

# EFFECT OF NONI (*Morinda citrifolia*) EXTRACT ON TREATMENT OF ETHYLENE GLYCOL AND AMMONIUM CHLORIDE INDUCED KIDNEY DISEASE

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## Abstract

*Morinda citrifolia* is one of the important medicinal plants having a lot of phytochemicals, which plays very important role in medicines. In this present investigation, the animals are induced to kidney stone by giving ethylene glycol mixed with water and given orally, and 0.5 % ammonium chloride are mixed with water and given orally for 28 days. The induced rats are treated with Noni (1 in 10 ml ) mixed with H<sub>2</sub>O and given to the rats for 28 days, and induced to kidney stone + ammonium chloride is treated with Noni extract mixed with water and given to the rats for 28 days. After the treatment the urinary parameters like creatinine, protein, calcium, oxalate, phosphate are decreased except magnesium, its level is increased and serum creatinine level is decreased. The results are shows the good medicinal properties of noni extract of *Morinda citrifolia*.

**Key words:** Kidney stone, Nephro-protective activity, noni, *Morinda citrifolia*, Creatinine

## 1. Introduction

Kidney is the major organ of our body playing an important role in the excretory system. There are lot of diseases are affect the kidney is kidney blockage and kidney stones. Majorly four types of stones are affecting the kidney are Calcium stones, Stuvite stones, Uric Acid stones, and Cystine stones. Different types of treatments are applied for kidney diseases such as medicine, changing of life style, treatment by surgery etc.

### 1.1 *Morinda citrifolia*

There is a lot of plants are used for the nephro protective activity [1]. *Morinda citrifolia* is the one of the important medicinal plant and commonly known as great Morinda and in Tamil nunaakai. It is coming under the family of Rubiaceae [2]. There is more number of bio-chemicals are present in the Noni fruit extracts are macronutrients like carbohydrates (oligo- and polysaccharides) and dietary fibers, micronutrients like vitamin C, caprylic acid, niacin (vitamin B3), iron, Pottasium, vitamin A, calcium and sodium [3] alkaloids and flavonoids [4]. It contains lot of amino acids like alanine, arginine, cystine, phenyl alanine, glycine, isoleucine, leucine, lycine, methionine, proline, tyrosine, tryptophan, and valine. It has lot of medical properties like prevents many type of cancers, reduction of heart diseases, role on blood pressure, improving of immune power, controlling of diabetes mellitus, reduction of asthma, good antioxidant, strengthening of neurons due to good nervous system, controls the cholesterol level which reduce the weight and get better skin. Noni is one of the herbal formulations of *Morinda citrifolia* it removes the water insoluble calcium oxalate and converts it into water soluble calcium. The parts of *M. citrifolia* such as fruit, leaves and root extracts removes the lipids from animal model are proved. Now it gets excreted in urine easily and prevents the stone formation. This is an herbal formulation and hence it does not cause any side effect [5-13].

In this present investigation, we performed the nephro-protective activity by using noni extract of *Morinda citrifolia*. In this in-vivo study the animals are divided in to 5 groups (A-E). the effect of noni extract for kidney diseases was analyzed. For that the different parameters such as creatinine, protein, calcium, oxalate and phosphate were analyzed in urine and creatinine in analyzed from blood samples. The histological changes in the kidney also examined.

## 2. Materials and methods

All the chemicals used were of analytical grade. Ethylene glycol purchased from fisher scientific, Mumbai. Ammonium chloride purchased from merck india ltd, Mumbai. The plant material was collected from the Arcot, TN, India.

### 2.1 Experiment design

The experimental rats were divided into five groups such as normal, kidney stones induced, and kidney stone treated. Age matched 30 male rats of the body weights around 240g are selected. They have been divided into 5 respective groups (i.e) Group-A, B, C, D and E. Ethical clearance was obtained from the Institutional Animal Ethical Committee, CPCSEA, India (Reg No.282/ac/09/CPCSEA). Group-A is normal diet, Group-B are induced to kidney stone by giving 0.75% of ethylene glycol mixed with water and given orally for 28 days, Group-C are all induced to kidney stone by giving 0.75% of ethylene glycol +0.5 % ammonium chloride are mixed with water and given orally for 28 days, Group- D are induced to kidney stone by giving 0.75% of ethylene glycol orally along with water simultaneously, treated with Noni (1 in 10 ml ) mixed with H<sub>2</sub>O and given to the rats for 28 days, Group-E are induced to kidney stone + ammonium chloride and treated with Noni extract mixed with water and given to the rats for 28 days.

Urine samples were collected from each rat one day before sacrifice, using a metabolic cage. The parameters Urinary like creatinine, total protein, calcium, magnesium, oxalate, phosphate are analyzed. At the end of 28<sup>th</sup> day, one animal from every group is taken and the rats were sacrificed, the blood is collected and the serum is separated, the Urinary and Biochemical parameters and the Histopathological analysis were done.

### 2.2 Paper chromatography

The amino acids present in the *Morinda citrifolia* fruits are analyzed using paper chromatography.

### 2.3 Histopathology

A portion of kidney tissue in each group was fixed in 10% formalin and preceded for histopathology studies. After paraffin embedding and block making, serial section of 5  $\mu$ M thicknesses were made and stained with haematoxylin and eosin and examined under microscope. For Calcium Oxalate induced stone, special stain for Calcium Oxalate like PAS and MSB are done. It gives the true result only for calcium oxalate not for other types of stones.

## 3. Result and discussion

Fig-1 represents the level of creatinine different group of rats in 28<sup>th</sup> day. The group-II and group III animals were treated with ethylene glycol and ethylene glycol +NH<sub>4</sub>Cl which induces kidney stones shows elevated level of creatinine when compared with Noni control. The two hydroxyl group of ethylene glycol oxidized to form aldehyde and acid, which binds with calcium to form calcium oxalate kidney stone, the creatinine level get elevated in high level on 28<sup>th</sup> day. On treatment with Noni in group-IV and group V rat, it shows almost reduced level of this biochemical marker at 28<sup>th</sup> day. It shows the highly significant value of 0.02 at 28<sup>th</sup> day. Creatinine is one of the waste products excreted by the kidney. The level of creatinine is increased under severe muscle wasting condition and excreted in the urine leads to creatinuria. In kidney stones condition the filtration and excretion of creatinine may affected and raise the level of creatinine in serum.

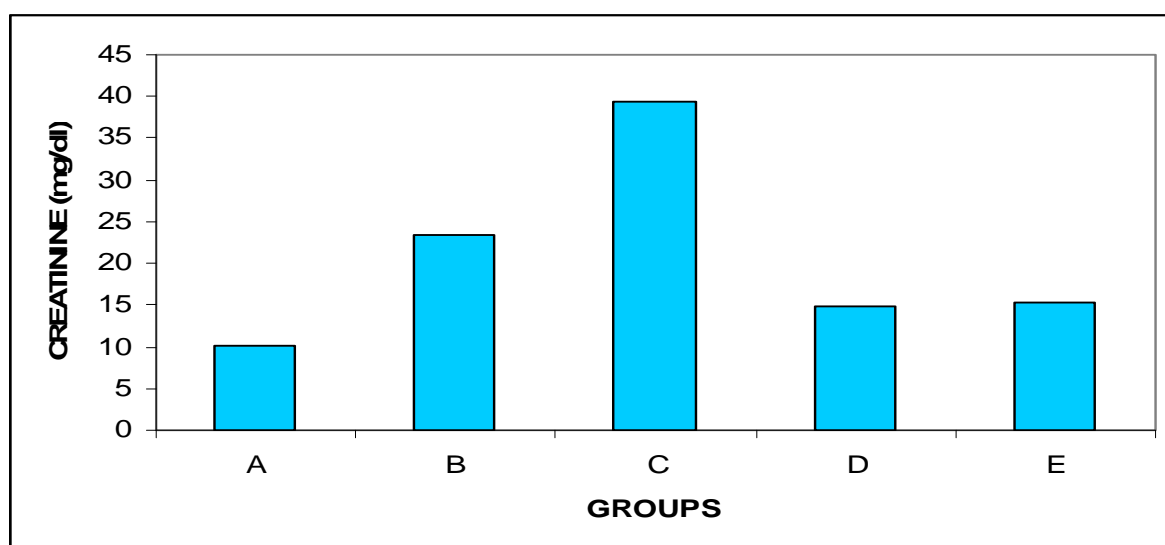


Fig - 1: Graphical representation of urine creatinine (mg/dl) on (A) Normal, (B) Ethylene glycol (Renal stone), (C) Ethylene glycol +NH<sub>4</sub>Cl (Renal stone), (D) Ethylene glycol and treatment with noni, (E) Ethylene glycol + NH<sub>4</sub>Cl and treatment with noni.

Fig-2 depicts the level of protein in different groups of rats. The group-II and group III animals were treated with Ethylene glycol and Ethylene glycol +  $\text{NH}_4\text{Cl}$  have elevated level of protein due to the induction of kidney stones. It comes to the almost normal on comparing with group-I control. On treating with noni, in group-IV and group V rats the level of significance was 0.03 in 28<sup>th</sup> day.

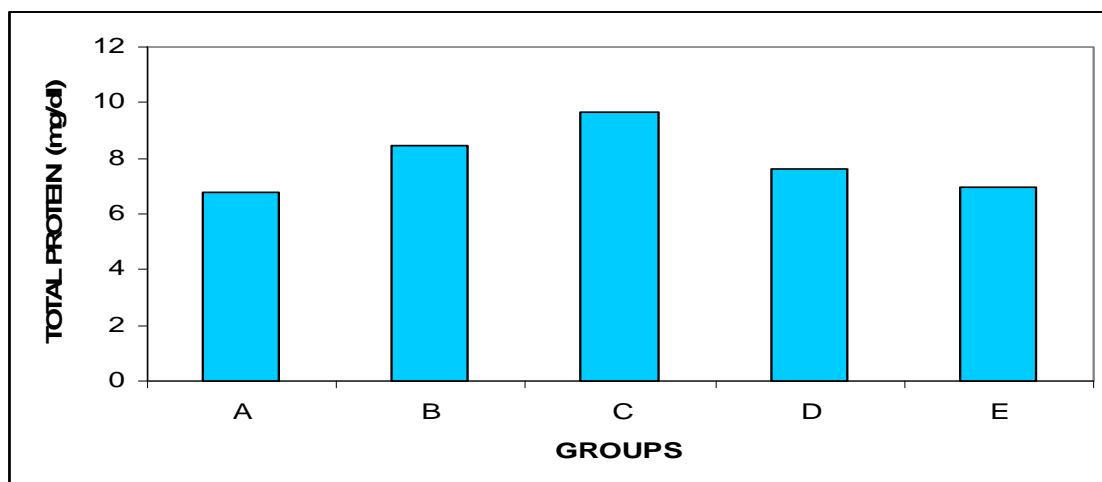


Fig 2: Graphical representation of urine total protein (mg/dl) on (A) Normal, (B) Ethylene glycol (Renal stone), (C) Ethylene glycol +  $\text{NH}_4\text{Cl}$  (Renal stone), (D) Ethylene glycol and treatment with Noni, (E) Ethylene glycol +  $\text{NH}_4\text{Cl}$  and treatment with Noni.

Fig-3 Represent the level of calcium in different groups of rats. The Calcium level is evaluated in group-II and group III, Ethylene glycol and Ethylene Glycol +  $\text{NH}_4\text{Cl}$  treated rats when compared to control group-I. Noni treated group IV and group V shows the reduced level of calcium in the urine with the level of significance of 0.03 in 28<sup>th</sup> day. On Ethylene glycol induction produces oxalic acid which leads to the acidosis with ultimate reduction of pH [14]. Due to the acidosis condition 0.5% of removable calcium on the extra cellular fluid causes the calcium oxalate stones in the kidney this may elevate the level of  $\text{Ca}^{2+}$  in the serum on group-II and group III rats.

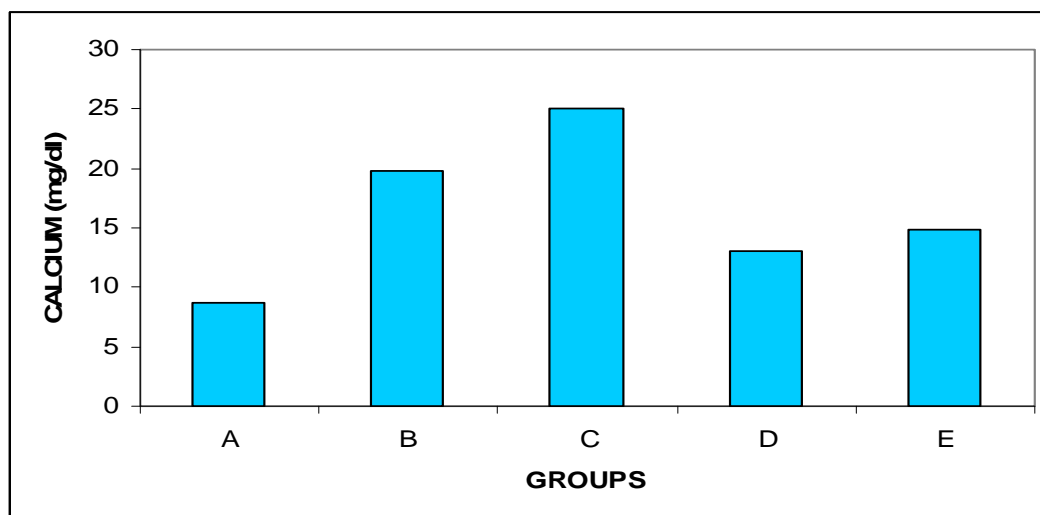


Fig 3 Graphical representation of urine calcium (mg/dl) on (A) Normal, (B) Ethylene glycol (Renal stone), (C) Ethylene glycol +  $\text{NH}_4\text{Cl}$  (Renal stone), (D) Ethylene glycol and treatment with noni, (E) Ethylene glycol +  $\text{NH}_4\text{Cl}$  and treatment with Noni.

On treatment with Noni on group- IV and group V rats the water insoluble calcium oxalate is converted to soluble calcium acetate, oxalate and all these excreted along with urine and reduces the calcium level in the urine. After treatment of group-IV and group V with noni decreases the level of  $\text{Ca}^{2+}$  almost near to the normal with the significance of 0.05 in 28<sup>th</sup> day.

Fig-4 Depicts the level of the  $\text{Mg}^{2+}$  in different group of rats. The kidney stone group-III animal shows a markedly decreased level of magnesium on 28<sup>th</sup> day. When compared to control group-II noni treated rat. Thus, ethylene glycol administration induces stone formation by raising urinary calcium, oxalate and phosphate, and by lowering magnesium as noted in G-II and Group III.

The different parameter for kidney function test usually increases on treating with Ethylene glycol except the magnesium which decreases in group-IV and V rats, When compared with normal group-I and control group-II noni treated rats. After treatment with noni the diminished value of magnesium is reverted back to the normal level in group- III and IV rats.

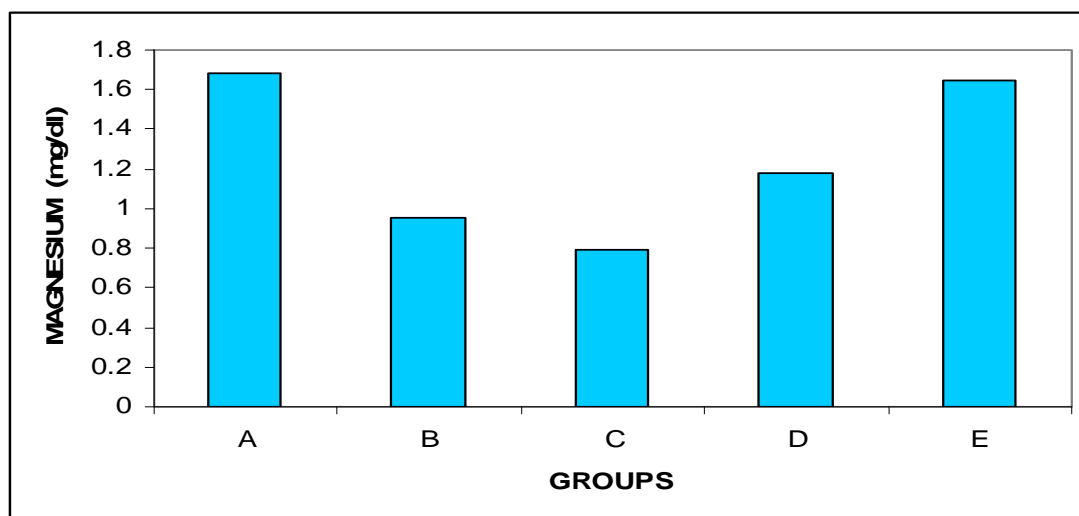


Fig 4: Graphical representation of urine magnesium (mg/dl) on (A) Normal, (B) Ethylene glycol (Renal stone), (C) Ethylene glycol +NH<sub>4</sub>Cl (Renal stone), (D) Ethylene glycol and treatment with Noni, (E) Ethylene glycol + NH<sub>4</sub>Cl and treatment with Noni

Fig-5 Shows the level of oxalate in the urine on the different groups of rats. The group-II and group III animals were treated with Ethylene glycol and Ethylene glycol + NH<sub>4</sub>Cl have elevated level of oxalate due to the induction of kidney stones. It comes to the almost normal on comparing with group-I control, on treating with noni, in group-IV and group V rats the level of significance was 0.05 in 28<sup>th</sup> day.

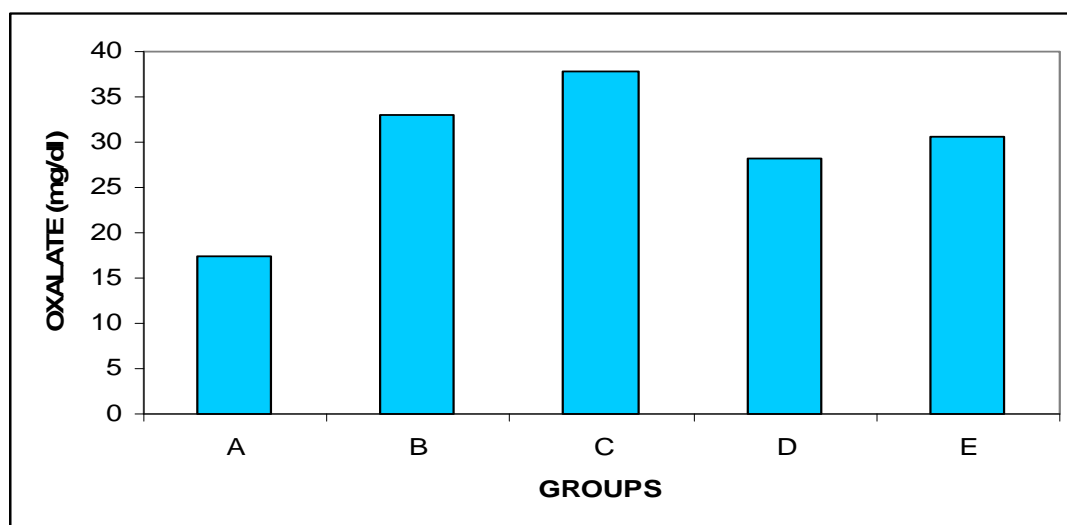


Fig 5: Graphical representation of urine oxalate (mg/dl) on (A) Normal, (B) Ethylene glycol (Renal stone), (C) Ethylene glycol +NH<sub>4</sub>Cl (Renal stone), (D) Ethylene glycol and treatment with Noni, (E) Ethylene glycol + NH<sub>4</sub>Cl and treatment with Noni.

Fig-6 Shows the level of phosphate in the urine on the different groups of rats. The different parameter for kidney function test usually increases on treating with Ethylene glycol and ammonium chloride, likewise phosphate level gets decreased on group-IV and V rats, after treatment with Noni and returns to normal control rats.

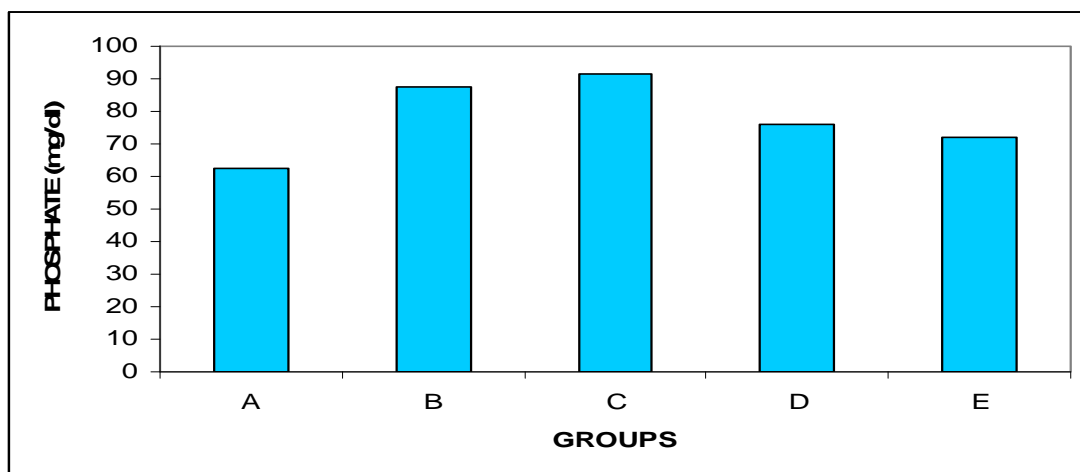


FIG 6: Graphical representation of urine phosphate (mg/dl) on (A) Normal, (B) Ethylene glycol (Renal stone), (C) Ethylene glycol +NH<sub>4</sub>Cl (Renal stone), (D) Ethylene glycol and treatment with Noni, (E) Ethylene glycol + NH<sub>4</sub>Cl and treatment with Noni

Fig-7 Shows the level of creatinine level in serum on the different groups of rats. The level of creatinine is increased in the ethylene glycol induced rats, it may cause the creatinuria. On treatment with noni, may be the causative factor for reduction in the level of creatinine in group-IV and V rats decreases the excretion of creatinine and maintains to the level almost normal.

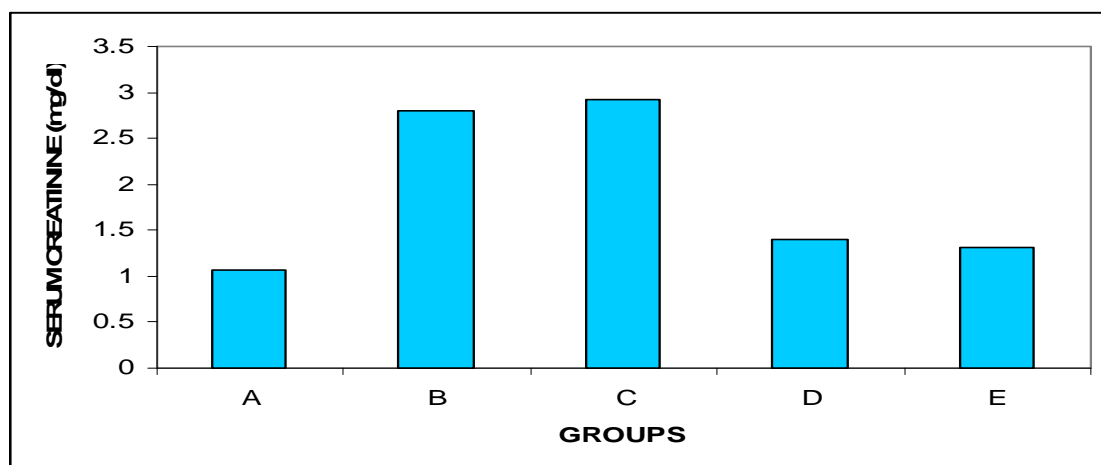


Fig 7: Graphical representation of blood creatinine (mg/dl) on (A) Normal, (B) Ethylene glycol (Renal stone), (C) Ethylene glycol +NH<sub>4</sub>Cl (Renal stone), (D) Ethylene glycol and treatment with Noni, (E) Ethylene glycol + NH<sub>4</sub>Cl and treatment with Noni

### 3.1 Histopathological studies on kidney:

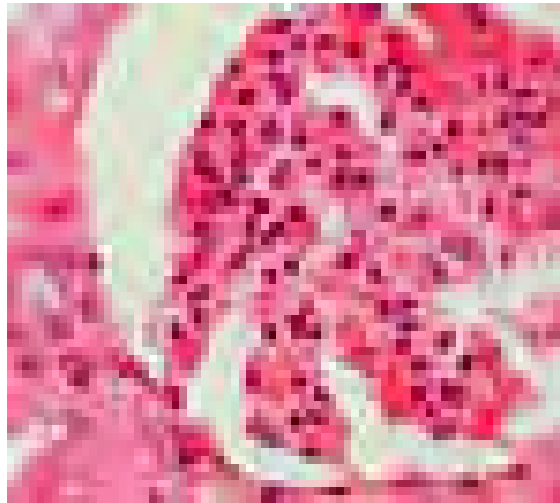


Fig 8: Normal Control

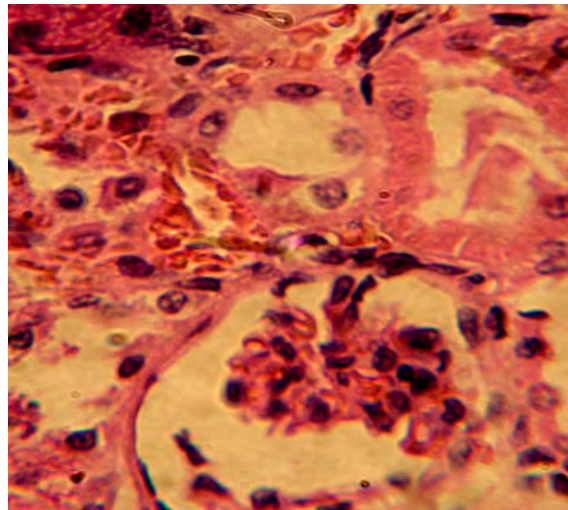


Fig 9: Ethylene Glycol Induced Renal Stone

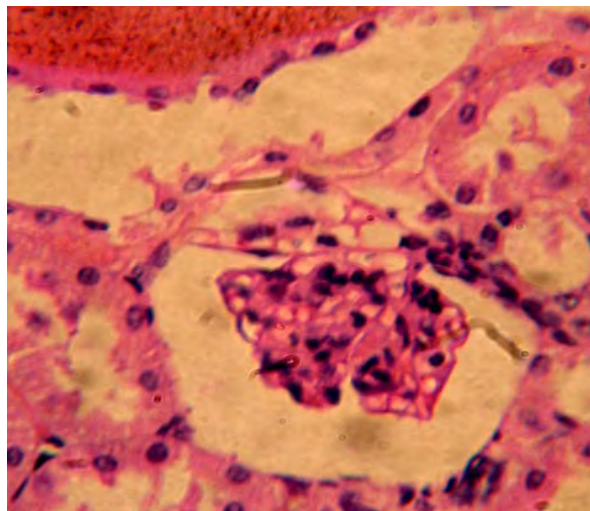


Fig 10: Ethylene glycol induced renal stone and treated with Noni

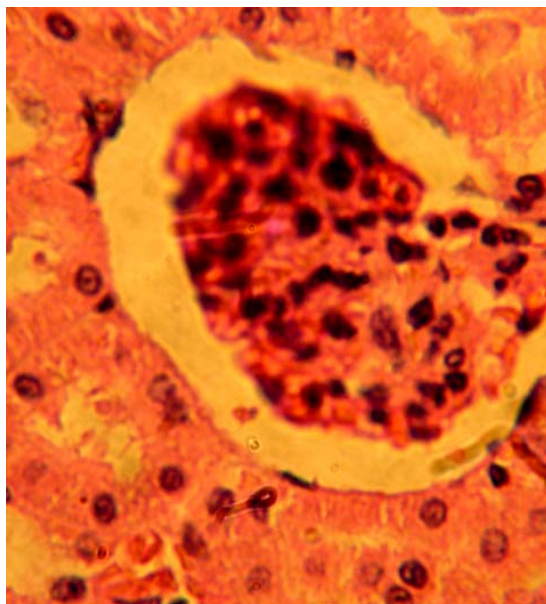


Fig 11: Ethylene glycol + ammonium chloride induced renal stone

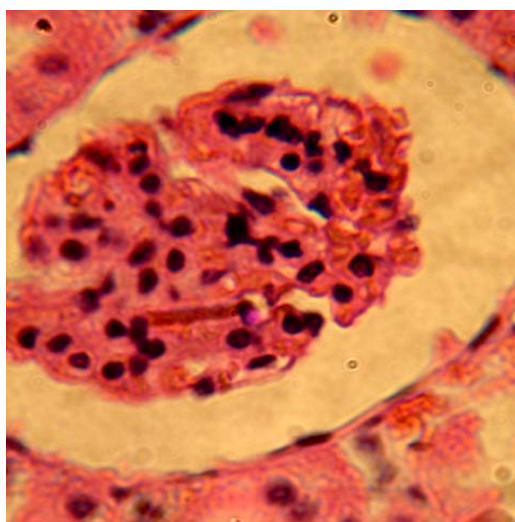


Fig 12: Ethylene Glycol + Ammonium Chloride Induced Renal Stone & Treated with noni

Histopathological studies showed that Ethylene glycol +  $\text{NH}_4\text{Cl}$  administration in rats cause acute renal stones on kidney. This almost comes to normal appearance with mild change in severe renal stones of rats treated with Noni which indicating the administration of Noni decreased the renal stone induced by Ethylene glycol +  $\text{NH}_4\text{Cl}$ . Normal rat showed the normal appearance of kidney without any histological alteration.



### Paper chromatography results

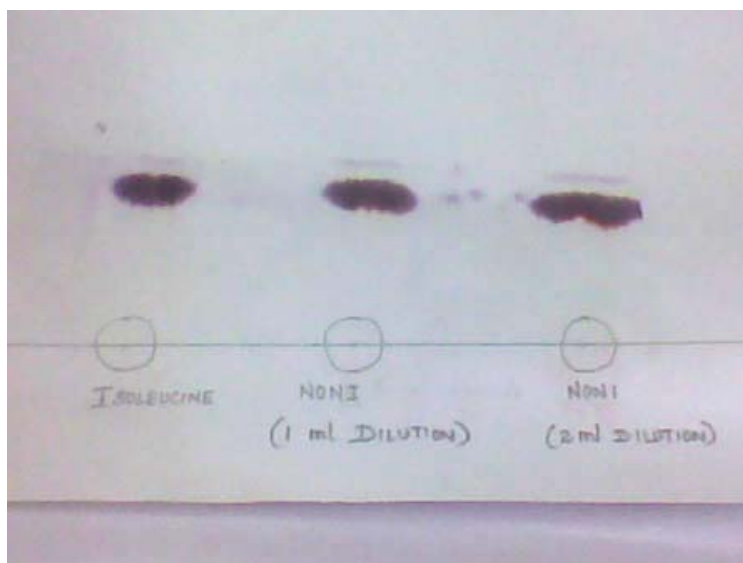


Fig: 13 Detection of Isoleucine in NONI

### 4. Conclusion

Kidney is the major organ for excretory system in our body. And kidney stone formation is the major problem for kidney and millions of peoples are affected by these problem. Because of low intake of water and unwanted food taking are major causes and apart from so many factors may cause the kidney stone formation. Curing of this problem is very important. There is a lot of natural medicines are used for these disease. In our study the product of *Morinda citrifolia* play vigorously against the kidney diseases. After the noni administration the urinary parameters like creatinine, protein, calcium, oxalate, phosphate are decreased except magnesium, its level is increased and serum creatinine level is decreased. Thus it has been concluded that Noni-formulation has inhibitory potential on ethylene glycol induced nephrolithiasis.

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