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Evaluation of *In-Vitro* Anti-Urolithiatic Activity of Methanolic Leaf Extract of *Ricinus communis*

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Abstract: In the current scenario herbal medicines became the main system for the treatment of many diseases. Because of the advancement in science and technology a huge number of plants were discovered having therapeutic capabilities to treat many deadly diseases and have gain importance and acceptance within the medical community. Among various diseases urolithiasis has been a common problem from various centuries. For the treatment of urolithiasis many synthetic drugs are available in the market but because of their side effects herbal remedies are widely accepted. Our present study aimed to evaluate the therapeutic potential to treat urolithiasis (kidney stones) where cystone was used as a standard drug. The present study revealed that the leaves of *Ricinus communis* possess mild antiurolithiatic activity which might be due to the presence of various phytochemical constituents such as flavonoids, alkaloids, triterpenes etc.

Keywords: *Ricinus communis,* urolithiasis, treatment, antiurolithiatic.

INTRODUCTION

Herbal medicines are defined as a plant or plant part or an extract or mixture of these used in herbal treatment. The pharmacological treatment of many diseases started long ago with the use of herbs [1]. Recently herbal medicine has become the main scientifically based system of many diseases. Because of public as well as medical establishments, studies leading to the scientific explanation of plant therapeutic capabilities are allowing this practice to gain increasing credibility and acceptance within the medical community [2].

Rapid changes in lifestyles have occurred with industrialization, urbanization, economic development and market globalization over the past decade have increased the growing epidemic of chronic non communicable diseases including obesity, diabetes mellitus, cardiovascular disease (CVD), hypertension, stroke and some types of cancer which are significant causes of disability and pre mature death in developing and newly developing countries, placing additional burdens on already overtaxed national health budget [3].

The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are combing the Earth for phytochemicals which could be developed for treatment of various diseases [4].

At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants. In many medicinal and aromatic plants (MAPs), significant variations of plants characteristics have been ascertained with varying soil traits, and the selective recovery and subsequent release in food of certain elements have been demonstrated. Great attention must be paid to choose soil and cropping strategies, to obtain satisfactory yields of high quality and best-priced products, respecting their safety and nutritional value [5].

Many of the medicines currently available to the public that have a long history of use as herbal remedies, including aspirin, quinine, opium, digitalis etc. According to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants. Among the 120 active compounds currently isolated from the higher plants are widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived [6].

MATERIALS AND METHODS

In vitro anti-urolithiatic activity test was carried out by turbidity method, in this method the *in vitro* anti-urolithiatic activity of the extract was tested in terms of inhibition of calcium oxalate formation by the

method of Prachi Khare *et al*. with modification [7]. The inhibition of calcium oxalate formation in the presence of the extract was compared with the inhibition of calcium oxalate formation in the presence of the standard (Cystone). The precipitation of calcium oxalate at 37°C and pH 6.5 was studied by the measurement of turbidity at 620nm using UV/Vis spectrophotometer. The turbidity caused due to formation of calcium oxalate by the reaction of calcium chloride ($CaCl_2$) with sodium oxalate.

In the control, turbidity due to the formation of calcium oxalate was determined in the absence of any inhibitor. For this, a volume of 0.95 ml of 50mM $CaCl_2$ (in Tris buffer pH 6.5) and 1ml of water were added in a test tube. Then 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5) was added. Formation of the turbidity results immediately after mixing of above chemicals. The measurement of turbidity was done by measuring the absorption by UV/Vis spectrophotometer at 620 nm after shaking the mixture for 1 min. Then the measurement of the absorbance was carried out after 1 min interval up to a period of 5 min and absorptions were noted down.

The study was continued to know the effect of plants extracts against stone nucleus formation (formation of calcium oxalate) *in vitro*. In this experiment the effect of the extract on inhibition was carried out in two concentrations of the extract. For this, in one test tube a volume of 0.95 ml of 50mM $CaCl_2$ (in Tris buffer pH 6.5) and 1ml of 100 µg/ml extract in water were added, and then 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5) was added. In another test tube a volume of 0.95 ml of 50mM $CaCl_2$ (in Tris buffer pH 6.5) and 1ml of 250 µg/ml extract water were added, and then 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5) was added. The measurement of turbidity was done by measuring the absorption by UV/Vis spectrophotometer at 620 nm after shaking the mixture for 1 min. Then the measurement of the absorbance was carried out after 1 min interval up to a period of 5 min and absorptions were noted down.

After that the effect of the standard (cystone) on the inhibition of the formation of calcium oxalate was studied. The standard drug is a poly herbal formulation. For this a volume of 0.95 ml of 50mM $CaCl_2$ (in Tris buffer pH 6.5) and 1ml of 100 µg/ml of standard in water were added, and then 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5) was added. The measurement of turbidity was done by measuring the absorption by UV/Vis spectrophotometer at 620 nm after shaking the mixture for 1 min. Then the measurement of the absorbance was carried out after 1 min interval up to a period of 5 min and absorptions were noted down.

Thus, test tubes were divided into the following groups:

Control: 0.95 ml of 50mM $CaCl_2$ (in Tris buffer pH 6.5) + 1ml of water + 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5)

Blank: 0.95 ml of Tris buffer pH 6.5 + 1ml of water + 0.95 ml of Tris buffer pH 6.5

Test 100 µg/ml (A1): 0.95 ml of 50mM $CaCl_2$ (in Tris buffer pH 6.5) + 1ml of 100 µg/ml of extract in water + 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5)

Test 250 µg/ml (A2): 0.95 ml of 50mM $CaCl_2$ (in Tris buffer pH 6.5) + 1ml of 250 µg/ml of extract in water + 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5)

Standard 100 µg/ml (A3): 0.95 ml of 50mM $CaCl_2$ (in Tris buffer pH 6.5) + 1ml of 100 µg/ml of standard in water + 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5)

Standard 250 µg/ml (A4): 0.95 ml of 50mM $CaCl_2$ (in Tris buffer pH 6.5) + 1ml of 250 µg/ml of standard in water + 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5)

Product Control for 100µg/ml (A5): 0.95 ml of Tris buffer pH 6.5 + 1ml of 100 µg/ml of extract in water + 0.95 ml of Tris buffer pH 6.5

Product Control for 250µg/ml (A6): 0.95 ml of Tris buffer pH 6.5 + 1ml of 250 µg/ml of extract in water + 0.95 ml of Tris buffer pH 6.5

Product Control for Standard 100µg/ml (A7): 0.95 ml of Tris buffer pH 6.5 + 1ml of 100 µg/ml of standard in water + 0.95 ml of Tris buffer pH 6.5

Product Control for Standard 250µg/ml (A8): 0.95 ml of Tris buffer pH 6.5 + 1ml of 250 µg/ml of standard in water + 0.95 ml of Tris buffer pH 6.5

Inhibition in stone nucleus formation was calculated by the graphical method using the following mathematical formula:

$$\text{Inhibition \%} = \{1-[Si / Sc]\} \times 100$$

Where;

Si: Slope of graph in the presence of inhibitor (drugs/extracts).

Sc: Slope of graph without inhibitor (Control).

RESULTS AND DISCUSSION

Results

In vitro anti-urolithiatic activity test by turbidity method i. e. inhibition of calcium oxalate crystal growth method. The results for this method are mentioned below (Table 1, and figure 1).

Table-1: Calculation of the percentage inhibition from the regression equation

Sample	Regression equation	Slope	% Inhibition
Control	y = 0.0218x + 0.3248	0.0218	-
Test 100 µg/ml	y = 0.0152x + 0.264	0.0152	30.27%
Test 250 µg/ml	y = 0.0155x + 0.2345	0.0155	28.89%
Standard 100 µg/ml	y = 0.0100x + 0.204	0.0100	39.44%
Standard 250 µg/ml	y = 0.0132x + 0.072	0.0132	54.12%

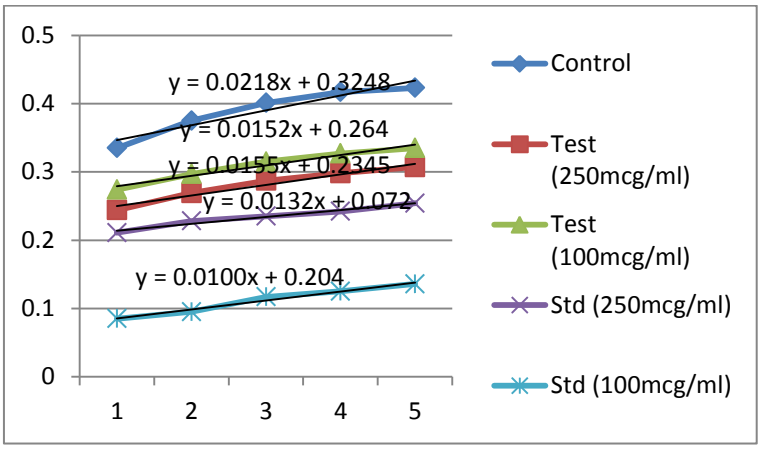

Fig-1: Figure showing change in turbidity without and with plant extracts and standard at 620nm

Calculation

Calculation of the % inhibition in the presence of extract and standard were calculated by using the following formula:

% Inhibition= $\{1-(S_i/S_c)\}$ x100

S_i = Slope of graph in the presence of inhibitor (extract and standard)
S_c= Slope of graph without inhibitor (control)

Discussions

The *in-vitro* anti-urolithiatic activity of the extract was evaluated by using turbidity method (inhibition of calcium oxalate formation). In the turbidity method i. e; the inhibition of calcium oxalate formation was measured in terms of turbidity by using UV-Visible spectrophotometer. The formation of calcium oxalate crystals were indicated by the formation of turbidity in the solution. More the inhibition less is the turbidity and less absorbance will be observed in UV-Visible spectrophotometer. The inhibition of calcium oxalate formation in the presence of the extract was compared with the inhibition of calcium oxalate formation in the presence of the standard (Cystone). The study carried out at 37°C and pH 6.5. The measurement of turbidity was done at 620nm using UV/Vis spectrophotometer.

First of all, growth of stone nucleus *in vitro* in the absence of any inhibitor was done (control). The turbidity was formed immediately after mixing of chemicals according to the procedure of the method and then the turbidity formed was measured in terms of absorption at 620 nm in UV/Vis spectrophotometer for a period of 5 min at an interval of 1 min. Absorptions were noted down and data obtained was used as the un-controlled growth of the stone nucleus for the comparison of growth in the presence of the standard drugs and plant extracts. And then the study was continued to know the effect of plants extracts against stone nucleus formation *in vitro* according to the procedure mentioned in the materials and methods chapter. Inhibition in stone nucleus formation was calculated by the graphical method using the mathematical formula mentioned in materials and methods section.

As *in vitro* crystallization study was performed, since nucleation is an important first step for the initiation of crystals, which then grow and form aggregates. Extract of *Ricinus communis* inhibited the crystallization by inhibiting nucleation of calcium oxalate through disintegrating into smaller particles with increasing concentrations of the fraction. From the results of the nucleation assay confirmed that the extract contained nucleation-preventing agents.

In this study it is observed that the low dose of test (100 µg/ml) has shown 30.27% percentage of inhibition of calcium oxalate formation whereas higher dose (250 µg/ml) of test has shown 28.89%, while the standard drug has shown 39.44% percentage inhibition for lower dose (100 µg/ml) and higher dose (250 µg/ml) has shown 54.12% (Table 1, and figure 1).

The observed anti-urolithiatic activity of the methanolic extract of the leaf of *Ricinus communis* might be due to the various phytoconstituents present in it. The plant *Ricinus communis* contains several

phytochemical constituents belonging to categories such as alkaloids, carbohydrates, glycosides, tannins, triterpenes and flavonoids etc. [8]. Previous studies on triterpenoids have been shown to possess antiurolithiatic effect. The lowering of oxalate excretion on treatment with the triterpene indicates the synthesis of oxalic acid from the glycollic acid is somehow inhibited [9]. Flavonoids containing plants have been reported to be associated with anti-urolithiatic activity [10].

CONCLUSION

From this study, it can be concluded that methanolic leaf extracts of *Ricinus communis* have moderate antiurolithiatic activity i. e. ability to inhibit the formation of calcium oxalate crystals *in vitro*. This is only a preliminary study and to make final comment the extract should be thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

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