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Therapeutic Effect of Epigallocatechin-3-Gallate and Quercetin on Renal Stone Formation in Rats

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Authors' Contribution
ZC contributed to the conception of the study. YL performed the experiment. XL performed analysis and prepared manuscript. SAH performed the data analyses and wrote the manuscript. JRD helped perform the analysis with constructive discussions.

Key words
Kidney stones, EGCG, Quercetin, Anti-oxidants, Serum biochemistry, miR-21

ABSTRACT

In the present study, anti-urolithic potentials of epigallocatechin gallate (EGCG), a constituent of green tea polyphenols and quercetin, individually as well as in combination, was evaluated in animal model. *In vivo* evaluation was carried out on male Sprague-Dawley rats, which were divided into seven groups i.e., Group 1 animals (controls) were fed regular chow and drank water ad libitum; group 2 animals were fed chow containing 3% sodium oxalate with the administration of gentamicin (40 mg/kg) and drank water ad libitum; group 3-7 animals were fed the same diet as group 2 with gentamicin administration and administered with standard drug i.e., allopurinol, EGCG, quercetin and EGCG: quercetin (1:1). All the study animals were sacrificed after 4 weeks of treatment after a 24-hour urine collection, and blood and kidneys were removed for urine and serum biochemical analysis, tissue anti-oxidant, miR-21 as well as morphological examination. We found that administration of Sodium oxalate induced induced oxidative stress, alteration in the serum as well as urinary biochemical alterations as well as spiked expression of miR-21. The alterations were reversed in all the treatment group especially EGCG and EGCG-Quercetin. The reversal potentials of combinational therapy were found to be more effective than the standard drug, Allopurinol. The histopathological analysis also confirmed the same. Although, EGCG has a potent inhibitory effect on urinary stone formation, and the antioxidative action of EGCG is considered to be involved. The combinational activity was enhanced along with quercetin.

INTRODUCTION

It is widely reported that across the globe more than 10% of the population are affected by urinary calculi or also known as kidney stones or urinary tract stone diseases, which is the 3rd most prevalent disorder of the urinary tract (Park *et al*., 2023). Kidney stones or the urinary calculi causes a wide spectrum of damage in the urinary tract like obstruction, hemorrhage or even infection (Parks *et al*., 1997; Hadjzadeh *et al*., 2007). The counter-measures including non-surgical methods as well as surgical intervention have been widely explored and are known to be effective but do have wide range of side effects and in some cases have severe complications.

Although, the mechanism of induction of kidney stones may significantly vary from case to same, but urinary and serum biochemical profiling of the major electrolytes still remains the easiest strategy for the diagnosis of formation of kidney stones (Terlecki and Triest, 2007).

MicroRNAs or commonly known as miRs are a group

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small non-coding RNAs that are involved in the regulation of various gene expressions (McGeary *et al*., 2019). miR-21 is one the most common oncogenic miRNA which is involved in a wide range of cancer such as lung, stomach, colon, prostate, breast and pancreas (Markou *et al*., 2008; Slaby *et al*., 2007; Yan *et al*., 2008). Faragalla *et al*. (2012) reported an upregulation of miR-21 in the renal cell carcinoma, which can potentially be used as a prognostic marker. In the present study, we tried to correlate the expression of mir-21 in the kidney stone induced animal model.

Epigallocatechin gallate (EGCG) and quercetin are probably some of the most widely studied active compounds from plants with significant *in vitro* and *in vivo* data to support (Mukhtar and Ahmad, 2000; Venables *et al*., 2008). In the present study, we evaluated the anti-urolithic potentials of EGCG, quercetin and compared the same with allopurinol, as the standard drug, individually as well as in combination, for their protective activity against cellular toxicity by oxalate and whether it attenuates the development of nephrolithiasis in an animal model.

MATERIALS AND METHODS

Experimental animals

Male albino rats weighing 150-200 g were procured from Zydus Research Centre. All the animals were fed with standardized animal chow and water ad libitum. The rats were acclimatized for a week prior to grouping and subsequently induction. The animals were housed as per the guidelines of CPCSEA. The experimental protocol was approved by Institutional Animal Ethical Committee, Nirma University Ahmedabad under the CPCSEA guidelines of Ministry of Environment and Forest, New Delhi (Protocol No: IS/PHD/21/037).

The animals were divided into six groups of 6 animals each. Group I served as normal control and received regular rat feed and drinking water ad libitum. Sodium oxalate (3%) in drinking water was fed to Groups II - VI for induction of renal calculi for 28 days. Group II served as lithiatic control and received vehicle (1% Tween 80); Group III received the standard anti-urolithiatic drug, allopurinol (50 mg/kg bw, po) from the 15th to 28th day. Group IV received the EGCG (150 mg/kg/day) from the 15th day till 28th day. Group V received the quercetin (10 mg/kg/day) from the 15th day till 28th day. Group VI received combination of EGCG and quercetin with ECGC (1): quercetin (1). All the dosing were given once daily by oral route using gastric tube.

Urine analysis

On the last day of the completion of the respective dosing, all the animals were individually kept in the metabolic cages for the collection of 24 h urine. The urine samples were subjected to light microscopic analysis for the detection of calcium oxalate crystals and subsequently were analysed for various parameters such as urea, creatinine, calcium, uric acid, phosphorus and electrolytes like sodium, potassium and chloride. Urea, creatinine and uric acid was analysed using diagnostic kits, while the electrolytes were determined using biochemical automat.

Serum analysis

At the end of the study, the animals were sacrificed using high dose of Diethyl ether and autopsy was performed. Blood was collected by heart puncture and used for the serum biochemical analysis after separation of serum. The blood collected was centrifuged to separate the serum and was used for various parameters such as urea, creatinine, calcium, uric acid, phosphorus and electrolytes like sodium, potassium and chloride. Urea, creatinine and uric acid was analysed using diagnostic kits, while the electrolytes were determined using biochemical automat.

Malondialdehyde and catalase assays

Lipid peroxidation analysis was performed in the form of analysis of its product i.e., malondialdehyde (MDA) concentrations, as a product of lipid peroxidation. MDA was analyzed as per the protocol provided by Moreno *et al*. (2005).

The catalase activity (CAT) was carried out as per the protocol provided by Hill and Signal (1996).

Histopathological analysis

During autopsy, both kidneys were removed, cleared of any visible fat. Small piece of tissue was sliced and was fixed in 10% formaldehyde and subsequently subjected to histopathological analysis under light microscope.

miR-21 expression

Fresh kidney samples were used for the expression on miR-21 with U6 and RNU48 as housekeeping miRNA. The data were expressed as relative expression.

Statistical analysis

GraphPad Prism v.6 was used for performing the statistical analysis. All the values are expressed as mean± SEM of 6 animals. * In the data indicates significance comparison with the control group and # for comparison with the diseased group, * or # p<005, ** or ## p\0.01, *** or ### p\0.001, **** or #### p<0.0001.

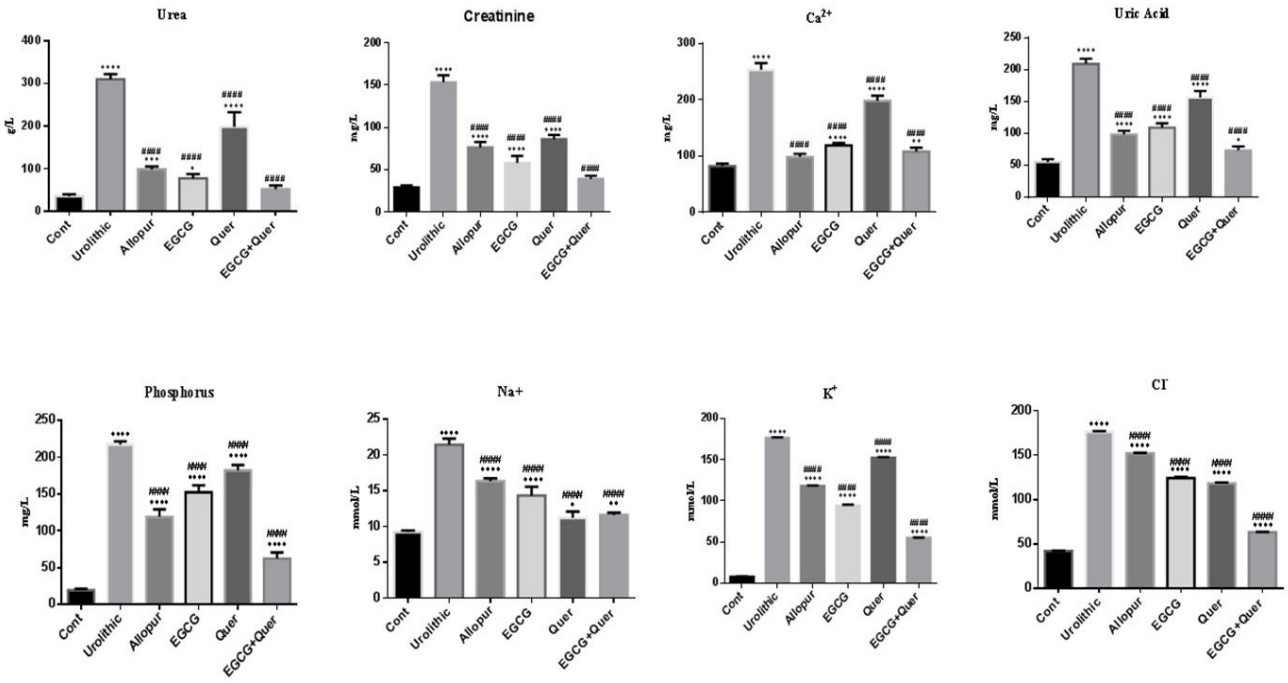

Fig. 1. Feefect of EGCG and quercitive on the various urine parameters (urea, creatimine Ca^{++}, Uric acid, P Na^{+}, K^{+}, Cl^{-}) in male albino rats. Group I: normal control group; Group II: lithiatic and 1% Tween 80; Group III: allopurinol; Group IV: EGCG; Group V: quercetin; Group VI: EGCG and quercetin. All the values are expressed as mean±SEM of 6 animals. * In the data indicates significance comparison with the control group and # for comparison with the diseased group, * or # p<005, ** or ## p<0.01, *** or ### p<0.001, **** or #### p<0.0001.

RESULTS

Administration of 3% sodium oxalate in aqueous solution to the rats resulted in significant alteration in the urine as well as serum parameters. Significant elevation in the urea, creatinine, uric acid, calcium, phosphorus, sodium, potassium and chloride levels were observed in the induced group. Although, all the four treatment groups showed significant reduction comparable to that of the induced group. EGCG and quercetin combinational group provided the best results (Fig. 1).

Similar trend was observed in the serum biochemical analysis of the same parameters (Fig. 2). Among the four groups, EGCG and quercetin combination provided the best results even better than the allopurinol treated animals.

The histological analysis of the kidneys of the control group showed normal histoarchitecture without any abnormality (Fig. 3a). In Sodium oxalate induced animal's histopathological sstudy showed presence of crystals and highly dialated tubules (Fig. 3b). The histoarchitecture was restored back comparable to the control group in all the reversal groups. Reversal similar to that of the control architecture was observed in the EGCG+Quercetinn treated group (Fig. 3c-f) .

The anti-oxidant assay also confirmed the histopathological studies with elevated lipid peroxidation or MDA and decline in the catalase activity in the kidney stone induced group. The changes are reversed significantly in all the four reversal groups, which highly encouraging results similar to that of the control was found in the EGCG+Quercetin group (Fig. 4).

The miR-21 expression was found to be very significantly elevated in the kidney stone induced group. Upon reversal strategy, the expression was significantly reduced (Fig. 5).

DISCUSSION

In the present study, kidney stone was induced in the animal by oral administration of sodium oxalate in drinking water for a fortnight. The rats were the most commonly used animal model for the study of hypercalciuria. Currently there are many experimental models for the induction. Some of the drug induced models includes administration of ethylene glycol (EG) or sodium oxalate (SO) or l-hydroxyproline (HP) (Lee *et al*., 1996; Chen *et al*., 2020).

The animals showed significant oxalate crystals

comparable to that reported by others (Ravindra and Sunil, 2016). The experimental showed significant histopathological damages in the kidneys with particular damage on the tubules which was completely disoriented. Similar results are also reported by Grases *et al*. (2015) and Ghelani *et al*. (2016). Following the treatment the histopathological observations were found to be restored back to control animal's morphological conditions. The EGCG and quercetin combination provided the most satisfactory results. The results were at par to other previously reported findings (Grases *et al*., 2015; Ghelani *et al*., 2016; Marhoume *et al*., 2021) (Fig. 3).

The induction of kidney damage resulted in significant elevation of oxidative stress. The antioxidants, thus would prevent any such damage or enhance the tussue regeneration. In the present study, there was a significant alteration observed in the MDA as well as catalase levels and all the reversal groups showed significant reversal in the altered functioning. The results thus observed were in line with other reported findings (Katalini *et al*., 2006; Grases *et al*., 2015; Ghelani *et al*., 2016; Marhoume *et al*., 2021). In the present study both EGCG as well as EGCG along with Quercetin showed high anti-oxidant levels inducing maximum reversal potentials (Fig. 4).

During kidney stone induction, there are significant increase in the serum and urinary levels of urea, uric acid and creatinine (Divakar *et al*., 2010; Ghelani *et al*., 2016; Pawar and Vyawahare, 2017). In the present study, we also found similar trends, confirming the induction of kidney stones in the animal models. Upon reversal, all the elevated parameters were restored back to control levels (Figs. 1, 2).

One of the primary effect of kidney stones after the obstructive glomerular filtration is the alteration in the electrolyte levels in the form of Ca^{2+}, Na^{+}, K^{+} and Cl^{-}. Elevated electrolytes have been reported in both serum as well as urine indicated significant kidney damage (Blachley and Hill, 1981). In the present study, all the four electrolytes showed significant elevation both in the serum as well as in the urine and reversal group showed significant reversal comparable to that of the control group (Figs. 1, 2).

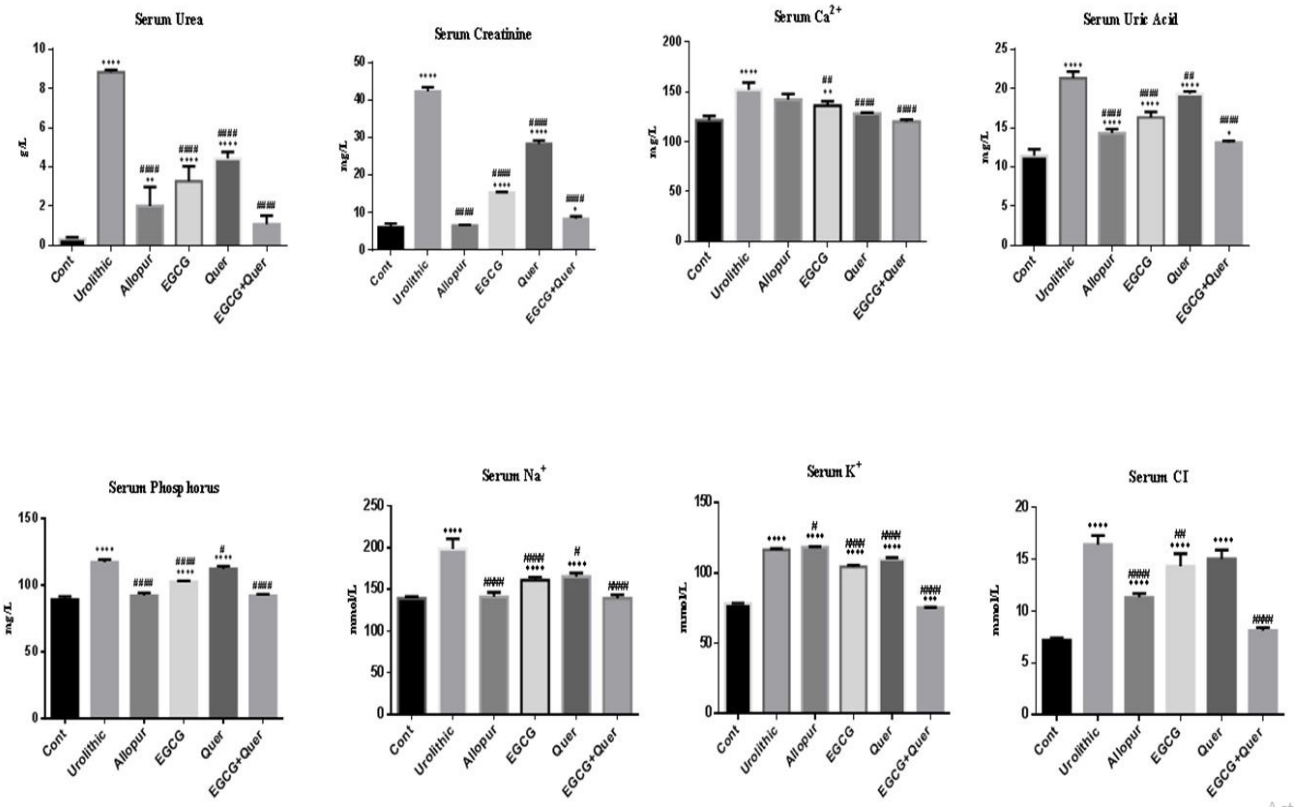

Fig. 2. Effect of EGCG and quercition on the various seral parameters (serum urea, serum creatinine, serum Ca++, serum uric acid, serum phosphorus, serum Na^{+}, serum K^{+}, serum Cl^{-}) on different treatment of the male albino rats.
For details of various groups and statistical details, see Figure 1.

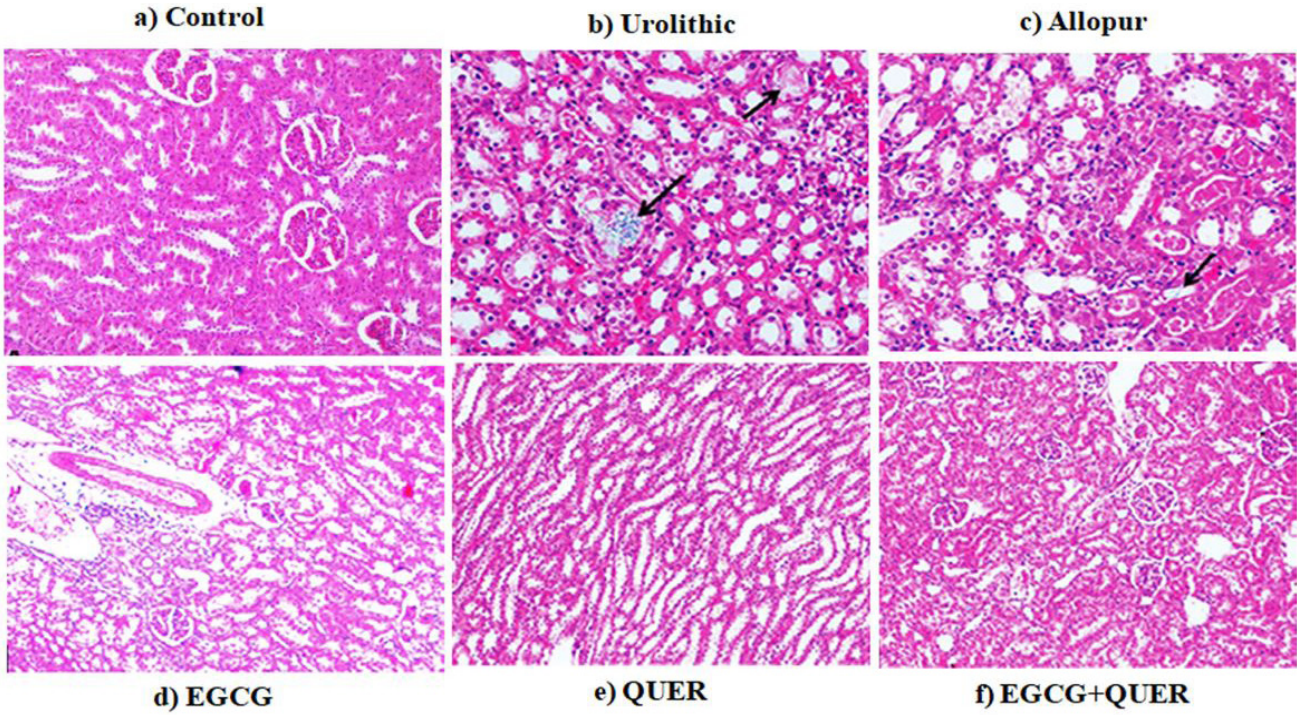

Fig. 3. Effect of EGCH and quercition on histopathology of the male albino rats kindney. For details of various groups and statistical details, see Figure 1.

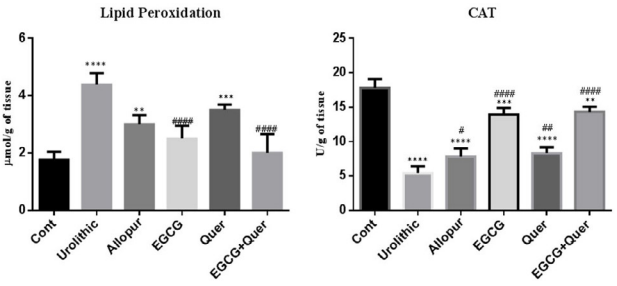

Fig. 4. Effect of EGCH and quercition on tissue antioxidant activity in the male albino rats.
For details of various groups and statistical details, see Figure 1.

MiR-21 has been reported to be identified as a prognostic marker for a variety of tumors along with in renal cell carcinoma (Pavkovic *et al*., 2016; Zununi *et al*., 2017). In the present study there was a significant increase in the tissue miR-21 expression, The results confirmed the tissue damage and elevated oxidative stress. As the reversal groups showed tissue recovery with respect to the histopathological studies as well as reduction in the oxidative stress, the miR-21 also showed a significant decline in all the treatment groups (Fig. 5) (Gomez *et al*., 2015).

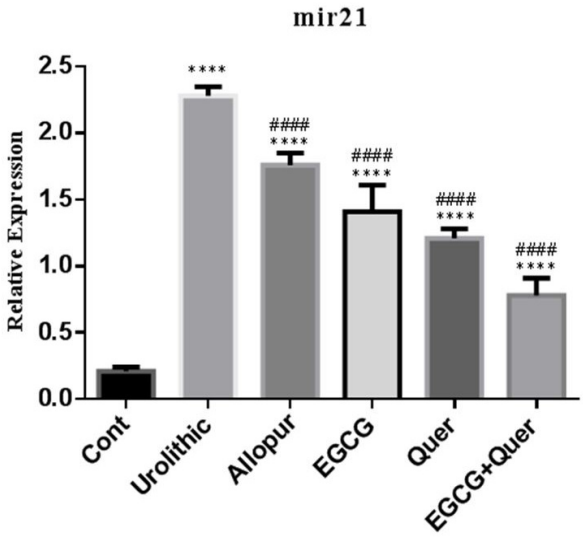

Fig. 5. Effect of EGCH and quercition on tissue expression of miR-21 in the male albino rats.
For details of various groups and statistical details, see Figure 1.

CONCLUSION

In the present study, the efficacy of EGCG and quercetin was compared to that of the standard drug,

allopurinol. The data suggests that quercetin showed reversal at par or in some instances less than Allopurinol. On the contrary, EGCG and EGCG in combination with quercetin provided more robust reversal. It still needs to be investigated the exact mode of action and other tissue expression with could in future would benefit in developing this as a potential treatment strategy for kidney stones.

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IRB approval

The study was approved by the Xi'an Zhongkai Experimental Animal Co., Ltd Ethical Committee (Ethics approval number: No. 2021-3-106)

Ethics statement

This study and included experimental procedures were approved by the institutional animal care and use committee of Xi'an Zhongkai Experimental Animal Co., Ltd (Approval No. 2021-3-106). All animal housing and experiments were conducted in strict accordance with the institutional guidelines for care and use of laboratory animals.

Statement of conflict of interest

The authors have declared no conflict of interest.

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