

Antiuro lithiatic effect of *Cymbopogon Proximus*, *Alhagi Maurorum*, on Sulfadimidine induced urolithiasis in male New Zealand rabbits.

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ABSTRACT

The present study aimed to investigate the prophylactic effect of *Cymbopogon proximus* and *Alhagi maurorum* on Sulfadimidine_ induced urolithiasis in rabbits . Thirty New Zealand male rabbits were allocated into six equal groups (each of five): Group (1) was used as a negative control. Group(2) were administered sulfadimidine (200mg/kg) by intramuscular injection. Groups(3) and (4) were administered sulfadimidine(200mg/kg) by intramuscular injection and 330mg/kg of *Cymbopogon proximus* alcoholic and aqueous extracts respectively orally. Groups(5) and (6) were administered sulfadimidine(200mg/kg) by intramuscular injection and 400mg/kg of *Alhagi maurorum* alcoholic and aqueous extracts respectively orally. The period of experiment was 10 days. Blood and urine samples were collected from rabbits on the 10th day. The results recorded a significant decrease in serum creatinine, urea, uric acid and crystalluria in *Cymbopogon proximus* and *Alhagi maurorum* groups compared to sulfadimidine treated group. We conclude that *Cymbopogon proximus* and *Alhagi maurorum* have a nephroprotective and antiuro lithiatic effects against sulfadimidine induced crystalluria.

Keywords: Sulfadimidine, *Cymbopogon proximus*, *Alhagi maurorum*, Crystalluria, rabbit.

1. INTRODUCTION

Sulfonamides are the main form of antibacterial group permitted for extensive use. They have bacteriostatic effects against a broad spectrum of pathogens (Zessel et al., 2014). They are also effective against some protozoa, including *Toxoplasma* and coccidian (Yazici et al., 2009; Fox et al., 2002). Sulfonamides are a proper choice of antibiotic for infected rabbits because of their little toxicity, availability and easiness of administration. They have an unwanted side effects on the physiological function of the kidneys, so can cause harmful effects, that effects limits its use (Mustafa et al., 2014).

The administration of sulfonamides can be complicated by aggregation of crystalline of these drugs beginning Crystalluria (Dorfman and Smith, 1970) which is characterized by the occurrence of crystals in the urine. thus crystalluria represents a hazard factor for kidney stones. Nephrolithiasis (also known as kidney stones, renal stones) is a common disease with an increasing rate of recurrence (Khan et al., 2016; Alelign and Petros, 2018) also it can cause kidney failure, urinary tract infections (UTIs) and severe abdominal pain, blood in urine and flank pain so prevention of stone formation is highly recommended . Presently available possibility for prevention or treatment of urolithiasis are ineffective, costly and with side effects. So alternative treatments especially from herbal

medicines and therapeutic plants were considered as origins of safe, economical, effective and publically believed treatments (Atmani et al., 2003; Johri et al., 2010; Bennett and Brown 2000). Medicinal plants are utilized by 80% of the world people as the simply available medicines mainly in developing countries.

Cymbopogon proximus belongs to Family Poaceae is a traditional Egyptian medicinal plant generally known as "Halfa Bar". In the Egyptian traditional medicine, it is recognized as an efficient diuretic and renal antispasmodic (Askary et al., 2003). In South Egypt this plant is used as a diuretic, increase urin flow, colic pain destroyer, help for expulsion of tiny bit stones which present in the urinary tract, and antipyretic, hypotensive, antiemetic, anticonvulsant (El Tahir and Abdel Kader, 2008) antioxidant and antibacterial properties (Selim, 2011). According to phytochemical analysis of *Cymbopogon proximus*, the occurrence of saponins, Flavonoids, glycosides, Terpenes and tannins may be a rationale for the use of this plant in medicine preparations.

Alhagi maurorum belongs to family Fabaceae has been seen in different regions of Egypt, especially the north to the south of Sinai deserts, According to traditional medicine *Alhagi* has diuretic property and avoids kidney spasms, therefore, since ancient times, it has been used to relieve kidney pain from kidney stones and urinary tract stones

expulsion. In addition, it is efficient to attenuate UTI and renal colic (Zargari, 1997). It has been used as laxative, purgative, diaphoretic, expectorant and diuretic (Boulous,1996). Alhagi phytochemical tests have shown that the plant has bioactive and active pharmaceutical ingredients for example flavonoids, flavone glycosides, Alhagidin, Alhagitin, proanthocyanidins, triterpenes, tannins and catechines (Eskalieva and Burasheva, 2002 ; ALimova et al.,2010).

The current study was proceeded to evaluate the effectiveness of *Cymbopogon proximus* and *Alhagi maurorum* as a preventative agents against sulfadimidine-induced crystalluria and urolithiasis.

2. MATERIALS AND METHODS

Preparation of plants Extracts

The fresh two plants aerial parts (2kg) were collected from western desert and Sinai deserts in Egypt in March 2018, and recognized by Department of Pharmacognosy, Faculty of Pharmacy; Zagazig University. Sulfadimidine 33.3% injection (Sulfa 333)[®] was obtained from Interchemie werken 'De Adelaar' company.

Ethanollic Extract

Cymbopogon proximus extract: according to (El-Nezhawy et al., 2014).

The air dried powdered aerial parts of *Cymbopogon proximus* (Halfabar, 2 kg) were extracted with ethanol alcohol under reflux conditions and the ethanolic extract was vaporized under reduced pressure to obtain the dried total ethanolic extract (140g) The obtained dried extract was freshly prepared as suspensions in saline immediately before use for oral administration.

Alhagi extract: according to (Marashdah and AL-Hazimi 2010) aerial parts (2 kg) powder were extracted with 1000 ml of absolute ethanolic alcohol under reflux for 2 h and the solution was filtered and the solvent was isolated under vacuum distillation to provide 60 g of precipitate.

Aqueous Extract

Cymbopogon proximus extract: according to (Warrag et al., 2014) the aerial parts of the plant were well washed, put in air to be dried and then milled to a fine powder. Then the powder was suspended at 5% concentration of distilled water (D.W). The suspension was saved at continuous mixing for 24 hr. After settlement, the supernatant was taken and used for animals' administration.

Alhagi extract: according to (Neamah,2012) the aerial parts of the plant were washed neatly with tap water then put the plant parts to boiled in 3 L of distilled water (DW) for 1hr, dark brown solution was gained, then solution dried by

reduced pressure, the powder was preserved in sterile container to be used for animals' administration.

*The ethanolic and Aqueous extracts of *Cymbopogon proximus* and *Alhagi maurorum* were dissolved in distilled water (D.W) at a doses 330mg/kg (Warrag et al., 2014; Paget and Barnes, 1964) ,400 mg/kg (Neamah,2012; Paget and Barnes,1964) body weight of rabbit respectively then shacked until completely dissolved and were given by using a stomach tube. *sulfadimidine were given to rabbits intramuscular at dose 200mg/kg (Carpenter et al., 2001)

Experimental animals

Thirty male New Zealand rabbits weighing between 1.5 to 2 kg were used in this study. The whole experiment was carried out in the same environmental conditions at room temperature in laboratory experiment. Animals was stayed 2 weeks prior to experiment as adaptation period and the bedding of the animal cages changed every 24hrs. Rabbits were allocated into six equal groups each of five.

Experimental design

Group (1): Negative control, were nourished with normal diet and water ad libitum.

Group (2): Received water ad libitum and normal diet with therapeutic dose of sulfadimidine (200mg/ kg body wt I.M) for 10 days and serve as positive control.

Group (3): Received the same dose of sulfadimidine as group-2 and 330mg/kg of *Cymbopogon proximus* (half bar) alcoholic extract orally for 10 days.

Group (4): Received the same dose of sulfadimidine as group-2 and 330mg/kg of *Cymbopogon proximus* (half bar) Aqueous extract orally for 10 day.

Group (5): Received the same dose of sulfadimidine as group-2 and 400mg/kg of *Alhagi maurorum* (camel thorn) alcoholic extract orally for 10 days.

Group (6): Received the same dose of sulfadimidine as group-2 and 400mg/kg of *Alhagi maurorum* (camel thorn) Aqueous extract orally for 10 days.

Urine collection and analysis

All animals were fasted overnight then urine samples were collected from each rabbit only on the last day, after treatment with sulfadimidine and two medicinal plants. These animals were kept in individual cages; urine samples were collected and were examined for sulfa crystals.

Blood collection and analysis

Blood was collected from ear veins in the last day of the experimental period. Then serum was separated by centrifugation at 3,000 rpm for 15 minutes to investigate creatinine (Henry, 1974), urea, uric acid (Vassault et al., 1986),

electrolytes as sodium (Young, 2001), potassium (Young, 2001) and calcium (Burtis, 1999).

Histopathological

Fixation and tissue processing: The formalin preserved tissues from kidney; ureter and urinary bladder were processed in an automated tissue processor. Stained sections were examined for presence of urolithiasis and the accompanying tissue changes including degeneration necrosis, inflammation, proliferation and any other pathological changes (Suvarna,2013).

Statistical analysis

The obtained data in the current study are expressed as mean±SE. Statistical significance of the difference between groups was determined by. One way analysis of variance (ANOVA) followed by Tukey's test. The statistical software package used for analysis was Statistical Package for Social Sciences (SPSS 15). The values were considered to be significantly different when the P value < 0.05. (Zar,1996).

3. RESULTS

Urine samples obtained from rabbits intramuscularly (I.M.) injected with sulfadimidine at a dose of 200mg/kg b.wt. (gp. 2, positive control) daily for 10 days showed heavy sulfa crystals compared with the normal control fed with normal diet and water ad-libitum (gp. 1, negative control) as shown in Fig1, Fig2 (a,b,c,d).

The concurrent use of ethanolic or water extract of *Cymbopogon proximus* in a dose of 330 mg/kg b.wt (gps. 3 & 4) and *Alhagi maurorum* in a dose of 400 mg/kg b.wt. (gps. 5 & 6) orally in distilled water for 10 days resulted in significant decrease in urine sulfa crystals compared with sulfadimidine group, the samples appear amorphous with few crystals as shown in Fig 3 (a,b,c,d)

Biochemical results

Kidney function tests

Concerning evaluation of kidney functions in the current study, determinations of serum creatinine, urea and uric acid levels were demonstrated in Table (1)

There are a significant ($p < 0.001$) increase in the serum creatinine, urea and uric acid levels in sulfadimidine injected rabbits compared with the normal control. This increase was alleviated or decreased after treatment with ethanolic and water extracts *Cymbopogon proximus* and *Alhagi maurorum*, compared with the positive control (gp. 2).

b. Serum electrolytes

The serum electrolytes investigation in the current study was illustrated in Table (2)

There was a significant ($p < 0.001$) decrease in the serum sodium and calcium levels in sulfadimidine injected rabbits compared with the negative or normal control. On the other hand, serum potassium level showed a significant ($p < 0.001$) increase compared with normal rabbits.

Oral administration of ethanolic or water plant extracts, *Cymbopogon proximus*, *Alhagi maurorum* concomitantly with sulfadimidine in rabbits resulted in a significant improvement in all serum electrolytes concentrations.

Histopathological findings

A. Control negative group

Examined sections from kidney, urinary bladder, ureter and urethra revealed normal histomorphological structures (Fig 4, A,B,C,D)

B) Sulfadimidine treated rabbits, 200mg/kg B.Wt I.M

Kidney: Examined sections of the kidney showed mild glomerular mesangial proliferation, cloudy swelling of the renal tubular epithelium mild congestion and hyaline casts. Focal interstitial nephritis with dilatation of the collecting tubules and presence of amorphous bluish material in lumina of some tubules could also be detected Fig4 (E,F,G,H). Section from ureter of some cases showed normal mucosa with partial sloughing of the lining epithelium. Focal bluish amorphous deposit in the lumina accompanied by partial necrosis and sloughing of the transitional epithelium could be detected in other cases. (Fig .4. I,J,K,L). Sections from the bladder revealed focal necrosis and sloughing of the transitional epithelium and focal reactive hyperplasia of the epithelial lining. The muscle layer revealed dispersed bundles by slightly edematous fibrous tissue. Focal ulceration of the lining epithelium could be detected in some.

Cymbopogon proximus ethanolic extract 330mg/kg B.W. orally and sulfadimidine 200mg/kg B.W. I.M

Examined sections from kidney showed mild degenerative changes in the tubular epithelium, ureter and urethra revealed normal histomorphological structures meanwhile the Urinary bladder revealed focal desquamation of the transitional epithelial (Fig.5., A,B,C,D)

D. Cymbopogon proximus water extract 330mg/kg B.W orally and sulfadimidine 200mg/kg B.W. I.M)

Examined sections from kidney, urinary bladder, ureter and urethra revealed normal histomorphological structures. (Fig.5.E,F,G,H).

E. Alhagi maurorum ethanolic extract 400mg/kg B.W. orally and sulfadimidine 200mg/kg B.W. I.M

Examined sections from kidney showed shrinkage of some glomeruli, cystic dilatation of some collecting tubules and degenerative changes in the renal tubular epithelium. The

ureter revealed desquamated epithelial cells in the ureteral lumen. The Urinary bladder showed normal histomorphological structure.(Fig.6, A,B,C,D).

proliferation. The urinary bladder showed focal necrosis in their transitional epithelium.(Fig.6.E,F,G.).

F. Alhagi maurorum water extract 400mg/kg B.W. orally and sulfadimidine 200mg/kg B.W. I.M)

The examined sections from kidney and urethra revealed normal histomorphological structure. The renal pelvis denoted congestion of the blood vessel and focal fibrous tissue

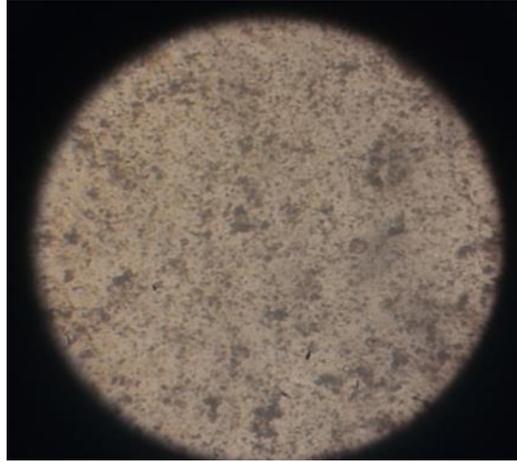


Fig 1

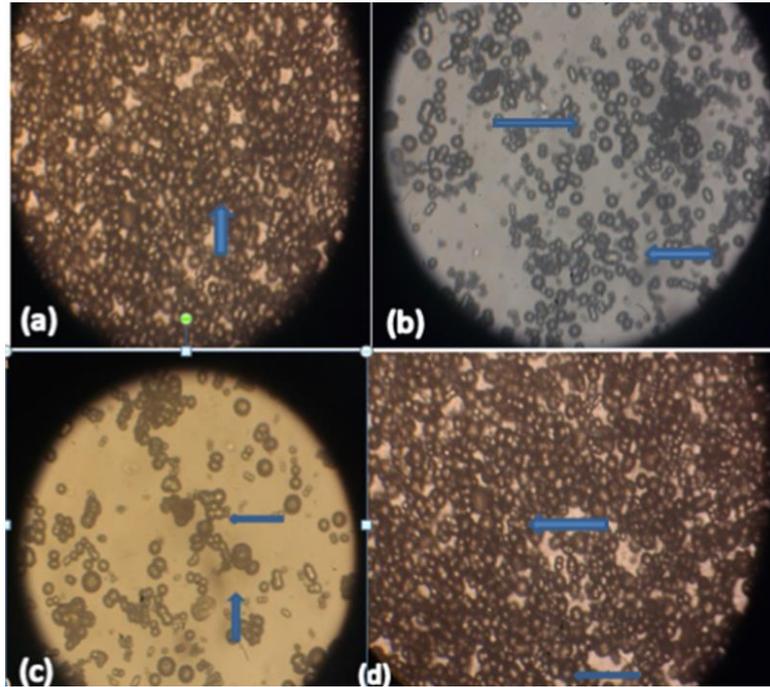


Fig 2: (a,b,c,d)

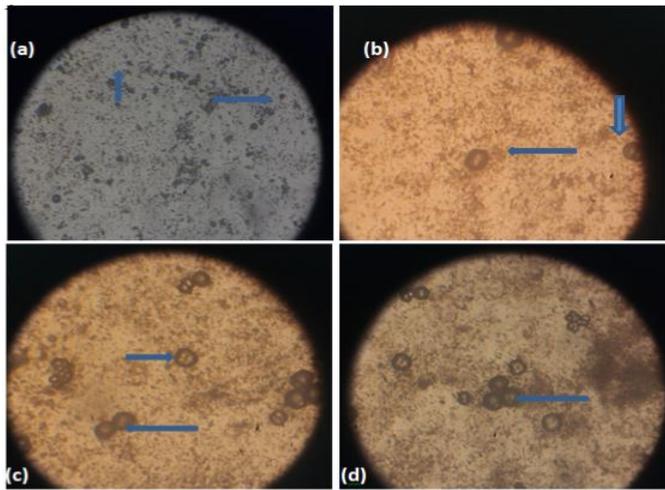
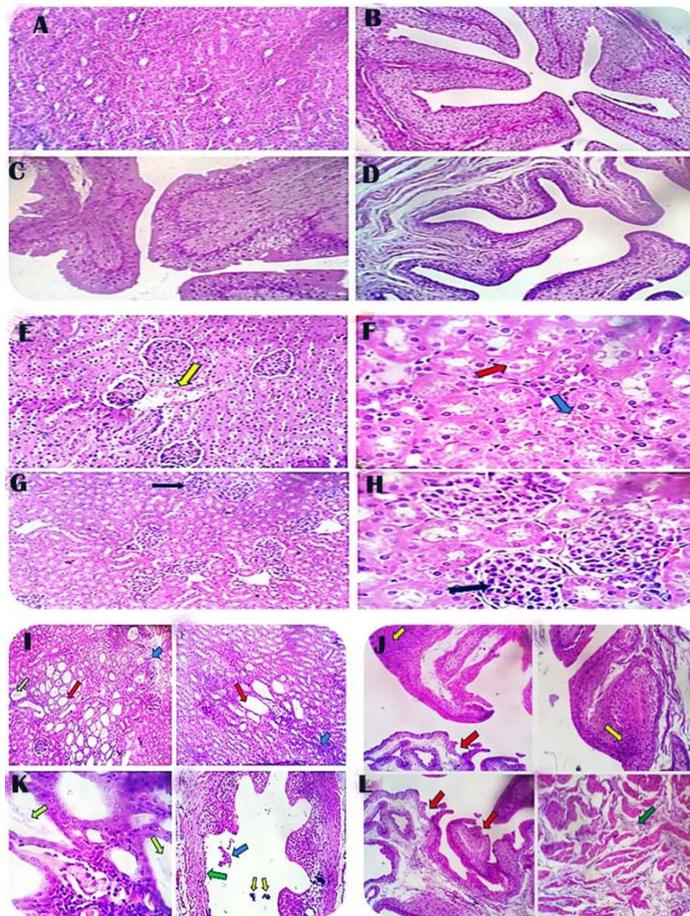


Fig 3(a,b,c,d)



Fig(4)

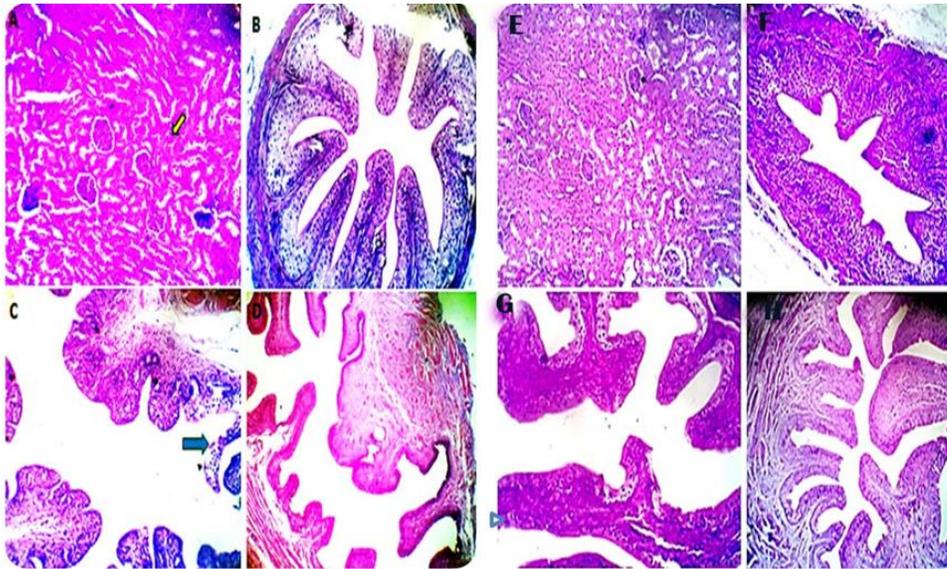


Fig (5)

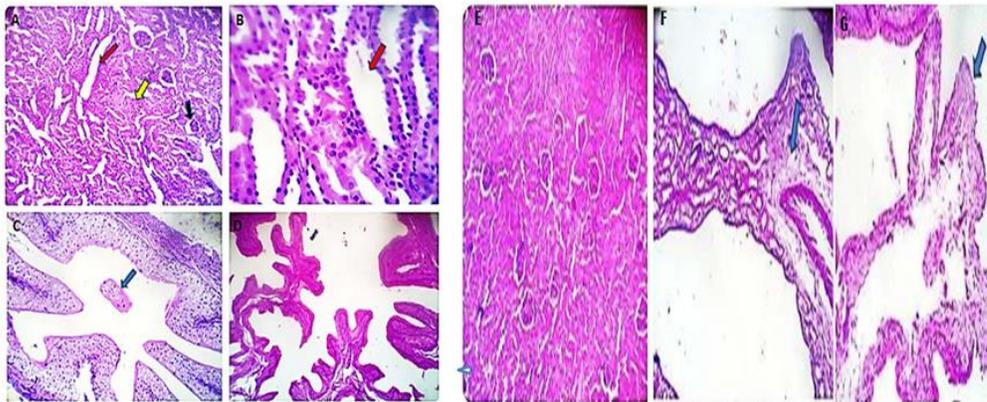


Fig (6)

4. DISCUSSION

In veterinary practice there is a rising need for the correct housing, feeding and treatment of small mammals such as rabbits, fur-bearing animals, rodents, and exotic species of birds. Bacterial infections of these animal species are quite frequent and antimicrobial treatment is regularly required (Kraft, 1994; Gabrisch and Zwart, 2001). Sulphonamides are synthetic compounds of a wide antimicrobial spectrum, which limit overgrowth and multiplication of numerous Gram-positive and Gram-negative bacterial species and some protozoa, for example, *Eimeria spp* and other coccidial organisms which can affect rabbits with a disease called coccidia, especially young and freshly weaned rabbits. (Sakar et al., 2005) Sulfonamides represented by sulfadimidine, which is sulfonamide, derived synthetic compounds.

Assessment of serum creatinine, urea and uric acid was carried out to test the renal function, and as markers of glomerular and tubular damage (Thamilselvan and Menon, 2005). Results revealed significantly higher levels of creatinine, urea and uric acid in sulfadimidine injected rabbits (200 mg/kg b.wt., i.m) for 10 days compared with the normal control ($P < 0.001$). The increase in the aforementioned renal markers was alleviated or decreased after treatment with ethanolic and water extracts of *C. proximus*, *A. maurorum* compared with the positive control. The highest decrease in serum creatinine and urea that restored towards the normal values was demonstrated in rabbits received ethanolic extracts of *C. proximus* (330 mg/kg b.wt.), followed by of *A. maurorum*(400mg/kg. Our findings were in agreement with Warrag et al (2014) who said that stone group of rats (given 0.75% ethylene glycol and 2% ammonium chloride in drinking water for 10 days) treated with 5% aqueous extract of *C. proximus* (1.5ml/100gb.wt./day for 10days) presented significantly lower levels of creatinine, blood urea nitrogen and calcium. They concluded that *C. proximus* has a significant protective effect against ethylene glycol- induced nephrolithiasis in rats. Changizi-Ashtiyani et al (2016) reported that *A. maurorum* (100 mg/ kg per day orally for 10 days after a single dose of 7 mg/ kg intraperitoneal cisplatin) was able to reduce the levels of the renal function markers (serum creatinine and urea nitrogen copared with cisplatin group.The histopathological findings in the kidney of treated rabbits support the previous renal function biomarkers. Examined sections from kidney, ureter, urinary bladder and urethra of sulfadimidine- injected rabbits and treated with aqueous extract of *C. proximus*, revealed normal histomorphological structures, while kidney of rabbits treated with ethanolic extract showed mild degenerative changes in the tubular epithelium, ureter and urethra revealed normal histomorphological structures, meanwhile the urinary bladder revealed focal desquamation of the transitional epithelial. These findings were nearly in correlation with those reported by Badr et al., (2012) who concluded that results indicated

that the kidney histopathological sections of the control and *C. proximus* groups showed normal renal glomeruli and tubules, as well as Warrag et al.,(2014) The authors encountered that the histopathology of *C. proximus* treated rats showed nearly normal renal architecture, with no tubular necrosis, no tubular lumen dilatation, no intraluminal stone and no interstitial edema. They added only mild interstitial inflammatory cell infiltrates were observed, while the glomerular morphology remained unchanged. These findings suggest a potent protective effect of the plant against sulfadimidine induced urinary damage. Examined sections from kidney and urethra of sulfadimidine- injected rabbits and treated with aqueous extract of *A. maurorum* revealed normal histomorphological structure. The Renal pelvis denoted congestion of the blood vessel and focal fibrous tissue proliferation. The urinary bladder showed focal necrosis in their transitional epithelium. However, rabbits treated with ethanolic extract of *A. maurorum* showed shrinkage of some glomeruli, cystic dilatation of some collecting tubules, degenerative changes in the renal tubular epithelium, and desquamated epithelial cells in the ureteral lumen. The urinary bladder showed normal histomorphological structure. Our results were in harmony with Marashdah and Farraj (2010). They recorded that a 2% aqueous extract of *Alhagi maurorum* powdered roots possess a spasmolytic action and a ureter relaxing action that can improve getting rid of renal stones and release of the accompanying pain

With regarding to electrolytes analysis in the present work, there were significant ($p < 0.001$) decrease in the serum sodium and calcium levels in sulfadimidine- injected rabbits compared with the negative or normal control. On the other hand, the serum potassium level showed a significant ($p < 0.001$) increase compared with normal rabbits Therefore, the results revealed that intramuscular injection of sulfadimidine to rabbits caused signs of nephrotoxicity manifested by significant increases in serum urea, creatinine and uric acid associated with decreases in serum levels of sodium and calcium, with increased serum potassium electrolytes. The diuretic effects of alcoholic and aqueous extracts of *Alhagi maurorum* was evaluated and noticed that increasing in urine volume and the sodium and potassium excretion rate and may due to it's higher phenolic and flavonoid which are responsible for and strong antioxidant activity (Parthasarathy et al., 2009)and according to (Faten and El-Khateeb, 2013) phytochemical tests of *Cymbopogon proximus* showed the presence of saponins, Flavonoids, glycosides and tannins may be a rationale for the use of the plant in medicine preparations.

To our knowledge this is the first paper to studying the nephroprotective and antiurolithiatic activity of *Cymbopogon proximus* and *A. maurorum* against sulfadimidine induced crystalluria in male rabbits and we hope we could focus the light on the proplem of sulfadimidine induced crystalluria in

male rabbits and the nephroprotective and antiurolithiatic activity of these important medicinal plants.

Conclusion

It could be concluded from the obtained results that ethanolic or water extract of *Cymbopogon proximus* and *Alhagi maurorum* in combination with sulfadimidine have a nephroprotective and antiurolithiatic effects against sulfadimidine induced urolithiasis and crystalluria in male rabbits. Therefore, after this study we advise to use ethanolic or water extracts of *Cymbopogon proximus* and *Alhagi maurorum* during long term treatment of sulfadimidine to avoid the proplem of crysalluria in rabbit.

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