PROTECTIVE EFFECT OF CHRYSIN IN SODIUM OXALATE INDUCED UROLITHIASIS IN RATS

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Abstract

Urolithiasis is a common urinary tract disorder which affects many people worldwide. Although there are many treatments available none of them are without any harmful effects and the chances of the relapse of the disease is also high. The present study explores the possible role of Chrysin, a polyphenol in the treatment of urolithiasis. This was based on the fact that many plants containing a considerable amount of flavanoid and other isolated flavonoids have shown to possess good activity against kidney stones. Sodium oxalate induced urolithiasis was taken as a model and different urine and serum biochemical parameters were estimated. The results indicated a beneficial and preventive role of Chrysin against the formation of kidney stones.

1.Introduction:

Urolithiasis commonly known as kidney stones is the third most common urinary tract disorder. It causes severe pain in the patients. Numerous treatment options including drugs as well surgery exist but none of them are without shortcomings. Limitations include the various side effects resulting from drug therapy and the high recurrence rate. So the need exists to look for alternate and better treatment options. A lot of research is being carried out to search for bioactive molecules from plant sources. There is already evidence of ayurvedic medicines and their components having antilithiatic property due to their ability to change the ionic composition of urine, antimicrobial properties , diuretic effect and, antioxidant potential. Flavonoids are a group of polyphenolic compounds obtained from plants and having a wide range of pharmacological activities. Studies have indicated that plants containing high content of flavonoids can inhibit the formation of calcium oxlate stones both invitro and invivo. This is attributed to the antioxidant, antibacterial, diuretic, antiinflammatory and free radical scavenging activity of flavonoids.

Chrysin is a flavonoid with several pharmacological properties that include diuretic,antioxidant, anti-inflammatory, anti- apoptotic activity and, anti microbial (Cherkaoui *et al*., 2008) (Nelida *et al.*, 2015). Plants containing chrysin as flavonoids e.g. Aerva lanata, Avocado leaf extract have been tested for their action against kidney stones and the results are promising (Adepu *et al.*,2013). (Adep *et al*., 2013, Amini *et al*., 2012. The present study therefore intends to find out the efficacy of Chrysin, a flavonoid in treatment of kidney stone.

2.Materials and Methods:

2.1 Animals:

Male wistar rats (250-350g) were employed for the study. Animals were provided free access to standard chow and drinking water and they were maintained at 24 ± 4°C in 12 hour light/dark cycle in animal house facility of Chandigarh College of Pharmacy, Landran, Mohali (Punjab). Animals were acclimatized to laboratory conditions at room temperature prior to the experiments. The experimental protocol was duly approved by Institutional Animal Ethics Committee (IAEC) and was carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.2 Treatment drug and chemicals:

All the chemicals including the drug Chrysin was obtained from Sigma Aldrich.

2.3 Experimental Protocol:

Thirty male wistar rats (200-250g) were maintained with regular chow and water ad libitum under normal temperature. Group I: Was maintained with regular chow and water ad libitum under normal temperature. Groups II-V was given sodium oxalate for 5 days. After 5 days biochemical estimations will be done to check for the development of urolithiasis. After 5 days treatment will be started.

Group I**:** Was maintained with regular chow and water ad libitum under normal temperature.

Group II: (Disease control group)70mg/kg sodium oxalate *i.p*.

Group III: (Standard Group) 750mg/kg Himalaya Cystone *p.o*.

Group IV: (Low dose Treatment Group) Chrysin 100 mg/kg *p.o*.

Group V: (High dose Treatment Group) Chrysin 200 mg/kg *p.o.*

Procedure was continued from 1st to 15th day. After this biochemical parameters were analyzed by using various methods and kits.

2.4 Biochemical Analysis

a) Collection and analysis of urine**:**

All the Rats were kept in the metabolic cage and urine was collected overnight. Urine urea, uric acid, sodium, calcium, magnesium, creatinine and urine microprotein levels were measured by using specific diagnostic kits. (Taylor *et al*., 2008).

b) Serum analysis: Blood samples from each animals were taken by cardiac puncture. Serum was separated and blood urea nitrogen , uric acid and urea were analysed by using diagnostic kits (Thangarathinam *et al*., 2013).

c)Kidney histopathology and homogenate analysis: Left kidney of each animal was used for histopathology using haematoxylin and eosin stain. Right kidney homogenate was used for assaying Malondialdehyde (MDA), Superoxide dismutase (SOD), Catalase assay, and reduced Glutathione (GSH) using chemical methods (Dash *et al*., 2007).

d) Statistical Analysis

Results were expressed as mean ± Standard deviation of mean (STDEV). The data obtained from various groups was statistically analysed using one way ANOVA followed by Tukey's multiple range test. The $p < 0.05$ was considered to be statistically significant.

3.Results and Discussion:

Table 1: Effect of Chrysin treatment on biochemical parameters of urine in Wistar rats

Group	Urine urea (mg/dl)	Urine uric acid (mg/dl)	Urine sodium (mmol/l)	Urine magnesium (mg/dl)

Normal Control	12.6 ±0.37	2.5±0.18	18.3±0.33	2.13± 0.35
Disease control	55.7±1.36^{b***}	6.8±0.18^{b**}	74.3±3.47^{b**}	0.7± 0.33^{b**}
Standard Control	16.8±0.41^{c***}	4.1±0.16^{c**}	26.2±0.45^{c**}	1.46± 0.44^{c**}
Low dose treatment group	20.9±0.99^{d1***}	6.08±0.19^{d1***}	29.8±1.75^{d1**}	1.21± 0.44^{d1**}
High dose treatment group	24.7±0.71^{d2***}	5.2±0.16^{d2**}	51.6±0.60^{d2***}	1.0±0.089^{d2***}

(^{b} group compared with normal control group, ^{c} group compared with disease control group, ^{d1} group compared with disease control group, ^{d2} group compared with disease control group), *: $p< 0.05$, **: $p< 0.01$, ***: $p< 0.001$. A difference in the mean value was considered to be statistically significant.

Table 2: Effect of Chrysin treatment on biochemical parameters of urine in Wistar rats

Group	Urine calcium (mg/dl)	Urine creatinine (mg/dl)	Urine microprotein (mg/dl)
Normal Control	4.2 ±0.15	4.9 ± 0.75	4.9± 0.68
Disease control	7.6±0.35^{b*}	10.2 ± 1.15^{b**}	10.2± 0.44^{b**}
Standard Control	4.4±0.15^{c*}	6.4 ± 0.59^{c**}	5.5± 0.70^{c**}
Low dose treatment group	5.3±0.14^{d1***}	9.7 ± 1.44^{d1***}	8.5± 0.46^{d1***}

High dose treatment group	5.0±0.15^{d2**}	7.9 ± 0.65^{d2**}	7.4± 0.91^{d2***}

(^{b} group compared with normal control group, ^{c} group compared with disease control group, ^{d1} group compared with disease control group, ^{d2} group compared with disease control group), *: $p< 0.05$, **: $p< 0.01$, ***: $p< 0.001$. A difference in the mean value was considered to be statistically significant.

Table 3: Effect of Chrysin treatment on biochemical parameters of serum in Wistar rats

Group	BUN	Uric acid	Urea
Normal Control	20.7 ± 1.82	5.9 ± 0.53	2.7 ± 0.22
Disease control	97.9± 2.0^{b**}	2.45 ± 0.36^{b*}	5.1 ± 0.40^{b**}
Standard Control	29.4± 2.50^{c**}	5.7 ± 0.47^{c*}	3 ± 0.86^{c**}
Low dose treatment group	54.2± 2.9^{d1***}	4.05 ± 0.25^{d1**}	4.5 ± 0.41^{d1***}
High dose treatment group	31.9± 2.1^{d2**}	5.3 ± 0.36^{d2*}	4 ± 0.28^{d2**}

(^{b} group compared with normal control group, ^{c} group compared with disease control group, ^{d1} group compared with disease control group, ^{d2} group compared with disease control group), *: $p< 0.05$, **: $p< 0.01$, ***: $p< 0.001$. A difference in the mean value was considered to be statistically significant.

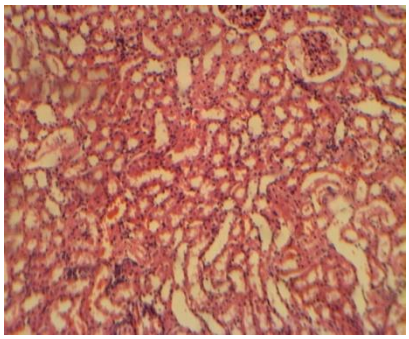
Table 4: Effect of Chrysin treatment on kidney homogenate analysis in Wistar rats

Group	TBARS(nmol/mg)	SOD(units/ml)	GSH(µmol/mg)	Catalase(mol/l)
Normal Control	3.43±3.53	321.32± 38.9	79.39± 0.9	63.28±0.37
Disease control	7.84±7.84^{b**}	161.19± 3.5^{b**}	39.19±0.3^{b**}	32.14±1.47**
Standard Control	4.77±4.77^{c**}	277.48± 25.7^{c**}	69.41± 0.9^{c**}	58.11± 1.56**
Low dose treatment group	5.88±5.98^{d1***}	199.48± 9.4^{d1***}	53.8 ± 0.8^{d1***}	45.17± 0.77***
High dose treatment group	5.15±5.05^{d2**}	267.14± 29.0^{d2**}	67.14± 0.7^{d2**}	46.28±1.47***

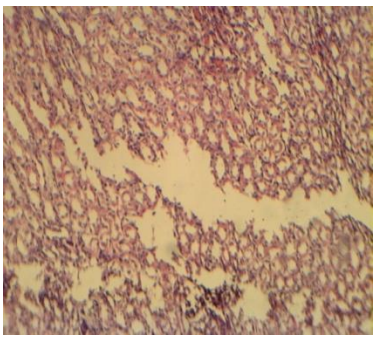
(^{b} group compared with normal control group, ^{c} group compared with disease control group, ^{d1} group compared with disease control group, ^{d2} group compared with disease control group), *: $p< 0.05$, **: $p< 0.01$, ***: $p< 0.001$. A difference in the mean value was considered to be statistically significant.

Results of kidney histopathology:

In histopathological observations gross examination of rat's kidney from normal control group showed a normal cortical structure of the kidney including no sodium oxalate depositions with normal glomeruli, distended tubules and proximal and distal convoluted tubules without any inflammatory changes (fig-a). In the disease control group inflammatory changes were seen. Although complete remission of stones did not occur in the standard and treatment groups the inflammation was less and the structure resembled that of normal group.



a) Normal Control



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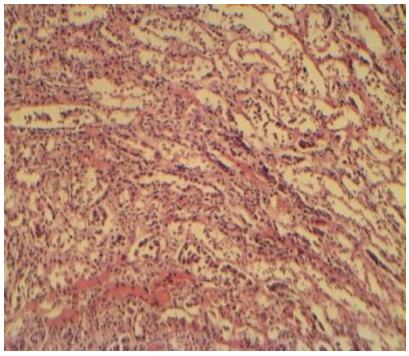
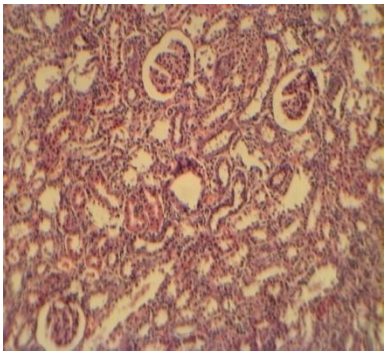
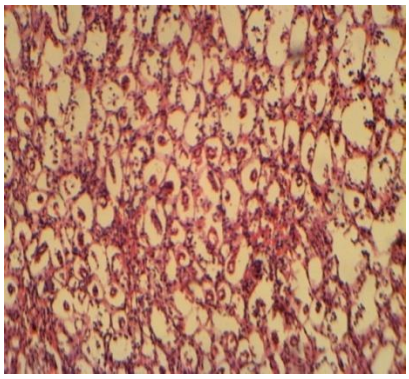


c) Standard Control



d) Low dose treatment group



e)High dose treatment group

Discussion

Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia. In this cycle, amino groups donated by ammonia and L-aspartate are converted to urea, while L-ornithine, citrulline, L-argininosuccinate, and L-arginine act as intermediates. Urea production occurs in the liver and is regulated by N-acetylglutamate. Urea is found dissolved in blood (in the reference range of 2.5 to 6.7 mmol/L) and is excreted by the kidney as a component of urine (Sakami W et al., 1963). The urine chemistry showed that urea levels in urine were increased significantly in the disease control group as compared to normal group. There was a significant decrease in standard control group as compared to disease control. There was also a marked decrease in two treatment groups with the decrease being more with the higher dose.

The increase in Urine potassium level was in accordance with the previous studies with Sodium oxalate in various animal species (Betanabhatla et al., 2009; Divakar et al., 2010). The increase in urine potassium level could be due to the fact that sodium oxalate leads to the acidosis which results in hyperkalemia because of shift of potassium from the intracellular to the extracellular compartment. The potassium level was found increased in disease control group and decreased in standard group and two of the treatment groups respectively. However the decrease was more significant in the low dose treatment group.

Urinary phosphorous level showed decrease in disease control group while increased in standard control group and significant difference was observed in high dose treatment group. The low dose did not show much improvement as of to disease control group.

The main determinant of uric acid stones is urine pH. A low urine pH has more insoluble uric acids concentration (Charles *et al*., 2002). Therefore, the uric acid level was also significantly

increased in disease control group as compared to normal control group. There was a significant decrease in uric acid level in standard control group. The high dose treatment group showed a considerable improvement.

If the patient continues to consume a high sodium diet, sodium will reach the distal nephron and increase the excretion of calcium and potassium along with citrate, resulting in a change in the urinary pH that will eventually increase the risk of stone formation (Haewook Han1 et al., 2015). The Urinary Sodium level was found increased in disease control group. While, decreased in standard control group. Moreover, low dose treatment group showed remarkably decreased level of sodium as compared to high dose treatment group.

An increased urinary level of calcium favours the nucleation and precipitation of Calcium oxalate crystal attachment and more centers for nucleation of new crystals (Lemann et al., 1991). Sodium oxalate induced significant Calcium oxalate crystalluria with larger size suggesting significant hyperoxaluria (Bashir & Gilani, 2011). The increase in calcium level in renal tissue might be due to the increased bioavailability of nitric oxide (NO) which in turns activates cGMP (30,50-cyclic guanosine monophosphate) that controls the increase in intracellular calcium levels (Divakar et al., 2010). The Urine calcium level showed increase in disease control group while, Standard group showed decreased calcium level. The calcium level was found to be decreased more in high dose treatment group as compared to low dose.

Magnesium complexes with oxalate and reduce the supersaturation of sodium oxalate by reducing the saturation and as a consequence reduces the growth and nucleation rate of calcium oxalate crystals (Selvam et al., 2001). The Urinary magnesium level was found decreased in disease control group, while in standard control group and two of the treatments groups showed increase excretion of magnesium as compared to disease control group. The increase was however more in the low dose.

The Urinary Creatinine level, was found increased in disease control group, although the Creatinine level was found more decreased in standard and high dose treatment group as compared to low dose treatment group. The decrease was more in the high dose treatment group.

Microproteinuria reflects proximal tubular dysfunction. The supersaturation of urinary colloids results in precipitation as a crystal initiation particle which when trapped acts as a nidus leading

to subsequent crystal growth. This is associated with Microproteinuria (Selvam et al., 2001). Urine Microproteinuria level was found increased in disease control group, whereas Standard control group showed Significant decreased level of Microprotein. Moreover the Microprotein level was found decreased in high dose treatment group and low dose as of to disease control group, with decrease being more in high dose..

In Urolithiatic condition, obstruction to the outflow of urine by stones in urinary system takes place and as a result nitrogenous substances such as urea, Creatinine and uric acid get accumulated in blood due to reduced excretion by the kidneys. The elevated serum levels of Creatinine, uric acid and BUN indicate marked renal damage in Calculi induced animals (Krishnaveni Janapareddi et al., 2013**)**. In serum BUN was increased in disease control group as of normal control group. And there was significant decrease in standard control group as of to disease control group. The BUN levels also decreased in low and high dose treatment group with decrease being more with high dose.

The Serum uric acid level was also significantly decreased in disease control group as compared to normal control group. There was a significant Increase in uric acid level in standard control group. While, the higher dose treatment group showed a considerable improvement as compare to low dose group.

The serum level was increased significantly in the disease control group as compared to normal group. In standard control group there was significant difference as of to disease control group. In low dose and high dose there was decrease in serum urea as of to disease control group and decrease was more in low dose.

Furthermore, levels of antioxidative enzymes such as Total protein, catalase, superoxide dismutase (SOD) and glutathione (GSH) in the renal cortex were found to be significantly decreased while TBARS was found increased. In the present study, other than TBARS all the biochemical tests was found of decreased level in disease control group and simultaneously increased in standard control group and two of the treatment groups. While in TBARS, It was observed that there was increased MDA level in disease control group and simultaneously decreased in standard control group and two of the treatment groups. So it can be concluded that,

biochemical estimations also showed promising results. There was also considerable improvement in oxidative stress with administration of Chrysin as seen with improvement in the assay of various parameters like superoxide dismutase, glutathione level (GSH) and catalase.

The histopathological findings showed that administration of Chrysin decreased the renal damage to glomeruli as compared to the disease control group. Histopathological picture of high dose treatment group resembled that of normal control group.

Conclusion:

The mechanism involved can be-

a) improving the renal tissue antioxidant status and cell membrane integrity.

b) inhibition of crystal nucleation, aggregation and growth. and c) regulation of oxalate metabolism.

Evidence suggests that in many sodium oxalate stone formers the earliest changes may be sodium salt deposition in the medullary interstitium, in marked hyperoxaluric states, primary hyperoxaluria directs calcium oxalate crystal adhesion to renal epithelial cells (Atmani *et al*., 2004). Sodium oxalate intake leads to increase in levels of promoters like calcium, oxalate, uric acid, and inorganic phosphate and decrease level of inhibitors like magnesium and citrate as observed in disease control groups(Kachchhi *et al*, 2012), and administration of treatment drug Chrysin, must have decreased the level of promoters and increased the level of inhibitors.

Chrysin also showed good antioxidant activity as oxidative stree is common in kidney stones. Chrysin is also beneficial by inhibiting oxidative stress. Chrysin also showed good diuretic action and improvement in biochemical parameters so from the results obtained it can be concluded that Chrysin has a protective effect against the formation of kidney stone.

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