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Original Article

CYTOPROTECTIVE EFFECT OF *OCIMUM* EXTRACT ON INJURED RENAL EPITHELIAL CELLS

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ABSTRACT

Objectives: Renal cell injury is one of the important factors affecting urolithiasis. Crystals which aggregate to constitute the kidney stones are formed in the supersaturation condition in kidney. Reactive oxygen species generated due to the injury of renal epithelial cell by crystal formation and the injured cells itself participate in kidney stone formation. In the present study antioxidant, anti-inflammatory and cytoprotective effect of aqueous extract of *Ocimum* leaves were studied.

Methods: Qualitative analysis of the plant extract was done using the basic phytochemical screening. DPPH (1, 1-diphenyl-2picrylhydrazyl) free radical scavenging activity test was done to estimate antioxidant activity of plant extract and protein denaturation method used to assess anti-inflammatory property of plant extract. The MTT (3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay was also done for the evaluation of cytoprotective effect of the plant extract on Vero cell line.

Results: The results indicated that different concentrations of aqueous extract had strong antioxidant (94.5%±1.8) and anti-inflammatory (80.4%±1.3) activity. The aqueous extract increased the cell viability of Vero cell lines in a dose dependent manner. 40µg of the *Ocimum* extract exhibited 88.35%±1.32 cell viability of Vero cells which were injured with calcium oxalate crystals.

Conclusion: Aqueous extract of *Ocimum* has cytoprotective effect on injured Vero cell lines. Anti-inflammatory and antioxidant property of *Ocimum* extract might be responsible for this cytoprotective nature of *Ocimum* extract.

Keyword: Urolithiasis, *Ocimum*, Antioxidant, Anti-inflammatory, Crystal, Vero cell line.

INTRODUCTION

Urinary stones have fascinated the scientific attention due to their high frequency of occurrence as well as their high recurrence rate. Considered as a multifactorial disease, incidence of nephrolithiasis is reported to be increasing across the world [1]. Along with other factors like diet, lifestyle and geographical distribution, oxidative stress is also one of the factors affecting kidney stone disease. Formation of kidney stones has also been linked to high oxidative stress and damage to the tubular cells of the kidney in humans. Non-obstructing stones produce no symptoms or signs apart from hematuria. Stones less than 5 mm in diameter have a high chance of passage; those of 5–7 mm have a modest chance (50%) of passage, and those greater than 7 mm almost always require medical intervention [2]. About 80% of stones are calcium based and about 80% of those are calcium oxalate stones [3].

Oxidative stress has a critical role in the pathophysiology of several kidney diseases, and many complications of these diseases are mediated by oxidative stress, oxidative stress-related mediators and inflammation [4]. Reaction Oxygen Species (ROS) and damaged cells provide the platformm for the nucleation of the kidney stone formation. Surgical intervention to treat kidney stone disease has several side effects. About 80% of the patients have the chances of recurrence of this disease. Surgical methods provide relief to the patient but have serious side effects.

Therefore, for the successful management of this disease it is necessary to explore the use of alternative treatment which has minimal or no side effects. Several medicinal plants have been used since ages to treat kidney stones in traditional medicine like *Bergenia ligulata, Rotula aquatic, Bauhinia racemosa, Coleus spp., Bryophyllum spp., Didymocarpus pedicellata, Ocimum basilicum* [5].

Ocimum sanctum, commonly known as *tulsi*, is being used in Ayurveda for thousands of years for its diverse healing properties. Different plant parts of *Ocimum sanctum* such as the leaves, stem, flower, root, seeds and even whole plant have been traditionally used for the treatment of several diseases. The decoction of leaves and flowers are used to treat cough and cold and eye diseases. The poultice prepared from the stem is applied on wound for quick healing. The plant has also been used as an anti-oxidant, anti-inflammatory, immuno-modulatory, anti-infective, anti-stress, antipyretic, anti-diabetic, cardio protective, and neuroprotective and hepatoprotective agent [6]. Chemically, the stem and leaves of *Ocimum sanctum* is known to contain a variety of chemical constituents including the alkaloids, phenols, tannins and the flavonoids [7].

These phytoconstituents may provide this herb its medicinal property against various diseases. Pharmacological studies have demonstrated that *Ocimum sanctum* possess protective effect against several diseases. However, the biological effect of the extract of leaves of *Ocimum sanctum* has never been studied for urolithiasis.

Therefore, to explore alternative treatments for the management of kidney stone disease we investigated the *in - vitro* anti- inflammatory and antioxidant activity of *Ocimum sanctum* leaf extract. The cytoprotective effect of leaf extract was studied on Vero cell lines.

MATERIALS AND METHODS

Plant collection

The leaves of *Ocimum sanctum,* were collected from the local areas of Delhi and Noida and were authenticated by a botanist at Delhi University and the specimen submitted to the Delhi University Herbarium of the Botany Department.

Extract preparation

250 grams of the dried powdered Ocimum leaves were used for extraction. Powdered sample was boiled in 1000 ml of distilled water until the volume was halved. The extract was then filtered using the muslin cloth and the filtrate was cooled and lyophilized. 10 mg of lyophilized sample was collected and stored at 4 °C until use.

Phytochemical screening of plant extract

Crude extract of the *Ocimum* leaves was subjected to various phytochemical analyses to establish the presence of different chemical components such as flavonoids, alkaloids, sterols and terpenoids, phenols and the tannins test [8].

Determination of total flavonoid content

The total flavonoid content of *Ocimum* extract was determined using the aluminium chloride colorimetric method [9]. Gallic acid was used as a reference standard for plotting the calibration curve. 1 ml of plant extract was mixed with 3 ml of methanol along with 0.2 ml of 10% aluminium chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water. Reaction mixture was incubated at room temperature for 30 min. Absorbance of the reaction mixture was measured at 420 nm with UV spectrophotometer. The total flavonoid content was determined from the linear equation of standard curve prepared with gallic acid.

Determination of antioxidant activity by DPPH-scavenging assay

1, 1–diphenyl–2–picrylhydrazyl (DPPH) (Himedia, Mumbai, India) radical scavenging method [10] was used to determine the free radical scavenging activity of *Ocimum* extract. The assay mixture contained 1 ml of 1 mM DPPH radical solution prepared in methanol and 1 ml of extract solution. The solution was rapidly mixed and incubated in dark at room temperature for 30 min. Absorbance was measured at 517 nm using UV spectro-photometer. The percentage inhibition (%) was calculated by the following formula:

% free radical scavenging activity = [(absorbance of control–absorbance of sample)/absorbance of control] X 100..... [Eq.1]

Evaluation of *in-vitro* anti-inflammatory activity

Anti-inflammatory activity was evaluated by protein denaturation method [11,12]. Diclofenac (non steroidal anti-inflammatory drug) (Salveo life care, Zirakpur, India) was used as the standard drug. The reaction mixture consisted of the plant extract (100-400 µg/ml) or standard diclofenac sodium (100-200 µg/ml) and was mixed with 2.8 ml of phosphate buffered saline (pH 6.4) and 2 ml of 1 mM egg albumin (Qualigens, Navi Mumbai, India) and incubated at room temperature for 15 min. Reaction mixture was then kept at 70 °C for 10 min to induce denaturation of protein. After cooling, the absorbance was measured at 660 nm. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = [(absorbance of test sample–absorbance of control)/absorbance of control] X100..... [Eq. 2]

Cell culture

Vero cells were obtained from National Centre of Cell Sciences (NCCS, Pune). They were cultured in 75 cm^2 tissue culture flask containing Dulbecco's Modified Eagle's Medium (DMEM) (Sigma Chemical Co., St. Louis, MO, USA); supplemented with 10% Fetal Bovine Serum (FBS) (Sigma Chemical Co., St. Louis, MO, USA) and antibiotics (streptomycin 100 mg/ml), penicillin (60 mg/ml), gentamycin (100 mg/ml). The cultured cells were maintained in a humidified incubator at 37 °C with 5% CO_2.

MTT assay

MTT test was conducted to study cell viability by the method described by Fulya Karamustafa *et al.* [13]. Vero cells were suspended in DMEM with serum and plated into the wells of 96-well tissue culture plates. Plates were incubated for 24 h at 37 °C in a humidified incubator containing 5% CO_2. Then the media was aspirated and 200 µl DMEM (without serum) containing calcium oxalate crystals were added into the wells. After 24 h, the media was aspirated and 200 µl DMEM (without serum) containing different volumes of *Ocimum* extract was added to each well. After 48 h, media was removed. Each well was then subjected to 20 µl MTT (Himedia, Mumbai, India) solutions, and incubated for a further 3 h. Then, MTT solution was removed and 100 µl DMSO was added to dissolve the formazan precipitate. The developed color was read at a wavelength of 570 nm with spectrophotometer.

Statistical analysis

The results are expressed as mean values of three independent experiments (each in triplicate, n=3). Data was analyzed by using the one way analysis of variance (ANOVA) to estimate the differences between values. Results were considered statistically significant if $p<0.05$.

RESULTS

Phytochemical screening

Phytochemical analysis of the leaf extract showed the presence of major phytochemicals such as alkaloids, phenols, flavonoids and tannins. Quantitative estimation of total flavonoid content of *Ocimum* was 26.7 mg/ml.

***In-vitro* anti-inflammatory activity**

Different concentrations (100-400µg/ml) of plant extract were studied to evaluate the inhibitory effect of *Ocimum* leaf extract on protein denaturation. 300µg/ml and 400µg/ml of extract showed significant inhibition of denaturation of egg albumin (fig. 1). Inhibition of denaturation of egg albumin by *Ocimum* leaf extract (100-400µg/ml) was in a dose dependent manner.

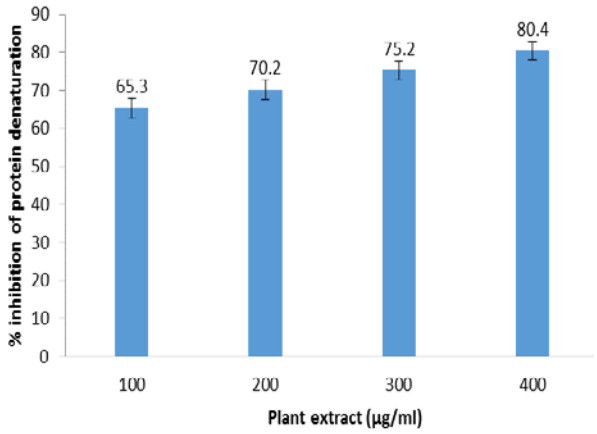

Fig. 1: Percentage inhibition of protein denaturation increased with increase in the concentration of the *Ocimum* extact. 80%±1.3 of maximum inhibition was observed with 400µg/ml of the extract. Results are expressed as mean±SD, n=3 ($p<0.05$)

Antioxidant activity by DPPH-scavenging assay

The antioxidant activity of different concentration of *Ocimum* leaf extract (100-400µg) in DPPH scavenging assay showed a dose dependent activity. The results were expressed as the percentage inhibition is illustrated in fig. 2. The scavenging ability of *Ocimum* leaf extract was also dose dependent. Maximum inhibition was shown by the 300µg/ml and 400µg/ml concentrations of the extract: 92.3%±1.22 and 94.5%±1.8 respectively.

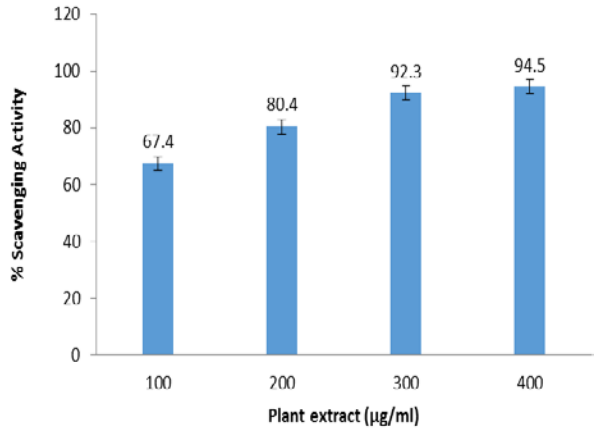

Fig. 2: Phytochemicals present in the *Ocimum* leaf extract showed 94.5%±1.8 of maximum scavenging activity. Results are expressed as mean±SD, n=3 ($p<0.05$)

Effect of *Ocimum* leaf extract on calcium oxalate injured Vero cell line

Different concentrations of the aqueous extract (4-40µg) of *Ocimum* leaf exhibited protective effect on injured Vero cell line. There was a marked decrease in the injury of the Vero cell line that was treated with calcium oxalate crystals (10µg) (fig. 3). As the concentration of the plant extract was increased there was an increase in the protective effect towards calcium oxalate injured epithelial cells. Again, with an increase in the concentration of the aqueous extract of *Ocimum* leaves, the percentage viability of the epithelial cells also increased.

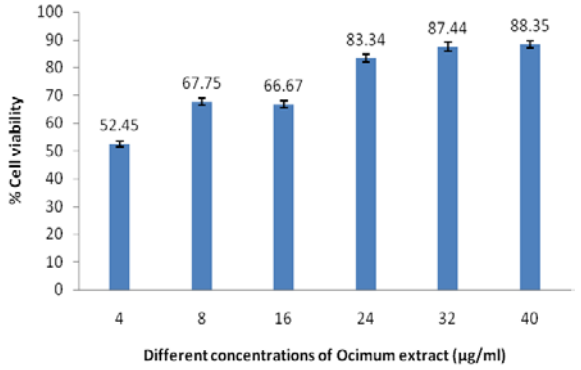

Fig. 3: Effect of various concentrations of *Ocimum* leaf extract on injured Vero cell line. Maximum percentage of cell viability (87.44±1.65 & 88.35±1.32)) was observed with 32 µg/ml and 40 µg/ml of *Ocimum* extract. Results are expressed as mean±SD, n=3 (p<0.05)

DISCUSSION

Supersaturation is the key source for crystallization in urine. Crystals that are formed in this condition serve as nidus for kidney stone formation. Oxidative stress has a critical role in the pathophysiology of several kidney diseases, and many complications of these diseases are mediated by oxidative stress, oxidative stress-related mediators, and inflammation [6]. Due to the side effects of medical intervention in this disease, phytotherapy is gaining popularity these days with several natural products being used to manage the condition. Medicinal plants are the source of natural antioxidant and they reduce the risk and development of many diseases by scavenging free radicals which are associated with the pathogenesis of numerous disease states [14]. *Ocimum sanctum* is rich in antioxidant phytochemicals and has been explored for decades for their protective effect for several diseases.

In the present work, phytochemical screening of *Ocimum* confirmed the presence of alkaloids, phenols, tannins and flavonoids in aqueous extract of its leaves. There are reports that confirmed that the pharmacological properties of *Ocimum* are due to the synergistic interaction of these phytochemicals [7].

In this study we have explored the antioxidant and anti-inflammatory effect of *Ocimum* leaf extract as well as its cytoprotective effect on Vero cell line. Different concentrations of *Ocimum* leaf extract inhibited the denaturation of egg albumin. Inhibition of protein denaturation by the plant extract was established to be dose dependent. Inflammation is the body's response to tissue injury which leads to denaturation of tissue proteins. Agents that can prevent protein denaturation therefore, would be important for the drug development against diseases where inflammatory action plays important role [15].

In addition to the anti-inflammatory activity, *Ocimum* extract also exhibited anti-oxidant activity. Maximum scavenging ability of the extract was 94.5%±1.8. The free radical scavenging ability of plant extract may be due to the presence of secondary metabolites like saponins, flavonoids, triterpenoids, tannins which are known for this biological activity [16]. In addition, the high phenolic content of *Ocimum sanctum* may have also contributed significantly to the antioxidant and anti-inflammatory activity reported [7, 17].

We also observed that the *Ocimum* extract has a cytoprotective effect on Vero cell lines which were injured with calcium oxalate crystals. Again, cytoprotective effect of different concentrations of *Ocimum* extract was dose dependent. Studies have shown that reduction in inflammatory response caused by the crystals help in restoring normalcy [18].

The increase in the cell viability proves that aqueous extract of *Ocimum* has the potential to protect the cells which were under stress due to crystal injury. There are reports suggesting that the *Ocimum* extract has protective effect on several diseases [7].

The results of our study suggest that aqueous extract of *Ocimum* leaves have alkaloids, phenols, tannin and flavonoids. Moreover, the leaf extract of the plant has good *in-vitro* antioxidant and anti-inflammatory activity that justifies its use in traditional medicine system.

CONCLUSION

In conclusion, aqueous extract of *Ocimum* has cytoprotective effects on injured Vero cell line. Anti-inflammatory and antioxidant property of *Ocimum* extract might be responsible for the cytoprotective nature of *Ocimum* extract. Our study throws light on the possible use of *Ocimum sanctum* as the therapeutic agent to treat kidney stone disease. However, further detailed study is required to ascertain the mechanism underlying its anti-oxidant, anti-inflammatory and cytoprotective effect against urolithiasis.

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CONFLICT OF INTERESTS

Declared None

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