}
A_1vs A_3	0.05^{ns}	0.00^{**}	0.31^{ns}
A_1vs B_1	0.05^{ns}	0.04^{*}	0.00^{**}
A_1vs B_2	0.05^{ns}	0.24^{ns}	0.00^{**}
A_2vs A_3	0.14^{ns}	0.17^{ns}	0.09^{ns}
A_2vs B_1	0.05^{ns}	0.16^{ns}	0.06^{ns}
A_2vs B_2	0.35^{ns}	0.00^{**}	0.00^{**}
A_3vs B_1	0.34^{ns}	0.02^{*}	0.00^{**}
A_3vs B_2	0.19^{ns}	0.00^{**}	0.00^{**}
B_1vs B_2	0.06^{ns}	0.01^{**}	0.16^{ns}

Data are expressed as Mean ± SD. Statistical analysis was done by ANOVA test and then Bonferroni test was performed to compare between groups.

Group A_1 =Baseline control group), **Group A_2** =Isoproterenol treated control group, **Group A_3**=Isoproterenol treated control group after propranolol treatment), Group B_1 = Isproterenol treated group afterpeanut treatment, B = Isproterenol treated group after combined treatment of peanut and propranolol. n: totalnumber of subjects., * : significant at $p < 0.05$, ** : significant at $p < 0.01$, ns: non-significant

Table II : Serum TC, LDL and HDL Levels in different groups of rats (n=50)

Groups	Serum total cholesterol (mg/dl)	Serum LDL (mg/dl)	Serum HDL (mg/dl)
A_1(n=10)	152.33 ± 34.80	113.11 ± 36.40	49.67 ± 4.55
A_2(n=10)	182.33 ± 35.28	130.22 ± 32.60	40.33 ± 8.22
A_3(n=10)	163.11 ± 39.06	109.55 ± 32.28	45.44 ± 5.81
B_1(n=10)	120.75 ± 34.41	87.75 ± 29.37	50.88 ± 3.44
B_2(n=10)	126.00 ± 31.91	89.67 ± 26.30	58.56 ± 4.61

Statistical analysis:

	p value		
A_1vs A_2	0.04^{*}	0.04^{*}	0.01^{*}
A_1vs A_3	0.27^{ns}	0.46^{ns}	0.05^{ns}
A_1vs B_1	0.04^{**}	0.48^{ns}	0.27^{ns}
A_1vs B_2	0.06^{ns}	0.48^{ns}	0.00^{**}
A_2vs A_3	0.14^{ns}	0.10^{ns}	0.07^{ns}
A_2vs B_1	0.00^{**}	0.01^{*}	0.08^{ns}
A_2vs B_2	0.00^{**}	0.01^{*}	0.00^{**}
A_3vs B_1	0.02^{*}	0.08^{ns}	0.05^{ns}
A_3vs B_2	0.02^{*}	0.09^{ns}	0.00^{**}
B_1vs B_2	0.38^{ns}	0.45^{ns}	0.00^{**}

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 significantly higher levels of serum TC, LDL and lower level of HDL were observed in isoproterenol treated control group in comparison to that of baseline control group. Again, serum TC and LDL levels were significantly lower in isoproterenol treated group after peanut treatment and isoproterenol treated group after combined treatment of peanut and propranolol, but non significantly lower in isoproterenol treated control group after propranolol treatment when compared to that of isoproterenol treated control group. Whereas, serum HDL level was significantly higher in

isoproterenol treated group after combined treatment of peanut and propranolol, but non significantly higher in isoproterenol treated control group after propranolol treatment and isoproterenol treated group after peanut treatment in comparison to that of isoproterenol treated control group. Almost similar findings were also reported by different investigators by using different herbal plants.[8,22] Again, serum TC and LDL levels were almost similar, but serum HDL level was significantly higher in isoproterenol treated group after combined treatment of peanut and propranolol in comparison to that of isoproterenol treated group after peanut treatment. Similar finding was also reported by other researchers.[23] On the contrary, some investigators observed reduced level of serum HDL after walnut consumption[24]. This discrepancy may be due to using different species of nut (walnut) in that study.

Exact lipid lowering effects of peanut and its combined action with propranolol in isoproterenol induced cardiotoxic rats are not clear. Various suggestions are proposed by different investigators. According to them, high dose of isoproterenol causes lipid peroxidation of myocardial membrane, enhanced lipid biosynthesis in the myocardium by cardiac cAMP formation, leads to myocardial necrosis,[25] mobilizes lipids from adipose tissue resulting in hypercholesterolemia.[23] Again, propranolol can prevent lipid peroxidation and maintain myocardial integrity[9] but long term use of propranolol causes a significant decrease in HDL-C levels and increase in LDL-C levels,[26] due to the lipoprotein lipase inhibitory activity and lecithin-cholesterol acyl transferase (LCAT) lowering activity of propranolol.[27] However, Oleic acid which is the predominant mono-unsaturated fatty acid in peanut prevents LDL oxidation and reduces risk of cardiovascular disease.[17] The flavonoids and phytosterols of peanut show cardioprotective effect by lowering blood cholesterol levels.[16] In addition to this, combined therapy of nut extract and propranolol caused synergistic cardioprotection to myocardium, by both increasing the endogenous antioxidant levels and also by scavenging free radicals.[22]

In the present study, dyslipidemia was observed in rats treated with isoproterenol as evidenced by higher levels of serum TC, LDL and lower level of serum HDL. This is further supported by an increase in body weight in cardiotoxic rats of present study which may be due to increased production of free radicals.

Again, lower levels of serum TC, LDL and higher level of HDL were observed in isoproterenol treated group after peanut treatment and isoproterenol treated group after combined treatment of peanut and propranolol of the present study suggested the protective effect of Peanut and its combined action with propranolol on dyslipidemia against isoproterenol induced cardiotoxicity. These effects are most likely due to mono-unsaturated fatty acid content and the free radical scavenging activity of peanut. Moreover, in this study combined therapy of peanut extract and propranolol showed synergistic effect in the improvement of dyslipidemia than when they were used alone.

Conclusion

From this study, it can be concluded that peanut alone can maintain blood lipid level by decreasing TC and LDL-C levels and by increasing HDL-C level in isoproterenol induced cardiotoxic rats. However, the combined therapy of peanut with propranolol showed synergistic effect on preventing dyslipidemia. So, combined therapy of peanut and propranolol showed synergistic cardioprotective effects by reducing the cardiovascular risk factors.

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