Antithrombotic effect of tender coconut water

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Thrombolytic therapy has become standard treatment for coronary heart disease. The decreased level of fibrinogen, fibrin, factor V and increased 6 keto PG F1α and prothrombin in TCW administrated animals, suggests that TCW can be used for thrombolytic therapy.

Coronary heart disease (CHD) is the major cause of death in most developed countries and is rapidly increasing in prevalence in developing countries. Heart disease is caused by two processes, atherosclerosis and thrombosis. Atherosclerosis is the condition in which the lining of the arteries is thickened in places by raised plaques. This leads to the narrowing and hardening of arteries, which can ultimately result in a blockage. This proceeds through the accumulation of material known as atheroma in the inner arterial lining, which is effectively a combination of cholesterol and modified fatty material (lipids) surrounded by fibrous material. At the site of damage, lipid material, include LDL especially the oxidised lowdensity lipoprotein (LDL) (Holvoet P. 1998) cholesterol would accumulate on the walls of the blood vessels and form blood clot, making the blood vessels narrow and thereby restricting blood circulation. The result is higher risk of formation of thrombosis. Thrombi in the arteries are composed of platelet aggregates bound together by intrinsic fibrin protein strands mix with oxidized LDL and fibrinogen to occlude the vessel, and narrowing the lumen of the involved coronary artery (Drouet L,1998). The formation of a thrombus, a stationery blood clot, which forms at the site of damage is initiated by a tear or split through the plaque. These plaques can then block the artery completely, preventing blood flow to the heart, depriving the muscle of its oxygen supply. The end result is the death of heart muscle, leading to a heart attack or myocardial infarction. Most myocardial infarctions result from atherosclerosis of the coronary arteries usually with superimposed thrombosis. The development of thrombosis is different in its occurrence to atherosclerosis as it happens more or less spontaneously rather than over a period of time.

Underlying Causes of Thrombosis

1. *High Level of Low-density Lipoprotein (LDL)*

Low-density Lipoprotein (LDL) is responsible for carrying cholesterol to body cells. Excess LDL increases the risk of formation of thrombosis.

2. *High Blood Pressure*

High blood pressure is one of the most common causes of thrombosis. High blood pressure will cause the accumulation and hardening of fat on the wall of blood vessels. High blood pressure will also damage the inner layer of the blood vessels resulting in blood clots and thereby increasing the risk of thrombosis

3. *Obesity*

Excess fats accumulate inside the cardiac blood vessels narrowing them and thereby reducing the amount of blood flow. This increases the risk of thrombosis. In addition, obesity, high blood pressure and high cholesterol level are closely related in bringing about thrombosis.

4. *Age*

In general, our bodily functions deteriorate as we age. The cardiac blood vessels gradually lose their elasticity and become thickened and hardened, thereby making them narrow, and therefore increasing the risk of thrombosis.

5. *Smoking*

People who smoke have a higher risk of developing heart diseases. This is because nicotine and other chemical substances in tobacco increase the pulse rate and blood pressure. They also cause increase in the chance of blood clot formation.

6. *Lifestyle*

Busy lifestyle results in an increase in both pulse rates and blood pressure, and therefore increases the risk of thrombosis.

7. *Heredity*

People with parent who die of coronary diseases at a younger age have higher risks of developing thrombosis.

Natural plant products and Coronary Heart Disease

Overwhelming epidemiologic evidence indicates that a natural plant product affords protection against development of certain chronic diseases. The macro - and micronutrients (eg. protein, fat, carbohydrates, free amino acids, vitamins and minerals) necessary for normal metabolism, play an important role in health enhancement. Consumption of fruits and vegetables, olive oil, red wine and tea is inversely correlated with heart disease rates (Giugliano D. 2000). Herbal supplements from garlic, ginkgo, psyllium, guggul, fenugreek, green tea, ginger and turmeric and endosperm of whole grains are reported to prevent or potentially reduce cholesterol and other lipid levels in the blood. Natural products rich in vitamin E, arginine and magnesium are used to prevent abnormal clot formation (Bottecchia and Fantin, 1973; McCarty, 1986). The richness of macro and micronutrients in water of fresh tender coconut are reported to have hypolipidemic and antioxidant effect (Anurag, P 2003) (Anthony Loperito Loki 2003).

The water of fresh tender coconut which is the liquid endosperm, is the most nutritious wholesome beverage that the nature has provided for the people of the tropics. Tender coconut water (TCW) is not merely a thirst quenching drink but a mineral drink that cures most of the diseases of human being. Numerous medicinal properties of tender coconut water were reported (Adams and Brett, 1992; Macalalag and Macalalag, 1987; Kuberski et al., 1979). TCW is a diuretic, effective in the treatment of kidney and urethral stones and cures malnourishment (Macalalag and Macalalag, 1987). Previous studies in our lab clearly proves that cardioprotective action and hepatoprotective effect of TCW may be due to several factors viz., L-arginine, vitamin C, potassium, calcium and magnesium (Anurag,P 2003 and Anthony Loperito Loki 2003). Supplemental L-arginine's antiatherogenic activity may be accounted by its role as the precursor to nitric oxide. L-arginine, a physiological substrate for the production of endothelium derived relaxing factor, nitric oxide (NO), which plays an important role in regulation of vascular tone and homeostasis (Boger RH 1995). Since TCW shows hypolipidemic and cardioprotective effect, studies were carried out to investigate the anticoagulant properties of TCW in rabbits induced myocardial infarction (MI) and in rats induced experimental hypercholesterolemia.

Tender coconut water and thrombosis in experimental myocardial infarction

In this study, Newzeland strain rabbits weighing 2-2.5 kg were divided into two batches of 16 each. One batch was kept as the control and the other was infused with tender coconut water. 10 ml TCW /kg body wt, was given intravenously through the marginal ear vein at a rate of 10 drops/minute. Control rabbits received the same volume of physiological saline. Three hours after the administration of TCW and saline the rabbits in each batch were randomly subdivided into two groups and isoproterenol was administered for the induction of myocardial infarction to one group of the control and one group of TCW administered rabbits. Two hours after isoproterenol administration, the rabbits were subjected to second infusion of TCW (10 ml/kg body wt.). Control rabbits received the same volume of

saline. The same experimental protocol was repeated for the next day. Isoproterenol at a dose of 2 mg/kg body wt, dissolved in 10 ml of saline was administered through the marginal ear vein and a second dose was given 24 hours after the first dose. Four groups consisting of 8 rabbits each were maintained.Group 1-Control rabbits, Group 2 - Isoproterenol administered rabbits, Group 3-Rabbits given TCW, Group 4-Rabbits given TCW two hours prior and two hours after isoproterenol administration. Rabbits were sacrificed on the second day, 3 hours after the second dose of isoproterenol and blood was collected in anticoagulated tubes for the following haematological estimations- WBC, RBC, platelet count, Hb content, fibrinogen, fibrin, prothrombin time, factor V and 6 keto PG $F_1\alpha$.

Tender coconut water and thrombosis in experimental hypercholesterolemia

Male albino rats (Sprague Dawley strain, weight 140-150g) were segregated into three groups of 6 rats. They were treated as follows, Group 1 - rats fed laboratory diet, Group 2 - rats fed high fat diet, Group 3 - rats fed high fat diet and TCW. High fat cholesterol diet contains 0.5 per cent cholesterol and 10 per cent ground nut oil. TCW was given orally by intragastric gavage at a dose of 4 ml/100 g body wt, daily. After 90 days rats were sacrificed and blood was collected in anticoagulated tubes and above mentioned hematological estimation was done.

Results and discussion

The results indicate that isoproterenol administration and hypercholesterolemia results in abnormal blood clotting in rabbits and rats respectively. Isoproterenol administration and hypercholesterolemia significantly increased the levels of fibrin, fibrinogen, factor V and decreased the level of 6 keto PG $F_1\alpha$ and prothrombin time. Intravenous administration of TCW to isoproterenol treated rabbits and in hypercholesterolemia rats caused significant decrease in concentration of fibrin, fibrinogen, factor V and increased concentration of 6 keto PG $F_1\alpha$ and increased prothrombin time.

Isoproterenol administration and high fat cholesterol diet caused high levels of WBC and platelet count while in RBC and Hb levels showed no significant change. Leukocyte accumulation and fiber deposition in thrombi mediates adhesion of monocytes and platelets, leading to initiation of coagulation (Cheuk-Kiet et al., 2003). A high total WBC count is commonly found with the major predictors of atherosclerosis (Prentice RL, 1982 Friedman GD 1990 Nieto FJ1992). The higher total WBC count was mainly due to an increase in the number of neutrophils and lymphocytes (Zei-Shung Huang 1996). L-arginine in TCW decreases the WBC count as Nitric oxide plays an important role in the regulation of the peripheral blood number of neutrophils and lymphocytes (Geffner JR1995).

Platelet aggregation is considered as a predisposing factor for thrombi formation and involved in initiating the process of atherosclerosis (Jacobi et al., 1981). Hypercholesterolemia reduces production of nitric oxide, a potent inhibitor of platelet aggregation in endothelial cells. An increase in platelet aggregability is associated with unstable angina and Myocardial infarction. L-arginine in TCW restores endogenous nitric oxide activity and inhibits platelet aggregation (Emerson M1999) (Wolf A1997).

Recent research has shown that fibrinogen level is a more reliable indicator of heart disease risk than total cholesterol level. Fibrinogen is converted in to fibrin in the formation of blood clot by the enzymatic action of thrombin. The rate of conversion of fibrinogen in to the insoluble product fibrin is a key factor in haemostasis (Brummel et al., 1999). The strands of fibrin bind the platelet together and tighten the clot to make it stable. There is a significant positive correlation between plasma fibrinogen and serum total cholesterol in diet induced hyperlipidemia. Plasma lipids lengthen the fibrinogen half life time, that is, plasma triglycerides and cholesterol decrease the clearance rate of fibrinogen (Verschuur M, 2001). Thus high cholesterol diet causes a pre-hypercoagulative state due to increase in plasma fibrinogen levels (Okazaki M 1994). Intravenous infusion of TCW lowered the levels of fibrinogen and fibrin, reducing the risk of clot formation. The increase in fibrinogen levels produced was reversed by L-arginine in rats (Kawabata, 1996).

Prothrombin, or factor II, is one of several clotting factors produced by the liver. Prothrombin time is an important coagulation test because it measures the presence and activity of five different blood clotting factors (factors I, II, V, VII, and X). During the clotting mechanism, prothrombin is converted to

thrombin, which transforms fibrinogen into insoluble fibrin clot. Lower prothrombin indicates hyper coagulability. High fat cholesterol diet causes shortened prothrombin time and increases factor V and fibrin. A cholesterol rich diet increases cholesterol, triglycerides and factor II, VII, IX, X, XII levels, shortens the occlusion time and increased fibrin deposition (Amalia De Curtis 2001). V was found to be an independent risk factor for Myocardial infarction. The role of factor V is to activate thrombin. Intravenous administration of TCW prolonged prothrombin and decreased the levels of factor V in rabbits and rats, demonstrating its anticoagulant potential.

The concentration of 6 keto PG $F_1\alpha$ was found to be decreased in isoproterenol treated and hypercholesterolemic control animals while treatment with TCW increased their levels. PG I_2 is a critically important local regulator of a variety of cellular processes and 6 keto PG $F_1\alpha$ a stable metabolite of PGI_2. Cholesterol-feeding enhances platelet aggregation and TXA_2 formation, and stimulates platelet endothelial cell interaction. These effects are probably due to impaired Nitric oxide elaboration, as indicated by decreased urinary nitrate excretion. Dietary supplementation with TCW which is rich in L-arginine elevates systemic Nitric oxide elaboration and significantly increases the PGI_2/TXA_2 ratio (Bode-Boger SM 1998). The release form endothelial cells PGI_2act as potent vasodilators and inhibitor of platelet aggregation (Vaneetal., 1990). It thus beneficially influences the homeostasis between vasodilator and vasoconstrictor prostanoids *in vivo*.

Thrombolytic therapy has become standard treatment for coronary heart disease. Thrombolytic therapy results in fibrinolysis and varying degrees of depletion of circulatory fibrinogen, factor V and fibrin (Rutsch and Schnautzler, 1994). The decreased level of fibrinogen, fibrin, factor V and increased 6 keto PG $F_1\alpha$ and prothrombin in TCW administrated animals, suggests that TCW can be used for thrombolytic therapy. TCW significantly influences blood clotting process contributing to a hypocoagulable state, and the antithrombotic effect is mainly mediated through L-arginine-Nitric oxide dependent mechanism.

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