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A Novel prospective for Treatment of Calcium oxalate stones: Simultaneous Administration of Probiotics and Herbal Medicines

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ABSTRACT

Background: Hyperoxaluria is the major risk factor for the formation of Calcium oxalate stones. The purpose of this study was to evaluate the *in vitro* effect of the simultaneous administration of oxalate-degrading bacterias, *TribulusTerrestris* and *Zea mays* extract in reducing urinary oxalate. 24 Male Wistar rats were randomly divided into 4 equal groups (n=6). The rats of group I received a normal diet (positive control group) and groups II (negative control group), III, IV rats received a diet containing ethylene glycol (3%v/v) for 30 days. Groups III rats received *Zeamays* and *TribulusTerrestris* extract (the *Zea mays* and *TribulusTerrestris* extract were used 200 mg/kgbody weight). Group IV rats received plant extracts and probiotics in combination for 30 days. Also, 4 strains of *Lactobacillus*, 2 strains of *Bifidobacterium* and 2 strains of *L.paracasei* (that showed capability in oxalate degrading in culture media) were used as probiotics. 24-hours urine samples were collected on day 0, 15, and 30. Urine volume, oxalate, calcium, and creatinine levels were measured. Animals were sacrificed and kidneys were harvested, weighed and histopathologically evaluated for calcium oxalate crystals. Finally, the plant extracts were analyzed by GC-Mass. The obtained results Showed that EG significantly increased urine oxalate, calcium, and creatinine levels, and CaOx deposits. Provision of extracts and probiotics combination resulted in significantly lower levels of urine oxalate, calcium, creatinine and CaOx depositions as compared to Group II. The use of herbal extracts with Probiotic can be effective in the prevention and treatment of urolithiasis.

Keywords: GC-Mass ,Probiotic, TribulusTerrestris, Urinary Oxalate, Zea mays

INTRODUCTION

Oxalate ion in calcium oxalate stones is a highly toxic, which accumulation in various tissues (such as kidney and liver) causes impaired and inadequate tissue (Kim etal.,2011). The high excretion of oxalate causes elevated urinary oxalate and ultimately calcium oxalate stones formation(Peng etal.,2014). Almost 70-80 % kidney stones are composed of calcium oxalate (CaOx) and it is more frequent in males as compared to females (70-81% vs.47-60%)(Zhangetal.,2012). However, hyperoxaluria (increasing urinary oxalate) is a risk factor for the formation of calcium oxalate stones. Unfortunately, there are no satisfactory

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medications in modern medicine that can reduce urine oxalate and methods of extracorporeal shock wave lithotripsy(ESWL) and percutaneous nephrolithotomy (PCNL), do not guarantee the prevention of calcium oxalate stone recurrence(Liang etal.,2014). Moreover, they cause side effects such as hemorrhage, tubular necrosis, subsequent fibrosis of the kidney, kidney tissue damage, acute or chronic renal failure. All of which can ultimately lead to kidney transplantation(Vanetal.,2009). Nowadays, extensive research on gastrointestinal bacteria suggests that probiotic bacteria (including lactobacillus) also show high potentials for oxalate decomposition. Oxalate-degrading bacteria in certain enzymatic pathways convert oxalate to formate and carbon dioxide(Kwaketal.,2010). Research on probiotics suggest that these bacteria significantly reduce urinary oxalate(Todorovetal.,2009). Besides, there are some reports on traditional herbs containing active substances with therapeutic effects on calcium oxalate stones and hyperoxaluria(Khodadadiet al.,2016). The traditional medicine has now gained recognition all over the world with several indigenous drugs forming an indispensable part of health care(Aggarwal et al.,2012). *Zea mays* and *TribulusTerrestris* are among those that have been recommend as traditional medicines for kidney stones(Chhatreet al.,2012). The extract from the *Zea mays* and *TribulusTerrestris* are traditionally used in India, China and Iran, for treatment of lithiasis and hyperoxaluria(Mohammadi et al.,2016). *TribulusTerrestris* is an annual plant in the caltrop family (Zygophyllaceae) and widely distributed around the world. It is adapted to grow in dry climate locations in which few other plants can survive. It is an invasive species in Iran and India(Raja et al.,2011). Also, *Zeamays* is an important herb used traditionally in the world. *Zea mays* is a member of the grass family Poaceae, or true grasses. *Zea mays* is thought to have originated 55–70 million years ago in what is now Central or South America and Asia(Al-Attar et al.,2010). It has been widely used as a treatment for diseases such as urinary stones, nephritis, diuretic, and urinary infections. The potential use is very much related to its properties and mechanism of action of its plant's bioactive constituents such as flavonoids and terpenoids(Kavithaet al.,2012). The purpose of this study was to evaluate the effect of the simultaneous use of oxalate-degrading bacteria, *TribulusTerrestris* and *Zea mays* extract in reducing urinary oxalate. The GC-MS analysis of bioactive compounds of *TribulusTerrestris* and *Zea mays* is also reported.

METHODS

Animal experimentation

Male Wistar rats weighing 200-250 g, were housed in a laboratory kept for 12 hours light-dark cycle, controlled room temperature (23±2ºC), and relative humidity (50±10%). Also, they were exposed to 3% of ethylene glycol with 1% ammonium chloride in their drinking water for 3 days. Later, 3% ethylene glycol in drinking water was continued for 30 days. Rats were divided into 4 Groups (n=6). Group I received a normal diet (positive control group). Groups II (negative control group), III and IV rats received 3% ethylene glycol containing diet for 30 days. Groups III received *Zea mays* and *T.terrestris* extract and Group IV rats received extracts+ probiotics for 30 days.

Preparation of hydroalcoholic extract of *Zea mays* and *TribulusTerrestris*

In this study, 60g of each powdered plant (the plants of *Zea mays* and *TribulusTerrestris* were used) with 300 mL of ethanol 80%, were placed in a soxhlet extractor for 48 hours. Then, the rotary dried the extract. The experiment, the concentrations of the *Zea mays* and *TribulusTerrestris* extract were used 200 mg/kg of b.w for each rat. Then they were dissolved in distilled water and oral gavage was performed twice a day.

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Preparation of probiotic culture

Bacterial strains used in the study were as follow *Lactobacillus acidophilus PTCC1643, Lactobacillus Delbrukii PTCC1737,Lactobacillus Plantarum PTCC1745, Lactobacillus casei PTCC1608, Bifidobacteriumbifidum PTCC1644, Bifidobacteriumanimalis subsp. Latics PTCC1736, Streptococcus salivarius subsp. Thermophilus PTCC1738, L.paracasei AKPL-IR (JF461540.1), and L.paracasei AKKL-IR(JF461539.1).* To prepare the bacteria, 1g of dried cultures was inoculated into 100 mL of MRS broth medium (Merck, Germany and placed at 37°C for 6 to 8 hours. 1 mL of medium was transferred into 99 mL of the new MRS broth medium and diluted to 1%. Then, it was placed at 37°C for 6 to 8 hours. The cultures transferred to fresh medium during the week for the number of cells needed. Finally, they were kept in a refrigerator at 4°C. The probiotic cells were isolated after centrifugation at 4500 rpm at 4°C for 10 minutes. Then, the separated cells were washed 2 times using the solution of 0.1% peptone(Whittamoreet al.,2018).

Determining the oxalate-degrading capability of bacterial strains

All cultures were incubated in aerobic and anaerobic conditions at 37°C for 48 hours. Base media containing ammonium oxalate was prepared to determine the growth of each strain and its dependent on oxalate, as the energy source comparing the base media lacked ammonium oxalate. Finally, all selected-strains were cultured in an ammonium oxalate plate (20mM, 40 mM and 60 mM) to assess their oxalate degrading capability.

Collecting urine samples

On the 0, 15th, and 30th days of the study period, the rats were placed in metabolic cages and 24-hour urine samples were collected in tubes containing sodium azide (0.02%) to prevent bacterial growth. The specimens were aliquoted for various tests after determining their volume, pH, urinary oxalate, calcium, and creatinine were assayed using the commercial kit (DarmanKeve Res, Lab, Isfahan, Iran) in semiautomatic photometer according to manufacturer's protocol. Each week, 1-hour urine samples were collected before the start of 24 hours urine sample collections and were examined by light microscopy to analyze CaOx crystals.

In vivo urinary oxalate levels using selected probiotics

Prepared probiotics (~1011 CFU) in distilled water were given to the rats (intervention group) for 4 weeks (6 animals in each group). Rats were weighed weekly and urine samples were collected by placing the animals in metabolic cages for 24 hours.

Analysis of histopathology and CaOx crystal in kidney

In this study, CaOx crystals present in each kidney tissue was examined by staining methods. At first, the kidney tissue was fixed in 10% neutral buffered formalin, trimmed, processed, and set in paraffin. Sections from each kidney were stained with hematoxylin and eosin and examined under the light microscope for pathological analysis and polarized light microscope for visualizing CaOx crystals.

Chemical composition of the extract of *Zea mays* and *TribulusTerrestris*

Etheric extract of *Zea mays* and *TribulusTerrestris* were subjected to Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS analyses were performed using an Agilent 7890A GC coupled to 7000 Triple Quad mass spectrometer operating in full-scan EI mode at 70 EV with

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mass spectra in the range of 35–700 m/z. The GC was equipped with a DB-1 MS column (30 m× 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas with a flow rate of 1.2 mL/min(Salehpouret al.,2016). The injection volume and temperature was 0.2 μL and 250 °C in split mode (1:30) respectively. The oven temperature was programmed from 70 °C rose to 280 °C at 3 °C/min and kept for 4 min at the final temperature. The chemical structures of the detected compounds were determined by comparing their mass spectra and GC retention indices with those reported for the authentic compounds in the spectral databases including NIST and Wiley MS libraries(Farzanehet al.,2018).

Statistical analysis

The statistical analysis of the antibody level between different groups was performed based on one-way and two-way ANOVA using SPSS version16. Statistical significance was set at $p<0.05$

RESULTS

According to the results, all studied strains were able to degrade oxalate in oxalate-enriched medium, which caused further evaluation of these strains on animal models. Two bacteria of *L.paracasei AKPL-IR (JF461540.1), and L.paracasei AKKL-IR(JF461539.1),* which caused 100% oxalate degradation in medium, were registered as superior oxalate-degrading strains at NCBI site. The results also indicated that in the negative control group (EG treated group), there was a several fold increase in levels of urinary oxalate, urinary calcium, and urine creatinine over 30 days, compared to the positive control group (healthy group). At the end of the treatment period, severe weight loss (from a mean weight of 260 g to 150 g), inflammation, swelling, severe redness, bleeding of palms and eyes, a severe decrease in 24-hour urine volume and bloody urination were observed in rats of the negative control group. After removing the kidneys and assessing the precision-cut tissue slices, severe renal tissue necrosis, shrinking of the kidneys and a high accumulation of calcium oxalate crystals in all renal sections were observed. Meanwhile, rapid recovery in tissue and kidney complications and complete elimination of calcium oxalate sediments occurred in a shorter duration in groups treated with the simultaneous use of herbal extract and probiotic bacteria. In the group treated with concomitant use of medical extract and probiotics, there was a significant decrease in the levels of urinary oxalate, calcium, creatinine, which was similar to the positive control group (**Table 1).** At the end of the treatment period, inflammatory complications, necrosis, and redness of the eyes and tissues were completely improved in the treated group **(Figure 1-2).** In addition, the samples of this group reached a normal and balanced weight. In the two groups treated with *TribulusTerrestris* and *Zeamays*, there was a longer treatment and recovery period, compared to the group treated with concomitant use of plant extracts and oxalate-degrading probiotic bacteria.

The plant extracts were analyzed by GC-Mass chromatography, the chromatogram are depicted in **Figure 3-4**. A total of thirteen components were identified in the examined extract, accounting for 98.8% of the total composition. According to the results, there was a direct and significant correlation between the concomitant use of herbal extracts and probiotics and a faster decrease in urinary oxalate, urinary calcium, and the complete elimination of calcium oxalate crystals **(Figure 1-2)** ($P<0.05$). Therefore, it seems that adding ethylene glycol to drinking water increased urinary oxalate in rats. In addition, a significant reduction was observed in the level of urinary oxalate in prevention groups that received *TribulusTerrestris* extract, *Zeamays*, and oxalate-degrading bacteria in addition to ethylene glycol. It seems that herbal extracts caused ethylene glycol excretion in a non-oxalate

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manner, or plant compounds caused a lack of observing the compound as oxalate in urine through binding to the oxalate and eliminating it by urine. Moreover, probiotic consumption reduced inflammation and oxalate degradation. Nevertheless, while the exact mechanism of performance of *TribulusTerrestris* extract, *Zea mays*, and probiotics on metabolism and degradation of ethylene glycol and calcium oxalate deposits is not completely clear, the results showed that concomitant use of herbal extracts and probiotic bacteria significantly affected the decrease of urine oxalate.

DISCUSSION

One of the most important diseases of the genitourinary system is renal stone formation. In general, 80% of all kidney stones are composed of calcium oxalate(18Farzanehet al.,2018). Individuals with this condition might have diseases such as hyperoxaluria, which increases urinary oxalate(19Farzanehet al.,2018). Oxalic acid is an organic dicarboxylic acid formed in the primary and secondary process in plants and human being, respectively; in the latter, this compound is recognized as a toxic substance that can be increased through two endogenous and exogenous pathways(20Farzanehet al.,2018). Nearly 90% and a small amount of the oxalate is excreted through urine and feces, respectively. The fastest diseases caused by elevated oxalate are hyperoxaluria and oxalosis, which provide the foundation for sedimentation of calcium oxalate crystals and other stones in the kidneys and other body parts(21Farzanehet al.,2018).Sometimes the accumulation of calcium oxalate crystals in the urinary tract can cause inflammation of the pelvic and kidney tissues, which is associated with pain and bleeding(22Farzanehet al.,2018). In addition, kidney stones lead to many renal diseases, including renal dysfunction and kidney tissue destruction. Ultimately, kidney stones can cause acute or chronic kidney failure in individuals, all of which predispose a person to kidney loss, kidney transplantation and high healthcare expenditures(23Farzanehet al.,2018). Today, it is confirmed that oxalate-degrading bacteria in the gastrointestinal tract balance and degrade the oxalate. Extensive research on bacteria in the gastrointestinal tract shows that probiotic bacteria have great power in the degradation of oxalate(24Farzanehet al.,2018). Given the useful impacts of probiotics, current studies have more focused on the use of probiotic compounds in the treatment of renal stones. Researchers have employed various combinations of probiotic bacteria with different concentrations (containing a mixture of *lactobacilli*, *bifidobacteria*, and *Streptococcusthermophiles*) in order to reduce urinary oxalate(25Farzanehet al.,2018). In this regard, positive results have been obtained regarding the decrease of urinary oxalate and degradation of oxalate(23Farzanehet al.,2018). In terms of oxalate degradation by probiotic bacteria, the most effective types include *Lactobacillus acidophilus, L. Plantarum, L.casei, L.paracasei, L.Brevis, B.langum, B.infantis, B.breve, B.animalis, and Streptococcus thermophiles(*23Farzanehet al.,2018). The conventional treatment methods for primary hyperoxaluria and food regimens for patients with hyperoxaluria have not been successful regarding the decrease of urinary oxalate secretion, which necessitates the design of new treatment methods(Baradaranet al.,2017). On the other hand, the application of herbal and natural drugs has been considered by researchers due to the side effects and damages caused by chemical drugs. In search on the effect of *Zea mays* on kidney stones of lab rats, there was a decrease in the formation of calcium oxalate crystals. In another research by Mohsenzadeh*et al*., an increase of *Zea mays* inhibited the formation of the primary nucleus (nucleation) of renal stones(Mohsenzadehet al.,2016). Assessing the stone-breaking activity of *TribulusTerrestris* on rats with calcium oxalate stones, Hariprasath observed that the extract of the mentioned plant was able to decrease creatinine, uric acid, and urinary and serum urea. On the other hand, it reduced the amount of urinary oxalate in rats(Hariprasathet al.,2013). In 2009, Butterweck claimed that *TribulusTerrestris* is a thirst-

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quenching and diuretic compound that can break renal and urinary stones(Butterweckt al.,2009). *TribulusTerrestris* was found to inhibit stone formation in various models of urolithiasis using sodium glycolate and ethylene glycol(Raja et al.,2011). Glycolate oxidase (GOX) is one of the principal enzymes involved in the pathway of oxalate synthesis converting glycolate to glyoxylate by oxidation and finally to oxalate. The anti-urolithic activity of *TribulusTerrestris* is attributed to its GOX inhibition(Kavithaet al.,2012). The active ingredients of this plant include alkaloid, polyphenols, saponins, steroids, and glycosides, as well as steroid specific compounds.*Zea mays* has glucose, maltose, allantoin, sterol, saturated and unsaturated fatty acids, and cerebronic acid (Hirose et al.,2007) , (Shukla et al.,2013).This plant increases the level of daily urinary excretion and is among the diuretics that help the excretion of urinary sediments. Moreover, *Zea mays* contain proteins, vitamins, carbohydrates, volatile oils, steroids (e.g., phytosterol and stigmasterol), alkaloids, saponins, tannins, and flavonoids(Moradianet al.,2017) , (Eaidiet al.,2015).On the other hand, *TribulusTerrestris*encompasses a type of alkaloids, a small amount of volatile oil, some resin, tannin, vegetable sterols, sulfur, nitrogen and chlorine, flavonoids (e.g., quercetin), saponins (e.g., diosgenin and furostanol), glucose and galactose. Furthermore, *TribulusTerrestris* is a diuretic plant, excretes urinary stones, and treats infections and inflammation(Hariprasathet al.,2013; Fatourehchi., 2020).

In this study, four groups of male Wistar rats weighing 250-250 g were used (n=6). In the negative control group, the application of 3% ethylene glycol for 30 days was associated with several tissues and renal complications, including inflammation and necrosis of the eyeball, decreased urinary volume, increased by three-four times, and increased levels of urinary and serum parameters. In this respect, there was a significant relationship between the use of ethylene glycol and increased urinary and serum parameters. In the same group, there were abundant calcium oxalate and tissue necrosis in all kidney parts. In the positive control group (healthy group), rats had daily and normal access to food. The third group consisted of the use of herbal extracts of *TribulusTerrestris* and *Zea mays* twice daily, as well as the consumption of ethylene glycol for 30 days in drinking water. After the treatment period, there were a significant decrease in urinary oxalate, calcium and creatinine levels. Moreover, the amount of necrosis, tissue inflammation, and sedimentation of calcium oxalate crystals considerably decreased in this group .

There was a significant correlation between the use of herbal extracts and the reduction of urinary oxalate and the decrease of renal sediments, compared to the negative control group. In the fourth group, there was a concurrently use of herbal extracts and oxalate-degrading probiotic bacteria twice daily in the form of oral gavage. In addition, 3% ethylene glycol with drinking water was consumed in the mentioned group. The results were extraordinary since the daily concomitant use of the extract and probiotic significantly decreased urinary oxalate level, as well as excretion and removal of calcium oxalate sediments in less than 20 days. In other words, the consumption of probiotics with the extract accelerated the reduction of calcium oxalate sediments, in a way that complete elimination of all crystals, tissue inflammations, and necrotic lesions was observed in the renal tissue cut .Also, the GC/MS analysis of *Zea mays* cause to the identification and quantification of thirteen components represented around 97.4% identification. The most abundant compounds are fatty acids including n-Hexadecanoic acid (~33.5%) and 9,12-Octadecadienoic acid (Z, Z) (~28.4%). A total of thirteen components were identified in the examined extract, accounting for 98.8 % of the total composition. The most abundant compounds are Linoleic acid ethyl ester (~26%) and (E)-9-Octadecenoic acid ethyl ester (~13%). According to the results, significant differences were observed among the main composition of the extract.

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In the healthy control group, parenchymal components of the renal tissue (e.g., glomeruli and proximal and distal tubes), were histologically normal and removed any pathological lesions and calcium oxalate sedimentation. Meanwhile, glomeruli were damaged in the negative control group, which led to the sedimentation of proteins in blood observed in the urinary tract, especially in the cortical region, as red sedimentations. Microscopic examination was indicative of decreased severity of renal damage in the group treated with concomitant use of herbal extract and probiotic bacteria. Ethylene glycol produces toxic metabolites (e.g., glycolaldehyde and glyoxylate) in the body, which results in tissue damage and increased urinary oxalate. The formation of calcium stones increases by an excessive amount of oxalate in urine. In this regard, adhesion of calcium oxalate to the urinary tract surface leads to stone development in a way that they cannot be excreted(Eaidiet al.,2015) and (Van et al.,2007). According to the results of the present study, the prevention group experienced a decrease in the dilatation of the ureteric space of the Bowman's capsule, hydropic degeneration of epithelial cells of renal tubules, and lack of sedimentation of protein and calcium in the urinary tract, which demonstrated the lack of formation of renal stones.

CONCLUSION

Nowadays medicinal herbs are a good alternative to chemical drugs, one of the major reasons for this is low side effect compared to chemical drugs. Besides, Probiotics have been studied most of all in various fields. They are considered as one of the most important mechanisms for generating compounds that inhibit pathogenic bacteria in the intestines, and enhancing the immune system. However, considering the clinical problems and the treatment costs associated with increasing urinary oxalate and the formation of kidney stones, and given that probiotic bacteria, as well as plant compounds, can have a direct effect on the reduction of inflammation of the renal tissue, urinary oxalate and the formation of kidney stones.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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REFERENCES

1. Kim S , Kim R, Jung J, Kang S. In vitro antiviral activity of red alga, Polysiphonia morrowii extract and its bromophenols against fish pathogenic infectious hematopoietic necrosis virus and infectious pancreatic necrosis virus. The Journal of Microbiology.2011; 49: 102-6.
2. Peng Z, Chen W, Gao S , Su L , Li N, Wang L, et al. Therapeutic effect of XueNiao An on glyoxylate-induced calcium oxalate crystal deposition based on urinary metabonomics approach. J ClinBiochemNutr. 2014; 55(3): 184-190.
3. Zhang C Y, Wu J, Wang M. Lan B. Antioxidant properties of polysaccharide from the brown seaweed *Sargassumgraminifolium* (Turn.) and its effects on calcium oxalate crystallization. Mar. Drugs.2012; 10: 119-130.
4. Liang L, Li J, Tian O, Lee Q, Dang S. Androgen receptor enhances kidney stone-CaOx crystal formation via modulation of oxalate biosynthesis and oxidative stress. Mol Endo crinol. 2014; 28: 1291-1303.
5. Van D K, Wiesen J, Frank R. Urolithiasis in pediatric patients: a single center study of incidence, clinical presentation and outcome. Urol Res. 2009;177:2300.
6. Kwak C, Jeong BC, Ku JH, Kim HH, Lee J, et al. Prevention of nephrolithiasis by Lactobacillus in stoneforming rats a preliminary study. Urol Res. 2010; 34:265-70.

7. Todorov D, Bacteriocins from *Lactobacillus plantarum* production, genetic organization and mode of action. Braz J Microbiol. 2009;40: 209-221.

8. Khodadadi S, Rafieian-Kopaei M. Herbs, health and hazards; a nephrology viewpoint on current concepts and new trends. Ann Res Antioxid.2016; 1(1):05.

9. Aggarwal A, Tandon S, Singla SK, Tandon C. A novel antilithiatic protein from *Tribulusterrestris* having cytoprotective potency. Protein Pept Lett .2012;19:812-9.

10. Chhatre S, Nesari T, Somani G, Kenjale R, Sathaye S. Comparative Evaluation of Diuretic Activity of Different Extracts of *Tribulusterrestris* Fruits in Experimental Animals. Int J Res PhytochemPharmacol. 2012;3:129-33.
11. Mohammadi M, Parvaneh E, Zahra Tolou- Ghamari. Clinical Investigation of Levisticumofficinale (Lovage) Effectiveness' in Patients with Cystinuria. J Urol Res.2016; 3(6): 1071.
12. Raja M, Venkataram AR. Pharmacognostical studies on *Tribulusterrestris* and *Tribulusalatus.* Der Pharmacia Sinica 2011;2:136-9.

13. Al-Attar, A.M .Antilithiatic influence of spirulina on ethylene glycol-induced nephrolithiasis in male rats. Am. J. Biochem. Biotechnol. 2010; 6: 25-31.
14. Kavitha P, Ramesh R, Subramanian P. Histopathological changes in *Poecilialatipinna* male gonad due to *Tribulusterrestris* administration. *In Vitro* Cell Dev BiolAnim 2012;48:306-12.

15. Whittamore JM, Hatch M. The role of intestinal oxalate transport in hyperoxaluria and the formation of kidney stones in animals and man.Urolithiasis. 2018;45(1):89-108.
16. Salehpour Z, Jahantab E, Morshedloo MR, Iraji A, Mohamadi J. Essential Oil Composition of Aerial Parts of Vitex pseudo-negundo Populations Collected from Southwest of Iran. Journal of Essential Oil Bearing Plants. 2018;21(2):570-6.
17. Farzaneh Z, Kalantar K, Iraji A, Amirghofran Z. Inhibition of LPS-induced inflammatory responses by Satureja hortensis extracts in J774. 1 macrophages. Journal of Immunoassay and Immunochemistry. 2018;39(3):274-91.

18. Campieri C, Campieri M, Bertuzzi V. Reduction of oxaluria after an oral course of lactic acid bacteria at high concentration. Kidney Int. 2001; 60:1097–1105

19. Amini H, Jahantigh M , Galavi H R, Abdollahi A, Pirouzi A, Afkari R. Evaluation of oxalate-degrading activity and molecular Recognition of oxc, frc genes in lactic acid bacterium of inhabit in Human colon .International journal of pharmacy & technology. 2016;3:16055-66.
20. Kargar M,Afkari R,Ghorbani D S . oxalate-degrading capacities of gastrointestinal lactic acid bacteria and urinary tract stone formation, zahedan journal of research in medical sciences .2013; 15 : 54 - 58.

21. Borges NA, Carmo FL, Stockler-Pinto MB. Probiotic Supplementation in Chronic Kidney Disease: A Double-blind, Randomized, Placebocontrolled Trial. J Ren Nutr. 2018;28:28-36.
22. Guida B, Germano R, Trio R, Russo D, Memoli B, Grumetto L. Effect of short-term synbiotic treatment on plasma p-cresol levels in patients with chronic renal failure: a randomized clinical trial. NutrMetabCardiovasc Dis .2014;24:1043-1049.
23. Garabed E. Clinical Reviews in Bone and Mineral Metabolism. History urolIth. 2013 ; 2: 177.
24. Baradaran A. Administration of herbal drugs in geriatric individuals; trends on its helps and hazards. Geriatr Persia. 2017;1(1):01.

25. Mohsenzadeh A, Ahmadipour SH, Ahmadipour S, Asadi- Samani M. A review of the most important medicinal plants effective on cough in children and adults. Der Pharmacia Lettre. 2016;8:90-6.

26. Hariprasath K. Antiurolithiatic activity of Tribulus*Terrestris*fruits and Punicagranatum seeds in ethylene glycol induced rat models. Indo American Journal of Pharm Research. 2013:3(6).
27. Butterweck V, Khan V S. Herbal medicines in the management of urolithiasis: Alternative or complementary? Planta Med.2009; 75: 1095-1103.
28. Hirose M, Tozawa K, Okada S. Glyoxylate induces renal tubular cell injury and microstructural changes in experimental mouse. Urol Res. 2008; 36: 139-147.
29. Shukla D, Mandavia J, Barvaliya N, BaxiB. Anti-Urolithiatic Effect of Cow Urine Ark on Ethylene Glycol-Induced Renal Calculi. IntBraz J Urol. 2013; 39: 565-71

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 16458 - 16469
Received 05 March 2021; Accepted 01 April 2021.

30. Moradian A, Changizi-Ashtiyani S, Sharifi A. Protective Effects of Pomegranate Juice on Nephrotoxicity Induced by Captopril and Gentamicin in Rats. Iran. J Kidney Dis. 2017; 11, 422–429.
31. Eaidi F, Movahedian-Attar A , Nili N, Asgary S. Study zea mays anthocyanins on fatty streak formation in the renal arteries in hypercholesterolemic rabbits. Adv Biomed Res. 2015; 5:11-18.
32. Van Dervoort K, Wiesen R, Trachtman B. Urolithiasis in pediatric patients: A single center study of incidence, clinical presentation and outcome. J Urol. 2007; 177: 2300-2305.
33. Fatourehchi F. A Review on the Effect of Natural Antioxidants (Extracts and Nano Extracts) on the Oxidative Stability of Vegetable Oils. alkhass. 2020; 2 (4) :1-4

All values are expressed as Mean ,Std. Deviation, Minimum, Maximum; (n=6) animals in each group.

*C= Positive Control , **N= Negative Control

Table 1:Urinary Oxalate, Calcium and Creatinine levels in Positive and Negative Control groups in 0- and -30 days

Group		Oxalate(mm/day)	Creatinine(mg/day)	Calcium(mg/day)
*C-0	Mean	.3667	759.8333	1.7267E2
	Std. Deviation	.04131	40.42977	1.50155E1
	Minimum	.31	689.00	155.00
	Maximum	.41	800.00	189.00
C-30	Mean	.3533	715.6667	1.8417E2
	Std. Deviation	.04967	52.68649	6.24233
	Minimum	.29	677.00	177.00
	Maximum	.41	799.00	195.00
**N-0	Mean	1.2367	1412.8333	3.6567E2
	Std. Deviation	.17259	34.18430	5.35413
	Minimum	1.05	1356.00	358.00
	Maximum	1.50	1450.00	371.00
N-30	Mean	1.5967	1394.0000	4.6233E2
	Std. Deviation	.28994	16.13691	1.03473E1
	Minimum	1.08	1365.00	445.00
	Maximum	1.90	1412.00	471.00

All values are expressed as Mean ,Std. Deviation, Minimum, Maximum; (n=6) animals in each group.

*P.T.Z.0= probiotic, *T. Terrestris, Zea mays*

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Table 2 : Urinary Oxalate, Calcium and Creatinine levels inCo-treatment groups in 0- and -30 days				
Group		Oxalate(mm/day)	Creatinine(mg/day)	Calcium(mg/day)
T.Z.0	Mean	.8900	1295.5000	3.1117E2
	Std. Deviation	.06261	48.06142	7.25029
	Minimum	.81	1230.00	299.00
	Maximum	.98	1355.00	320.00
T.Z.30	Mean	.4980	847.0000	1.8717E2
	Std. Deviation	.05992	36.12755	6.17792
	Minimum	.38	785.00	179.00
	Maximum	.52	895.00	198.00
*P.T.Z.0	Mean	1.0033	1311.0000	2.8617E2
	Std. Deviation	.05750	62.64503	6.08002
	Minimum	.90	1238.00	275.00
	Maximum	1.06	1425.00	291.00
P.T.Z.30	Mean	.4050	900.0000	1.1950E2
	Std. Deviation	.10095	75.99474	3.44964
	Minimum	.35	789.00	113.00
	Maximum	.61	985.00	123.00

Table3: Chemical composition of etheric extract of *TribulusTerrestris*&*Zea mays* detected by GC-MS

Peak	Compound	(*Zea mays*) Area %	(*TribulusTerrestris*) Area %	RI[a]	Formula
1	2-Heptenal	0.49	0.38	927	$C_7H_{12}O$
2	n-Hexadecanoic acid	33.54	11.02	1950	$C_{16}H_{32}O_2$
3	Hexadecanoic acid, ethyl ester	2.22	12.44	1980	$C_{18}H_{36}O_2$
4	9,12-Octadecadienoic acid (Z,Z)-	28.40	12.92	2107	$C_{18}H_{32}O_2$
5	cis-Vaccenic acid	13.81	12.60	2115	$C_{18}H_{34}O_2$
6	Linoleic acid ethyl ester	3.93	26.32	2136	$C_{20}H_{36}O_2$
7	(E)-9-Octadecenoic acid ethyl ester	-	13.16	2141	$C_{20}H_{38}O_2$
8	Octadecanoic acid	9.30	4.33	2142	$C_{18}H_{36}O_2$
9	trans-13-Octadecenoic acid	-	0.63	2156	$C_{18}H_{34}O_2$
10	Octadecanoic acid, ethyl ester	0.74	2.83	2177	$C_{20}H_{40}O_2$
11	Unknown	1.47	-	2305	-
12	Unknown	-	1.17	2315	-
13	Eicosanoic acid	0.80	-	2343	$C_{20}H_{40}O_2$
14	Tetracosane (n-)	-	1.33	2378	$C_{24}H_{50}$
15	Pentacosane (n-)	2.26	0.88	2497	$C_{25}H_{52}$

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16	(E)-13-Docosenoic acid	0.77	-	2507	$C_{22}H_{42}O_2$
17	Docosanoic acid	1.19	-	2542	$C_{22}H_{44}O_2$

[a] RI: Retention indices based on the DB-1MS column

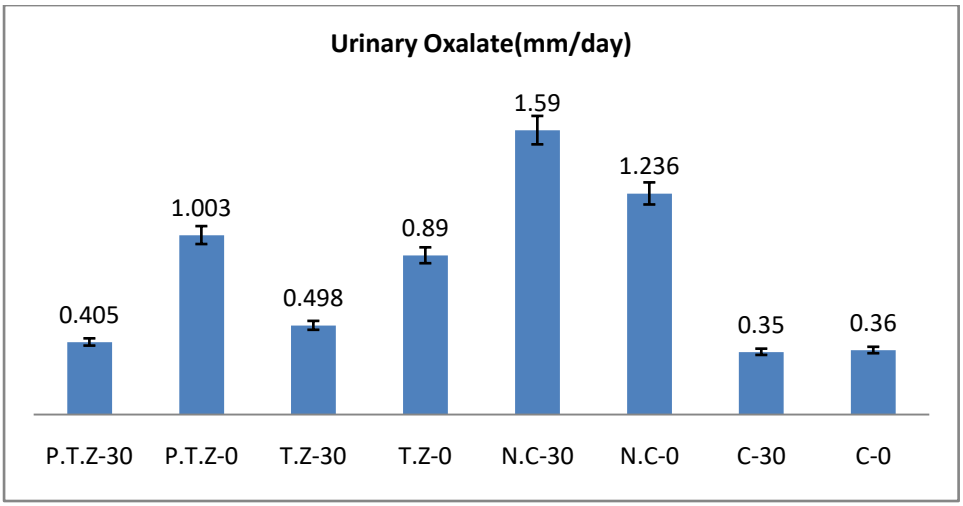

Figure 1:Urinary Oxalate level in all groups in 0- and -30 days

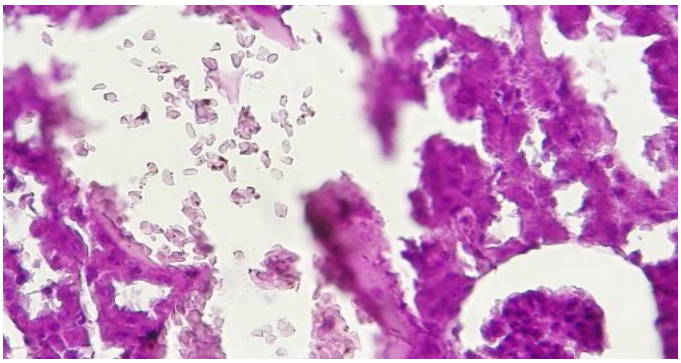

Figure 2: Accumulation of calcium oxalate crystals in kidney tubules in the negative group

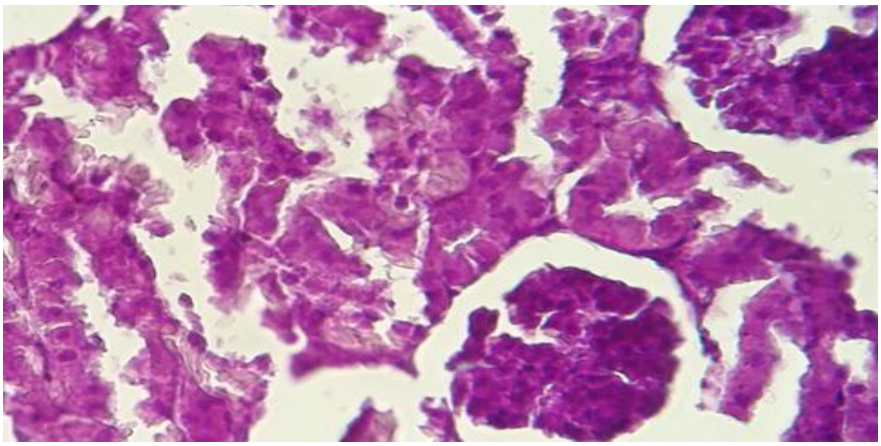

Figure 3:Accumulation of calcium oxalate crystals in kidney tubules in the cotreatment group (plant extracts with probiotic)

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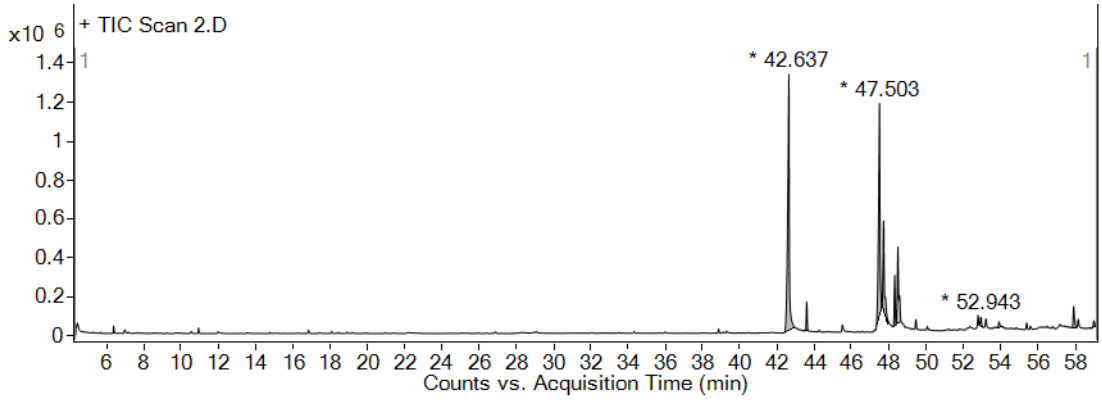

Figure 4: GC-MS chromatogram of etheric extract isolated from ***Zea mays***

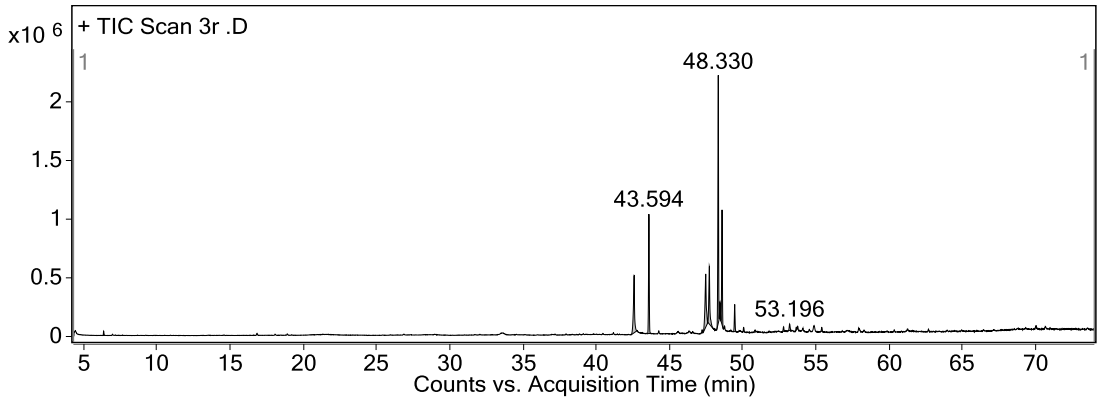

Figure 5: GC-MS chromatogram of *etheric* extract isolated from *TribulusTerrestris*