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"A STUDY ON IN VITRO ANTI-UROLITHIATIC ACTIVITY OF AQUEOUS EXTRACTS OF GREEN TEA & LEMON"

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ABSTRACT

A kidney stone, also known as a renal calculus is a solid concretion or crystal aggregation formed in the kidneys from dietary minerals in the urine. Urolithiasis is a complex process that occurs from series of several physicochemical event including super-saturation, nucleation, growth, aggregation and retention within the kidneys. Data from in-vitro, in- vivo and clinical trials reveal that phytotherapeutic agents could be useful as either alternative or an adjunct therapy in the management of Urolithiasis. Medicinal plants / natural products are more useful for body because they promote the repair mechanism in natural way. Plant species of *Green tea and Lemon*, have been reported to posses antiurolithiatic property. In this study aqueous, extracts of *Green tea plus Lemon* and standard for dissolving kidney stones- calcium oxalate by an in-vitro model. To check their potential to dissolve kidney stones- calcium oxalate by an in-vitro model for *Green tea and Lemon* and cystone as a standard compound collected from market. In the present study we found that Green Tea aqueous extract and Lemon juice are decreases formation of kidney stones due to presence of high rich contents of phenolic and flavanoids.

KEYWORDS: Kidney Stones, Urolithiatic, Calcium Oxalate, Green Tea and Lemon.

INTRODUCTION

Hemolysis is classically understood as the release of hemoglobin and other intracellular components from erythrocytes to the surrounding plasma, following damage or disruption of the cell membrane. Hemolysis may occur either in vivo or in vitro, and is a most undesirable condition that influences the accuracy and reliability of laboratory testing.[1] Along with pre

analytical causes, in vivo blood cell lysis can originate from hereditary, acquired, and iatrogenic conditions, such as autoimmune hemolytic anemia, severe infections, intravascular disseminated coagulation and transfusion reactions; it does not depend on the technique of the healthcare provider and is thus virtually unavoidable and cannot be resolved.[2] Visible hemolysis, as a hallmark of a more generalized process of blood cell damage, is usually not apparent until the separation of serum or plasma has occurred. It is commonly defined as an extracellular hemoglobin concentration of >0.3 g/L (4.65 mol/L), resulting in a detectable pink-to-red hue of serum or plasma with a visible appearance in specimens containing as low as 0.5% hemolysate.[3]

Medicinal plants are the rich source of medicinally important compounds and since ancient time, plants and plant derived products are used as medicine in traditional and folk medicinal system. Initially the herbal drugs were used in the form of dried powder, gums, extracts or formulations of more than one plant products. Advanced scientific techniques brought a revaluation in herbal medicine industry and all focus is concentrate on active principles (bioactive molecule). However, a lot of processing is required to develop a drug from the natural sources. Toxicity of the active molecule is a key factor during drug designing, and haemolytic activity represents a useful starting point in this regard, it provides the primary information on the interaction between molecules and biological entities at cellular level. Haemolytic activity of any compounds is an indicator of general cytotoxicity towards normal healthy cells (Da Silva et al., 2004).[4] In vitro haemolytic assay by spectroscopic method provides an easy and effective method for the quantitative measurement of hemolysis. This method provides the evaluation of the effect of different concentrations of biomolecules on the human erythrocytes.

Malaria is an endemic infectious disease causing morbidity and mortality in tropical and subtropical areas of the world. Most deaths from malaria occur among children living in Africa where a child dies every minute. Malaria is caused by Plasmodium parasites which are spread to people through the bites of infected Anopheles mosquitoes. Although an effective vaccine is the best long term control for malaria, it is still not available. The global strategy for malaria control mainly focused on treatment using antimalarials to reduce or eliminate parasites. However, the emerging of drug resistant malaria parasites and insecticide resistant Anopheles mosquitoes has limited adequate treatment of malaria.[4,5,6] Therefore, there is an

urgent need develop new antimalarials to fight with the parasites One way is to isolate new antimalarial compounds from plants that are not yet fully explored.

The effective antimalarial activity of the two plant based drugs, quinine and artemisinin[7], has generated much interest to explore other plant resources for their possible antimalarial efficacy. Tea (Camellia sinensis), originated in China, is a widely consumed beverage throughout the world. The growing interest in the health benefit of tea has prompted numerous investigations on their biological properties. There are two major kinds of tea, black and green tea. Both of them contain large amounts of phenolic substances consisting of catechin in green tea and the a flavin in black tea.[8] It was suggested that activities of tea polyphenol are mostly due to their powerful scavenging and antioxidant activity.[9]

Antioxidant tea components are reported to have beneficial protective effects against cancers and pathogenic microorganisms.[10,11] In addition to antioxidant, green and black tea extracts have been shown to improve erythrocyte survival in vivo during oxidative stress condition.[12,13] Moreover, green and black tea extracts have been reported to be more effective against oxidative stress-induced erythrocyte hemolysis.[14] It has been suggested that oxidative stress is able to induce hemolysis by increasing of permeability of erythrocyte membrane and polyphenolic content especially catechins and theaflavins have protective effects of hemolysis by maintain and reduce oxidative stress condition. Furthermore, correlation between Plasmodium parasite growth or parasitemia and anemia has been studied. Severe malarial anemia is a major complication of malaria infection and is multi-factorial resulting from loss of circulating erythrocytes from parasite replication, as well as immunemediated mechanisms.[15,16,17] Recently, antimalarial activity of green tea in both crude extract as well as some its major polyphenolic content has been observed in P. falciparum in vitro.[18,19]

The major phenolic content in green tea is catechins, and it has been previously demonstrated the antimalarial activity. It was found that antioxidant activity of green tea catechins correlate to antimalarial property, especially the interference with fatty acid biosynthesis may represent a primary mechanism to explain the observed in vitro growth inhibition effects.[20] In the present study we aim to evaluate the antihemolytic activity of Green Tea.

METHODOLOGY

MATERIALS AND METHODS

The following materials and methods are used in the present study.

Materials

Chemicals: Kidney Stones, Tris Buffer, Potassium Permanganate, Eggs, Hydrochloric Acid.

Apparatus and Instruments: Microcentrifuge tubes, Microcentrifuge, Micropipettes.

Plant Materials: Green Tea and lemon collected from local market.

Preparation of Green Tea Extract (10mg/ml)

Dried leaves of green tea (*Camellia sinensis* L.) were purchased from a Local Market s For extraction, 1 g of ground leaves of each tea sample was extracted with 100 ml of distilled water (DW) at constant temperature of 95 °C under continuous Stirring .The supernatant was Subsequently filtered through Whatman No. 1 filter paper to remove rough particles and then centrifuged at 3,000 rpm for 10 min. The supernatant, called green tea crude extracts (GTE) was stored at 2–4 °C until analyzed.

Preparation of Lemon fruit juice concentrates

The fruits were rinsed thoroughly with distilled water and were cut into halves. The juice was extracted from the fruits using a juice extractor. The fruit juices were then lyophilized and the concentrates obtained were preserved at 4°C in airtight containers until subsequent use.

Phytochemical screening

The Plant extracts were subjected for the presence of different phytoconstituents like alkaloid, steroid, flavonoid, tannin, Glycoside etc.

Evaluation for Anti-urolithiatic Activity

Collection of Kidney stones from Hospital.

Preparation of Semi-Permeable Membrane from Farm Eggs

The semi - permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Shell was removed chemically by placing the eggs in 2M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water and carefully with a sharp pointer a hole is made on the top and the contents squeezed out completely from the decalcified egg. Then egg membrane washed thoroughly with

distilled water and placed it in ammonia solution, in the moistened condition for a while & rinsed it with distilled water. Stored in refrigerator at a pH of 7- 7.4.

Estimation of Calcium oxalate by Titrimetry

Weighed exactly equal wt of the kidney stones and 10mg of the extract/compound/standard and packed it together in semi Permeable membrane by suturing. This was allowed to suspend in a conical flask containing 100ml 0.1 M TRIS buffer. One group served as negative control (contained only kidney stones). Place the conical flask of all groups in an incubator, preheated to 37^0 C for 2 hours, for about 7-8 hours. Remove the contents of semi-permeable membrane from each group into a test tube. Added 2 ml of 1 N sulphuric acid and titrated with 0.9494 N KMnO4 till a light pink colour end point obtained. 1ml of 0.9494 N KMnO4 equivalent to 0.1898mg of Calcium. The amount of undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually test substance(s) could dissolve.

In the present study we taken as total groups are following.

1. Group 1 (Negative Control it contains kidney stones only)
2. Group 2 (Positive Control it contains kidney stones plus Cystone standard drug)
3. Group 3 (Test I it contains kidney stones plus Green Tea Extract)
4. Group 4 (Test II it contains kidney stones ,Green Tea Extract and Cystone standard drug)
5. Group 5 (Test III It contains kidney stones, Green Tea Extract and Lemon Juice)

RESULTS AND DISCUSSION

Qualitative chemical tests indicated the presence of phenolic compounds, flavnoids, steroids and Saponin extracts of *Green tea and Lemon.* On basis of this fraction we performed *in vitro* Anti-Urolithiatic Activity by comparing extracts of *Green tea and Lemon* with standard. % Dissolution of Calcium oxalate table is given below.

Table 1: % Dissolution of Calcium oxalate.

S.No.	Groups	%Dissolution of Calcium Oxalate
1	Normal Control	0
2	Standard (Cystone)	59.08
3	GTE	19.03
4	GTE+Cystone (Std)	75.2
5	GTE+Lemon Juice	78.5

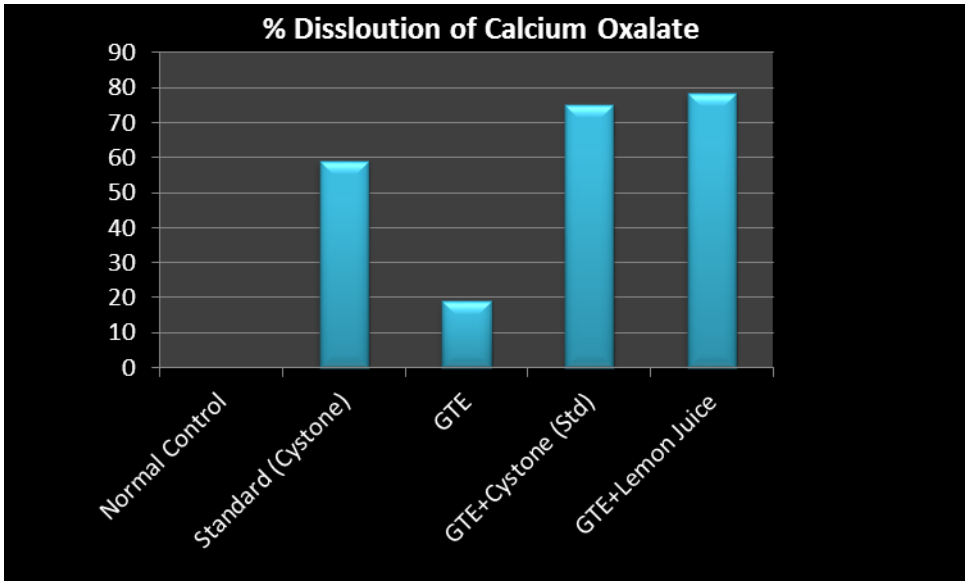

Table 2: Percentage reduction in Weight.

S.No.	Groups	Initial Wt (gms)	Final Wt (gms)	%Reduction in Wt(gms)
1	Normal Control	0.11	0.11	0
2	Standard (Cystone)	0.11	0.06	45.45
3	GTE	0.11	0.08	27.27
4	GTE+Cystone (Std)	0.11	0.05	54.54
5	GTE+Lemon Juice	0.11	0.04	63.63

Urolithiasis resembles arteriosclerosis in the mechanism, calcification composition, epidemiology and gene relationship. It has been reported that the vascular endothelial growth factor gene polymorphism is a suitable genetic marker of urolithiasis. Calcification in arteriosclerosis has been inhibited by antioxidants. Consumption of teas is generally known to increase urinary oxalate excretion. Antioxidant therapy with vitamin E has prevented calcium oxalate precipitation in the rat kidney and decreased urinary oxalate excretion in patients with kidney stones. The renal antioxidants vitamin E, ascorbic acid and glutathione were significantly decreased on oxalate challenge. Vitamin E administration in patients who underwent surgical stone removal rapidly restored antioxidant levels in the blood and decreased the urinary excretion of oxalate and calcium. A previous study of antioxidant enzyme levels in rats with stone formation showed that almost all antioxidant enzyme activities were attenuated except that of catalase. Sarica et al reported that calcium oxalate crystals and hyperoxaluria may be injurious to renal tubular cells, as indicated by apoptotic changes in a urolithiasis rabbit model. Sustained hyperoxaluria in association with calcium oxalate crystals induced apoptosis swell as necrosis. Although cell death by hypoxia is a well-known type of oxidative stress, which has been generally believed to manifest as

necrosis, recent biochemical observe NF-κB actions suggest the possibility of hypoxia induced apoptosis. Wu et al reported that NF-κB activation by oxidative stress induced human aortic endothelial cell death and apoptosis through the suppression of bcl-2, bax translocation and p53 induction. Apoptosis observed in this experiment was also thought to depend on the same mechanism. The blockade of NF-κB activation by antioxidants has been suggested to be an effective strategy for the treatment of urolithiasis and arteriosclerosis.

Lemon juice has a high antioxidant capacity due to the presence of citrate, vitamin C, vitamin E and flavonoids such as eriocitrin, hesperetin and limonoids. Vitamin E may prevent calcium oxalate crystal deposition in the kidney by preventing hyperoxaluria-induced per oxidative damage to the renal tubular membrane surface (lipid peroxidation), which in turn can prevent calcium oxalate crystal attachment and subsequent development of kidney stones.

In the present study we observed that due to presence of high rich contents of flavanoids and Phenolic compounds present in the Green tea and Lemon juice showed the much more effect when compared with the standard drug like cystone. From the above study elucidated that the possible mechanism might be due to the Lemon juice and green tea has a high antioxidant capacity due to the presence of citrate, vitamin C, vitamin E and flavonoids such as eriocitrin, hesperetin and limonoids.

CONCLUSION

Green tea has an inhibitory effect on urinary stone formation, and the antioxidative action is considered to be involved. The present study found that the administration of lemon juice effectively prevented the development of urolithiasis. These findings support the use of lemon juice and green tea as an alternative medicine to prevent urolithiasis. Further research is necessary to clarify the mechanism underlying this preventative effect of lemon juice and green tea.

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