Original Article

Cardioprotective Effect of the Methanolic Extract of Boiled Peanut (*Arachis hypogaea)* on Isoproterenol Induced Myocardial Infarction in Albino Rats

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ABSTRACT

Epidemiological studies revealed that Indians are at greater risk of getting myocardial infarction. On the other hand, India sports the highest per capita consumption of fresh peanuts globally, standing at 6.3kg in 2010. The boiled peanuts (*Arachis hypogaea*) is said to possess a bioactive phytoprinciple, Resveratrol, a potent cardioprotective agent. Hence this study was designed to investigate the cardio protective effect of the methanolic extract of boiled peanuts (*Arachis hypogaea)* against isoproterenol induced experimental myocardial infarction in albino rats. Wistar albino rats were pretreated with the methanolic extract of *A. hypogaea* (33mg / kg. of body weight, orally) for 15 days and then induced with isoproterenol (ISO 30 mg /kg. of body weight, sub-cutaneously for 2 consecutive days). On 18th day, blood was collected by cervical decapitation under anesthesia. Heart was removed for histopathological studies. Serum samples were used to estimate total cholesterol, triglycerides, HDL, LDL. The enzyme markers lactate dehydrogenase, SGOT and SGPT were estimated. In isoproterenol, induced rats levels of cholesterol, TG, LDL were increased significantly with a decrease in HDL level and the enzyme markers like LDH, SGOT, SGPT levels were increased. The pretreated group animals with *A. hypogaea* showed decreased marker enzymes level, cholesterol, TG, LDL and increased HDL level. The results suggested that the methanolic extract of *Arachis hypogaea* possess cardioprotective effect on experimentally induced myocardial infarcted albino rats. The activity might be because of the high content of resveratrol present in the boiled peanuts.

Keywords: *Arachis hypogaea*, isoproterenol, myocardial infarction enzymes; histopathology.

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INTRODUCTION

Myocardial infarction (MI) or acute myocardial infarction (AMI), commonly known as a heart attack, results from the interruption of blood supply to a part of the heart, causing heart cells to die[1]. 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 (cholesterol and fatty acids) and white blood cells (especially macrophages) in the wall of an artery. The resulting ischemia (restriction in blood supply) and ensuing oxygen shortage, if left untreated for a sufficient period of time, can cause damage or death (infarction) of heart muscle tissue (myocardium)[2]. Approximately one-fourth of all myocardial infarctions are silent, without chest pain or other symptoms. These cases can be discovered later on electrocardiograms, using blood enzyme tests or at autopsy without a prior history of related complaints[3]. Many of these risk factors are modifiable; so many heart attacks can be prevented by maintaining a healthier lifestyle. Physical activity, for example, is associated with a lower risk profile. Non-modifiable risk factors include age, sex, and family history of an early heart attack (before the age of 60), which is thought of as reflecting a genetic predisposition[4]. Socioeconomic factors such as least education and lower income (particularly in women), and unmarried cohabitation are also correlated with a higher risk of MI[5]. Treating MI after its occurrence would be a costlier affair for most people of low income group. Hence prevention would always be better, that too in the form of food is most welcome. Eating boiled peanuts is widespread among the people of India and it is regarded as a popular snack. The peanut, or groundnut (*Arachis hypogaea*), is a species in the legume or "bean" family (Fabaceae). The peanut was probably first cultivated in the valleys of Peru. It is an annual herbaceous plant growing 30 to 50cm (1.0 to 1.6ft) tall. *Hypogaea* means "under the earth", after pollination, the flower stalk elongates causing it to bend until the ovary touches the ground. Peanuts are known by many other local names such as earthnuts, ground nuts, goober peas, monkey nuts, pygmy nuts and pig nuts. Peanuts are rich in nutrients, providing over 30 essential nutrients and phytonutrients. Peanuts are a good source of niacin, folate, fiber, magnesium, vitamin E, manganese and phosphorus. Hot boiled peanuts are rich in protein, monosaturated fats and antioxidants. Peanuts are a significant source of resveratrol, a chemical studied for potential anti-aging effects and also associated with reduced cardiovascular disease and reduced cancer risk. Boiled peanuts are rich in the compound resveratrol, an antioxidant. Resveratrol also exists in high amounts in red grapes and peanuts. Boiled peanuts contain more resveratrol than raw peanuts, peanut butter, fresh grapes or red wine, according to the Linus Pauling Institute[6]. The name resveratrol presumably comes from the fact that it is a resorcinol derivative coming from a *veratrum* species[7,8]. Resveratrol (3, 5, 4'-trihydroxy-trans-stilbene) is a stilbenoid, a type of natural phenol, and a phytoalexin produced naturally by several plants when under attack by pathogens such as bacteria or fungi[9]. It is mainly found in 'seed producing' plants like grapes (a vine plant), yucca, eucalyptus, spruce, lily, blueberries, cranberries, bilberries, mulberries, peanuts and even chocolate. Resveratrol is a popular natural remedy said to offer a broad range of health benefits. Resveratrol is also similar to the phytoestrogens[10]. Hence the efficacy of the methanolic extract of boiled peanuts**,** found to be rich in resveratrol was tested in

experimentally induced myocardial infarction in albino rats.

MATERIALS AND METHODS

Plant material

Arachis hypogaea nuts were bought from the local market in Trichy. The peanuts were boiled, dried and powdered. The plant material was identified and authenticated by Rapinat Herbarium St. Joseph's college, Trichy.

Extraction

85^0C using menthol as solvent, since Tameshia *et al*., 2008 suggested that MeOH may be the best solvent for the extraction of resveratrol from peanut skins[21]. The solvent was filtered and filtrate was allowed to evaporate for one day to obtain dried extract.

Animals

Male & Female albino rats (100 - 120g) were obtained from the Venkateshwara Animal Breeders in Bangalore. The animals were maintained in the animal house with 12 hours of day and night cycle and were fed with standard pelleted rat feed. The experiment was carried out in accordance to the Institutional Animal Ethical Committee.

Experimental design

The rats were divided into five groups of six animals each. The methanolic extract of *Arachis hypogaea* was given orally for 15 days to the experimental groups by solublizing it in distilled water and Resvita, a commercially available grape seed resveratrol was taken as a positive control. The animals of Group I served as normal control and received normal diet and water. Animals of Group II were fed with normal diet and isoproterenol injected subcutaneously on 16th & 17th day. Group III animals were treated with methanolic extract of *Arachis hypogaea* (5mg/kg of body weight) extract with normal diet. Group IV animals were treated with the methanolic extract of *Arachis hypogaea* (5mg/kg. of body weight) extract with normal diet for 15 days. At 16^{th} & 17^{th} day isoproterenol was injected subcutaneously. The animals of Group V were treated with Resvita (grape seed resveratrol) along with normal diet for 15 days. At 16th & 17th day myocardial infarction was induced by subcutaneous injection of isoproterenol. At the end of the experimental period the rats were fasted overnight and sacrificed. Blood was drawn from the external jugular vein Blood was drawn from the external jugular vein. Serum was separated after centrifugation at 3000 rpm for 15 minutes for biochemical analysis; the heart was dissected out for histopathological studies and stored in 10% formalin. The activities of cholesterol and HDL[11], LDL[12], Triglycerides[13] and the marker enzyme activities like Aspartate amino transferase and Alanine amino transferase[14], Lactate dehyhdrogenase[15] were estimated. The heart sections were subjected to histological analysis to assess the changes in the cellular architecture.

Statistical Analysis

Results were expressed as mean ± SD. The significance of the difference the means of the tests and control studies was established by applying student's 't' test for independent samples.

RESULTS & DISCUSSION

The analyses of the serum parameters were tabulated. The infarction inducing chemical Isoproterenol hydrochloride is 3, 4 dihydroxy-α-[(isopropylamino) methyl] benzyl alcohol hydrochloride, a synthetic sympathomimetic amine that is structurally related to epinephrine but acts almost exclusively on β

receptors. Biochemical alterations in ISO-induced cardiomyopathy represent a complex pattern of changes in cardiac marker enzymes, lipid profile, lipid metabolizing enzymes, enzymatic and non-enzymatic antioxidants levels, glycoprotein levels, decrease in ATP store and changes in electrolyte levels in the blood as well as in the myocardial tissue[16 & 17]. In this present study, similar results were observed in case of the parameters taken for analysis. The level of cholesterol is elevated in group II when compared to normal group. The group IV animals exhibited reduction in the level of serum cholesterol when compared to group II due to the effect of methanolic extract of *Arachis hypogaea*. The group III animals exhibited the normal level of cholesterol but the group treated with methanolic extract of *Arachis hypogaea* and Group V showed reduced levels of cholesterol when compared to the group II (Table. 1). The levels of serum triglycerides are elevated in group II when compared to the normal group. The group IV animals exhibited a reduction in the level of serum triglycerides when compared to group II due to the effect of methanolic extract of *Arachis hypogaea*. The group III animals showed marked reduction in the levels of serum triglycerides which could be due to the effect of methanolic extract of *Arachis hypogaea*. Resvita standard group (V) also showed reduced levels of triglycerides when compared to the group II. The level of serum HDL-c is decreased in group II when compared to normal group. The group IV animals exhibited an increase in the levels of serum HDL-c when compared to group II due to the effect of methanolic extract of *Arachis hypogaea*. The group III animals showed a significant increase in the levels of serum HDL-c which could be due to the effect of methanolic extract of *Arachis hypogaea*. Resvita standard group (V) also showed increased level of triglycerides when compared to the group II (Table. 1). The level of serum LDL-C is elevated in group II when compared to normal group. The group IV animals showed a reduction in the level of serum LDL-C when compared to group II due to the effect of methanolic extract of *Arachis hypogaea*. In group III animals reduction in the levels of serum LDL-C was observed, which could be due to the effect of *Arachis hypogaea*. Group V showed the reduced level of LDL-C when compared to the group II (Table. 1). A previous study demonstrated resveratrol's short-term, direct influence on lipogenesis, lipolysis and the antilipolytic action of insulin in freshly isolated rat adipocytes and from the results provided it was evident that resveratrol affects lipogenesis and lipolysis in adipocytes contributing to reduced lipid accumulation in these cells[18 & 19]. Similar trend was observed in case of the lipid parameters of the animals treated with methanolic extract of boiled peanuts (A. hypogaea). The level of serum LDH is elevated in group II when compared to normal group. The group IV animals exhibited significant reduction in the level of serum LDH when compared to group II due to the effect of methanolic extract of *Arachis hypogaea*. The group III animal's reduction in the level of serum LDH was observed. Group V Resvita standard group also showed the reduced level of LDH when compared to the group II (Table. 1). The level of serum SGOT is elevated in group II when compared to normal group. The group IV animals exhibited reduction in the level of serum SGOT when compared to group II due to the effect of methanolic extract of *Arachis hypogaea*. The group III animals showed marked reduction in the level of serum SGOT which could be due to the effect of methanolic extract of *Arachis hypogaea*. Group V animals showed reduced level of SGOT when compared to the group II (Table. 1). The level of serum SGPT is

elevated in group II when compared to normal group. The group IV animals exhibited reduction in the level of serum SGPT when compared to group II due to the effect of methanolic extract of *Arachis hypogaea*. The group III animals also exhibited reduction in the level of serum SGPT which could be due to the effect of methanolic extract of *Arachis hypogaea*. Group V also showed reduced level of SGPT when compared to the group II (Table. 1). The data of a similar work showed a decrease in serum SGOT, SGPT and LDH levels in the resveratrol-treated rats20, which is in line with the present investigation. Histopathological examination of the cardiac section from the rats showed the extent and degree of necrosis, which was presented in the Figure 1. Several early events, such as ultrastructural changes, histological, biochemical, electrolyte and membrane changes, have been shown to occur within 48 h after the injection of isoproterenol. Glycogen depletion and fat deposition have been reported. Histological changes induced by excessive amounts of isoproterenol include degeneration and necrosis of myocardial fibres, accumulation of inflammatory cells, interstitial edema, lipid droplets and endocardial hemorrhage17. Control group hearts showed normal myofibrillar architecture with striations, branched appearance and continuity with adjacent myofibrils (Fig 1A). Rats induced with isoproterenol showed severe myocardial necrosis with separation of myofibers as compared to control group (Fig. 1B). *Arachis hypogaea* extract group hearts showed normal myofibers as compared to the control group (Fig. 1C). However, the isoproterenol induced, *Arachis hypogaea* methanolic extract pretreated rats exhibited decreased amount of myofibers fragments compared to isoproterenol induced group (Fig. 1D). The isoproterenol induced rats which received resvita pretreatment has exhibited the decreased amount of myofibers fragments compared to isoproterenol induced group (Fig. 1E). The preserved morphology of cardiac myofibers of control group indicates the cardio protective effect of *Arachis hypogaea*[17]

CONCLUSION

The cardio protective effect of methanolic extract of boiled *Arachis hypogaea* is probably related to its ability to reduce the elevated levels of cardiac enzyme markers and lipid profile. The activity might be due to the presence of resveratrol in the methanolic extract of *Arachis hypogaea*. Further studies are needed to prove the actual mechanism of action of *Arachis hypogaea*. The present results clearly emphasize the beneficial action of methanolic extract of *A. hypogaea* proved to be effective in reducing the myocardial damage. Hence this would be an easy and inexpensive way of preventing the incidence of myocardial infarction.

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Table 1. Effect of *A. hypogaea* extracts on serum cholesterol, TG, LDL, HDL and enzyme markers on experimentally induced myocardial infarction

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	LDH (IU/L)	SGOT (IU/L)	SGPT (IU/L)
G I	106.2 ± 1.5*	57.5±2.2*	144.1±9.1*	108.4±2.5*	306.1±4.0*	41.2±3.1*	56.5 ± 9.1*
G II	130.4 ± 1.9*	82.0±2.4*	108.3±2.2*	198.0±3.5*	557.0±9.1*	75.5±8.2*	89.7±6.2*
G III	118.7± 1.1*	64.5±4.1**	177.0±4.1**	115.7±7.1**	320.0±6.4**	58.4±3.5**	78.2±7.1**
G IV	115.0 ± 1.3*	67.5±7.1*	139.1±8.2*	144.0±3.1*	357.4±7.3*	50.5±7.4*	65.4±8.2*
G V	120.8 ± 1.8*	60.0±3.2*	159.2±6.3*	136.8±1.2*	352.8±8.3*	46.0±9.8*	63.5±4.5*

(Values are expressed as mean ± SD of 6 animals,*-statistically significant (P<0.05), **-statistically significant (P<0.001))

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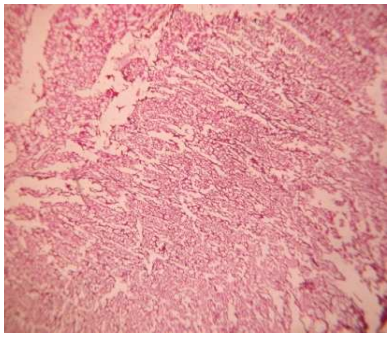
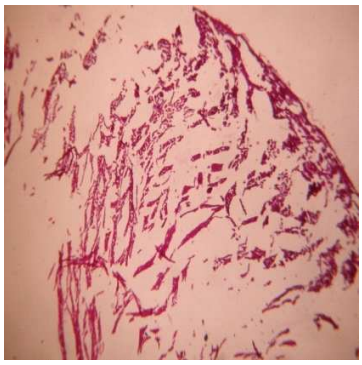
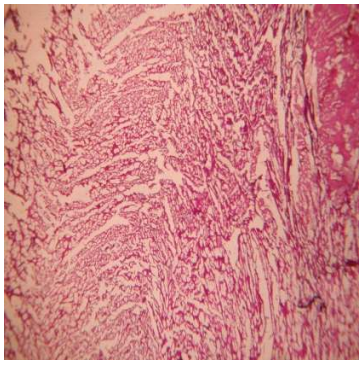


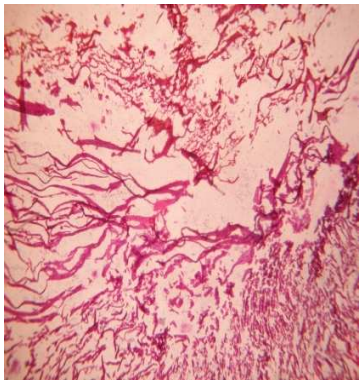
A) Control group



B) isoproterenol induced induced



C) *Arachis hypogaea* extract group



D) *Arachis hypogaea* extract+ isoproterenol group

E) Resvita isoproterenol group