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MODULATORY EFFECT OF NONI-HERBAL FORMULATION AGAINST ETHYLENE GLYCOL-INDUCED NEPHROLITHIASIS IN ALBINO RATS.

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Abstract

The effect of NONI-herbal formulation against ethylene glycol induced nephrolithiasis in albino wistar rats is summarized in this study. Lithiasis was induced in rats by administering 0.75% ethylene glycol in drinking water for 28 days and was manifested by high urinary calcium, phosphate, oxalate, and low urinary magnesium contents. Simultaneous administration of 1ml (1 in 10) NONI formulation orally for 28 days along with ethylene glycol (0.75% v/v) reduced urinary calcium, phosphate, oxalate and elevated urinary magnesium level. It also increased urinary volume thereby reducing the tendency for crystallization. The histopathological studies confirmed the induction as degenerated glomeruli, necrotic tubule and inflammatory cells was observed in section of kidney from animals treated with ethylene glycol. This was reduced; however after treatment with NONI formulation. These observations enable to conclude that NONI is effective against ethylene glycol induced nephrolithiasis.

Key words

Nephrolithiasis, Ethylene Glycol, NONI Formulation, Calcium Oxalate Crystals.

Introduction

Nephrolithiasis (renal stone formation) is a recurrent disorder prominent in males. The present day medical management of nephrolithiasis is either costly or not without side effects. Hence the search for Antilithiatic drugs from natural sources has assumed greater importance. Many Indian plants have been quoted to be useful as antilithiatic agents. They are effective with fewer side effects and are also inexpensive. Hence the Indian plants are constantly being evaluated for possible antilithiatic effects in a systemic manner. One such plant is *Morinda citrifolia* Linn. Belonging to family, Rubiaceae which is used as neuroprotector, anti-inflammatory [1], antitumor [2], antitubercular [3]. It also posses other activity such as anhelmentic action [4] and antibacterial action [5].

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It is reported to contain chemical constituents including one-hexenol (antiseptic), acetic acid(bactericide, fungicide), aucubin (antioxidant), scopoletin and euginol (analgesic, anti-inflammatory, cancer preventive), linoleic acid and myristic acid (cancer preventive) [6]. NONI is an herbal formulation of *Morinda citrifolia* available in local market as dietary supplement. The present study was designed to investigate the antilithiatic activity of NONI-herbal formulation in ethylene glycol induced nephrolithiasis.

Materials and Methods

Male albino Wistar rats (180-200 gm) were obtained from K.M. College of pharmacy, (Madurai, India). They were housed in well-ventilated cages, maintained at 25 ± 2° C under 12 hour dark / light cycle. They were fed standard pellet diet and had free access to water. The animals were maintained

in these conditions for one week before the experimental session. Our institutional animal ethical committee (IAEC) approved this study. The NONI formulation was collected from local market, Madurai.

Antilithiatic activity

The method of Christina et al., was followed to evaluate the antilithiatic effect [7]. The acclimatized animals were divided into three groups of six each designated as GI, GII and GIII. The animals of GI served as the normal control. The GII animals received 0.75% ethylene glycol in drinking water *ad libitum* for 28 days and served as the lithiatic control. The GIII group animals received 0.75% Ethylene glycol in drinking water *ad lib itum*, along with NONI formulation 1 mL (1 in 10) by oral route for 28 days.

The 24 hour urine samples were collected from rats housed in metabolic cages on 14^{th} and 28^{th} days and the volume noted. Urinary calcium, phosphate, oxalate and magnesium concentration were estimated using standard methods. Also, the serum and urine creatinine levels were estimated. To confirm the incidence of Lithiasis, the animals were sacrificed and there kidney were subjected to histopathological studies.

Histopathological studies

Kidney samples were weighed and fixed rapidly with 10% neutralized formalin (pH 7.4) section of kidney fixed in paraffin were prepared and stained with hematoxylin & eosin and observed for pathological changes.

Statistical analysis

The results are expressed as mean ±SEM. Statistical analysis was carried out using one way ANOVA, followed by Newman-keuls multiple range test. Differences below $P< 0.05$ implied significance.

Results

Urinary Data

The urinary excretion was increased significantly on the 14^{th} day in ethylene glycol treated rats (GII) compared with normal control rats (GI). Maximum oxalate excretion was observed with GII on 28^{th} day (31.01± 1.25 mg/24 hr per rat). However the oxalate excretion was reduced significantly (25.09±1038mg/24 hr per rat) in the NONI treated group (GIII), though normal values were not reached. The results are shown in table 1 & 2.

Likewise, ethylene glycol treatment increased urinary calcium (10.77± 0.24 mg/24 hr per rat) and phosphate (82.08± 3.19 mg/24 hr per rat) excretion significantly in lithiatic control group (GII) on the 28^{th} day. However after treatment with NONI, these values were reduced to 7.03± 0.34 mg/24 hr per rat and 66.68±0.49 mg/24 hr per rat, respectively in GIII.

The magnesium excretion on the 28^{th} day was reduced after treatment with ethylene glycol in GII (0.861± 0.076mg/24 hr per rat). Simultaneously, administration of extract to GIII, elevated the reduced magnesium level significantly (2.62± 0.35 mg/24 hr per rat), when compared with the lithiatic control group (GII).

Serum Data

The serum creatinine level after ethylene glycol treatment in GII was 1.8± 0.03 mg/dl which was brought down to 0.75 ±0.031 mg/dl in GIII after treatment with NONI. The results are shown in table 3.

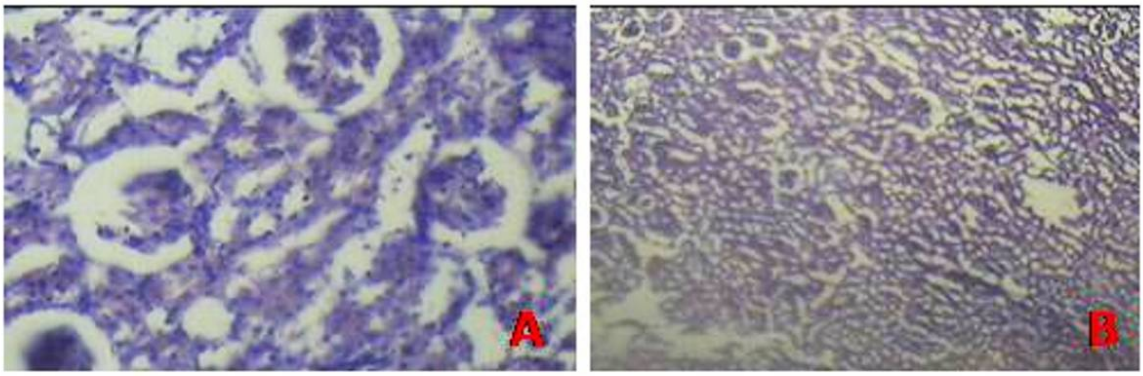

Fig 1: KIDNEY SECTION OF GI (NORMAL CONTROL) RATS
A-NormalGlomeruli B-Normal tubule

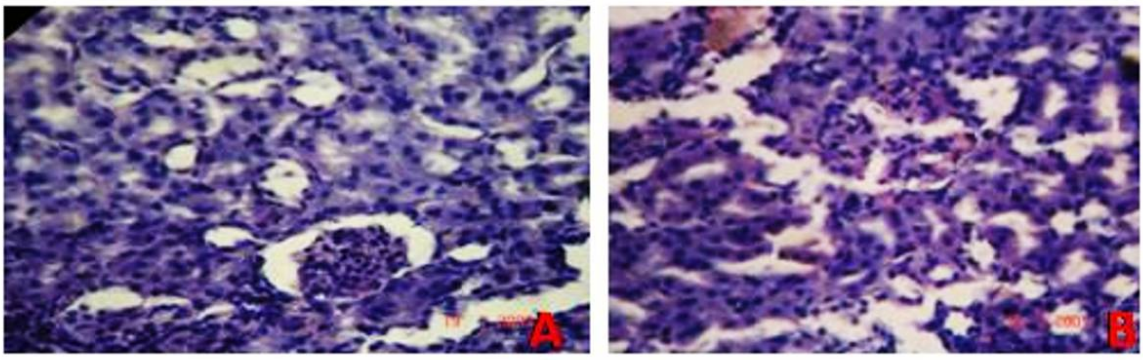

FIG 2 : KIDNEY SECTION OF GII (LITHIATIC CONTROL) RATS.
A- Degenerated Glomeruli B- Necrotic Tubules

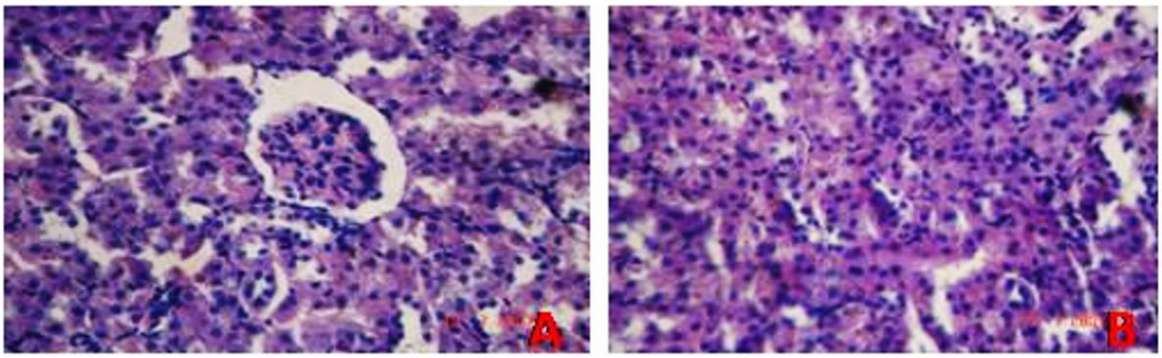

Fig 3: KIDNEY SECTION OF GIII (NONI+0.75% EG) RATS.
A- Improved Glomeruli B- Improved Tubules

Table 1: Effect of NONI on urinary biochemical parameters on the 14th day.

Groups	Calcium Mg/dl	Phosphate mg/dl	Oxalate mg/dl	Protein mg/dl	Magnesium mg/dl	Creatinine mg/dl
Control (G1)	6.04±0.35	63.46±1.38	18.26±1.70	3.54±1.004	1.80±0.158	58.25±5.12
Lithiatic control (GII)	8.88±0.29$^{\cdots}$	85.26±0.64$^{\cdots}$	26.18±1.02$^{\cdots}$	6.55±0.77$^{\cdot}$	1.07±0.070$^{\cdots}$	35.4±0.58$^{\cdot\cdot}$
Treatment with NONI (GIII)	7.4±0.45*	74.26±1.31***	14.48±0.65***	2.19±0.28**	1.82±0.138**	42.71±2.21^{NS}

Values are expressed as mg/24 hr urine sample. Values are expressed as mean ± SEM for six animals in each group. Newman-Keuls multiple range test ($p < 0.05$) was used.

$^{\cdots}$ Values are significantly different from normal control (G-I), $p<0.001$.

$^{\cdot\cdot}$ Values are significantly different from normal control (G-I), $p<0.01$.

$^{\cdot}$ Values are significantly different from normal control (G-I), $p<0.05$.

*** Values are significantly different from lithiatic control (G-II), $p<0.001$.

** Values are significantly different from lithiatic control (G-II), $p<0.01$.

* Values are significantly different from lithiatic control (G-I), $p<0.05$.

Table 2: Effect of NONI on urinary biochemical parameters on the 28th day.

Groups	Calcium mg/dl	Phosphate mg/dl	Oxalate mg/dl	Protein mg/dl	magnesium mg/dl	Creatinine mg/dl
Control (G1)	6.21±0.63	64.56±1.58	18.82±0.81	4.15±0.79	3.17±0.23	56.61±5.35
Lithiatic control (GII)	10.77±0.24$^{\cdots}$	82.08±3.19$^{\cdots}$	31.01±1.25$^{\cdots}$	12±1.15$^{\cdots}$	0.861±0.076$^{\cdots}$	5.66±0.28$^{\cdots}$
Treatment with NONI (GIII)	7.03±0.34***	66.68±0.49***	25.09±1.38**	14±1.78^{NS}	2.7±0.14***	14.38±3.62^{NS}

Values are expressed as mg/24 hr urine sample. Values are expressed as mean ± SEM for six animals in each group. Newman-Keuls multiple range test ($P < 0.05$) was used.

$^{\cdots}$ Values are significantly different from normal control (G-I), $p<0.001$

*** Values are significantly different from lithiatic control (G-II), $p<0.001$

** Values are significantly different from lithiatic control (G-II), $p<0.01$

Table 3: E ffect of N ONI, on serum creatinine level (mg/dl) on the 28th day.

Groups	Serum Creatinine (mg/dl)
GI	0.61±0.1
GII	1.8±0.22▪▪▪
GIII	0.75±0.029***

Values are expressed as mg/24 hr urine sample. Values are expressed as mean ± SEM for six animals in each group. Newman-Keuls multiple range test ($p < 0.05$) was used.

▪▪▪ Values are significantly different from normal control (G-I), $p<0.001$

*** Values are significantly different from normal control (G-II), $p<0.001$

Histopathological Studies

Section of kidney treated with ethylene glycol showed marked dilation of tubules, tubular damage and infiltration of inflammatory cells into the interstitial space. However kidney sections of animals treated with NONI showed improvement of the above symptoms and reduced crystal deposition as shown in fig 1, 2 and 3.

Discussion

Urolithiasis is the formation of stones in the urinary tract, causing pain and bleeding, and may lead to secondary infection. It is the third most common affliction of the urinary tract. Of many types of stones that are formed, the most common are calcium oxalate. Calcium oxalate stone disease is the most common human urinary stone disease in the Western Hemisphere. To understand different aspects of the disease, calcium oxalate urolithiasis in the rat is used as a model. Spontaneous calcium oxalate urolithiasis is very rare in rats. Thus the disease is experimentally induced and the rats are generally made hyperoxaluric either by administration of excess oxalate, exposure to the toxin ethylene glycol, or various nutritional manipulations. All the experimental models show renal injury associated with crystal deposition. One of the important phenomena that characterize urolithiasis is its high recurrence. Thus, a protective system is required including extracorporeal shock wave lithotripsy and medicament treatment. Unfortunately, these means remain costly and in most cases are invasive and with side effects. Therefore, it is worthwhile to look for an alternative to these conventional methods by using medicinal plants or phytotherapy. Therefore, it is highly recommended to explore new drugs coming from medicinal plants to treat and prevent the formation of kidney stones. Ideally, conventional and phytotherapy should supplement one another and have all the need available for lithiasis patients [8-11].

The present study showed that the ingestion of (0.75%) ethylene glycol (EG) can induce calcium-oxalate stone formation; other research also reported the same findings in this model [12-15]. In accord with this experiment, urinary calcium excretion, and urinary oxalate excretion were significantly higher in group fed with 0.75% EG (G-II), than those in the control group (G-I) and other treated group (GIII).

Changes in ionic pattern of urine are the major determinant of stone formation. In this study, the ionic pattern was found disturbed by treatment with EG. It has been reported that daily oral administration of EG for more than four weeks resulted in a significant increases in oxalate excretion and that the kidneys are the targets for the EG toxicities

which gets oxidized to oxalic acid leading to hyperoxaluria.

Hyperoxaluria is reported to be a more significant risk factor in the pathogenesis of stone formation. Likewise ethylene glycol administration increased the urinary calcium level. It has been stated that hyperoxaluria favours precipitation of calcium oxalate from urine. Thus the high oxalate and calcium ion concentration in urine tend to form calcium oxalate crystals. The growth of calcium oxalate crystals is further favoured by disturbances in the urinary levels of other ions like magnesium and phosphate. The available literatures states that, high urinary phosphate level with calcium forms calcium phosphate crystals, which induces further deposition of calcium oxalate on it [16]. In this study the high urinary phosphate level observed in ethylene glycol treated rats is likely to have formed calcium phosphate crystals.

Magnesium is considered as a potent inhibitor of calcium oxalate crystallization in-vitro, and binds to oxalate to form a soluble complex, consequently reducing the concentration available for calcium oxalate precipitation [17, 18]. Our study also revealed a similar observation. Thus, ethylene glycol administration induces stone formation by raising urinary calcium, oxalate and phosphate, and by lowering magnesium as noted in G-II. Our observations showed that NONI-formulation reduces the urinary calcium, oxalate and phosphate levels. It also raised the urinary magnesium concentration. The increase in urine volume may also minimize the tendency for crystallization. It was found that kidney function was impaired in the group of animals treated with ethylene glycol alone: however in the group treated with ethylene glycol and NONI-formulation, the kidney function was found to improve. Thus it has been concluded that NONI-formulation has inhibitory potential on ethylene glycol induced nephrolithiasis.

Conclusion

Biochemical analysis showed that the rats treated with EG alone had higher amounts of calcium in the kidneys compared to negative control rats. This EG-induced increase in kidney calcium levels was inhibited by the administration of NONI formulation. Histology showed that rats treated with EG alone had large deposits of calcium oxalate crystals in all parts of the kidney, and that such deposits were not present in rats also treated with NONI formulation. These data suggest that NONI formulation has a protective activity against urolithiasis.

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