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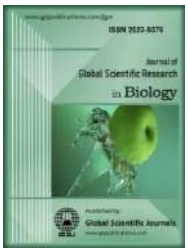

Effect of Boswellia Carterii gum Aqueous Extract on Experimental Pyelonephritis Caused by Escherichia Coli in Albino Rats

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ABSTRACT

The Therapeutic, nephrotoxic, and histological effects of Boswellia carterii gum aqueous extract, the infected rats challenged with experimental Escherichia coli UTI. were treated with 500mg/kg/bid B. carterii gum aqueous extract and Ciprofloxacin suspension with 7.5 mg/kg/bid, orally administered twice a day for five days. The animals were sacrificed on six day to determine bacterial count in kidney homogenates. Serum levels of urea, creatinine, alkaline phosphatase, and acid phosphatase. Where also evaluated to determine the nephrotoxicity potential of the extract is measured spectrophotometrically. The results of the treatment in animal models for E. coli UTI showed that the B. carterii gum aqueous extract produce a reduction in the bacterial load in kidneys homogenates by 17.17% compared with ciprofloxacin 33.5%. Nephrotoxicity studies showed evidence of nephrotoxic effect as acid phosphatase was increased after the treatment with B. carterii gum aqueous extract. A renal histological analysis indicates little histological changes.

1. Introduction

Urinary tract infections (UTI) are one of the most often seen bacterial infections in humans and represent a significant burden on health systems. More than 150 million physician visits worldwide have UTI infection. In United States, nearly 11 million physician visits were recorded, costing 6 billion USD per year (1). Most UTIs are not serious, but when kidney infection is present, they can cause damage to the kidney tissue (2).

Uropathogenic strains of Escherichia coli UPEC account for 30% of the nosocomial UTIs acquired and approx. 80% of the population acquired. UPEC can migrate from the colon to the urethra in children often because of the urinary tract blockages which result in pools of stagnant urine. The *E. coli* may have pili, which allow the bacteria to cling to the uroepithelial through enteric pathogens and then climb the bladder and kidney (3).

Medicinal plants are popular used due to their highly antibiotic resistance to UPEC and chronic UTIs (4,5,6), in the current study *Boswellia carterii* gum (Frankincense) or (olibanum), was chosen as it used to treat gastrointestinal, hormonal, and microbial diseases (7), diuretic, diarrhea, dysentery stomachic, cough, hemorrhage, urinary tubules piles, ulces, burns (8). It also have anti-

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inflammatory, antiseptic, antineurotic and analgesic effects (9).

Materials and Methods:

We used female rats from 6 to 8 weeks old and 150 ± 5 g in weight. The cage contained the five rats and was allowed to acclimate at least 24 hours before *E. coli* was inoculated. Free access to chow and water was permitted any time (10).

2. Experimental Design

Specimens of 20 rats were injected by E. Coli into the bladder to cause UTI and wait for 48 hours to guarantee the incidence, then treated for five days as shown in Table. (1), the rats were sacrificed to obtain kidneys and blood.

Table (1): Experimental Design

Groups	Number	Treatment
Group1: Zero	5	Zero
Group2: negative control (only bacteria)	5	1.5 ×10^9 *Escherichia coli*
Group3: Aqueous extract *Boswellia carterii* gum	5	500mg/kg animal body weight/ twice daily *Boswellia carterii* gum aqueous extract
Group4: Positive control (Ciprofloxacin)	5	7.5 mg/kg animal body weight/ twice daily Ciprofloxacin

***Bacteria*.** Ten *Escherichia coli* isolates were obtained from UTIs – patients attending Al-Zahrawy Hospital in Mosul, Iraq. They were identified according to the bacteriological and biochemical tests using Vitek 2 system, the most virulent isolate was chosen according to the results of the following two tests:

Test 1: Adhesion testing

Escherichia coli isolates were cultured in Nutrient broth and incubated overnight at 37℃. The cultures were centrifuged and resuspended in phosphate-buffered saline (PBS) PH 7.1. Uroepithelial cells were obtained from the sediment of a morning urine specimen from a healthy person without bacteriuria. They were washed and resuspended in PBS. To 10^5 epithelial cells were added 10^8 bacterial cells and PBS to a volume of 1ml. The mixture was incubated by rotation for 60 minutes at 37^0C, unattached bacteria were eliminated by repeated washing. For microscopic examination one drop of this suspension was transferred to a clean slide and stained with Giemsa stain, The number of bacteria adhering to epithelial cells was counted (11).

Test 2: Human serum bactericidal activity test

The Bacterial isolate transferred from stored slant to MacConkey agar and incubated overnight at 37℃ then three colonies were transferred to 5ml Nutrient broth and incubated overnight at 37℃ 1ml of the culture broth was transferred to 9ml Normal saline to obtained 10^{-1} dilution, this repeated three times to obtain the dilution 10^{-4}, 1ml of this dilution was transferred to 1ml of serum from healthy person and incubated for three hours after incubation, 10m1 of the mixture was transferred to the surface of Nutrient agar plate and spread using sterile L- shape rode, the plate was incubated overnight at 37℃, The colonies were counted on the plate and the findings interpreted according to : (12).

R (resistant): normal growth;
S (sensitive): ≤ 50 colonies;
I: intermediate growth

Inoculum Preparation:

The chosen isolate *E. coli* (isolate No.6) was transferred from Nutrient slant storage culture to MacConkey agar plates and incubated overnight at

37℃, after centrifugation at 2,000xg for 20 min, bacteria were resuspended in PBS to the concentration 1.51×10^9 CFU/ml as indicated by the OD at 597 nm. The exact concentration of bacteria in serial 10 fold dilution in PBS has been identified with viable counts (13).

Infection procedure (Ascending UTI):

Noting: the water dimension of the animals for 4 hours before and after injection.

The urinary bladders of the rats were emptied by a gentle bloating of the belly before infection. A urine drop was captured with a calibrated loop directly on the urethral orphan and applied to the MacConkey agar plate to measure the sterility. The animals were anesthetized with a mixture of ketamine hydrochloride and xylazine hydrochloride. A soft polyethylene catheter (external diameter 0.16 mm; Kebo Grave, Goteborg, Sweden) adjusted to 0.4 - 20mm needle on a tuberculin syringe was instilled into the urinary tract 50µL to inject prepared inoculum mentioned above (ASIK, Denmark).

The tubes were deliberately melted by gas flame until the ends were closed and flat, the tip must have been completely smooth to prevent damage to the tissue during inoculation with 50µL (14).

Bacteriology and Histopathology:

The animals were sacrificed by cardiac puncture under anesthesia with ketamine hydrochloride and xylazine hydrochloride, left kidneys were kept for histological examination while right kidneys were homogenized (automatic homogenization) with 1ml PBS, after serial dilutions to 10^{-6} of the homogenates, 1ml of each dilution was plated on MaCconkey agar to estimate the total number of bacteria in one gram of each kidney. Left kidneys were fixed with neutral formalin buffered 10%, embedded in paraffin, cut, and stained with heamatoxylin and eosin for histopathological analysis (HE) by light microscopy (15).

Nephrotoxicity Tests:

Blood Samples were collected from rats by capillary tubes in the inner corner orbital sinus puncture of the eye socket.

The blood was collected in anticoagulant – free tubes, the tubes were placed in a water bath for 10 minutes at 37℃, then centrifuged at 4000xg for 15 min (16). The serum was separated from the coagulated part, which must be non-decomposing, the serum was kept clear at -20℃, and the levels of urea, creatinine, and alkaline phosphatase were measured by Cobas C 311.

While the level of the acid phosphatase enzyme in the serum was measured by using the BioLabo kit (France).

Preparation of the crude aqueous extract:

Boswellia carterii gum were collected and dried in an electric oven at 40-45℃, milled with an electric grinder to obtain a fine powder, The powder was sieved through No.2 mesh, 100 grams of powder was suspend in 1L deionized water, leaved at 4℃ for 24 hours, then putted in a rotating flask in a water bath heated to 60℃ until a final volume of 100ml reached. The concentrated mixture was filtered using Whatman No.1 filterpaper with reduced pressure, lyophilized and kept in the refrigerator (17).

3. Results

Adhesion and serum susceptibility tests:

Ten *Escherichia coli* isolates were obtained from UTIs Patients attending Al-Zahrawy Hospital in Mosul, Iraq. Table (1) shows the results of the adhesion and serum susceptibility test of the *Escherichia coli* isolates according to the results isolate No.6 was selected.

Table 1: adhesion and serum susceptibility test results for *Escherichia coli* isolates

Isolate No	*Resistance to Serum Killing	**Adhesion to human uroepithelial cells
1	R	300
2	R	350
3	R	250
4	R	500
5	R	200
6	R	700
7	R	400
8	R	25
9	R	370
10	R	450

*R, Resistant: tested with human serum

**A, adhesion: the mean number of bacteria adhering to 40 epithelial cells was estimated for each isolate.

Recovery of bacteria from infected kidneys:

Treatment with *Boswellia carterii* gum aqueous extract reduced the CFU in kidneys by 17.17 % while treatment with Ciprofloxacin (positive control) decreased CFU in the kidneys by 33.5% (fig. 1).

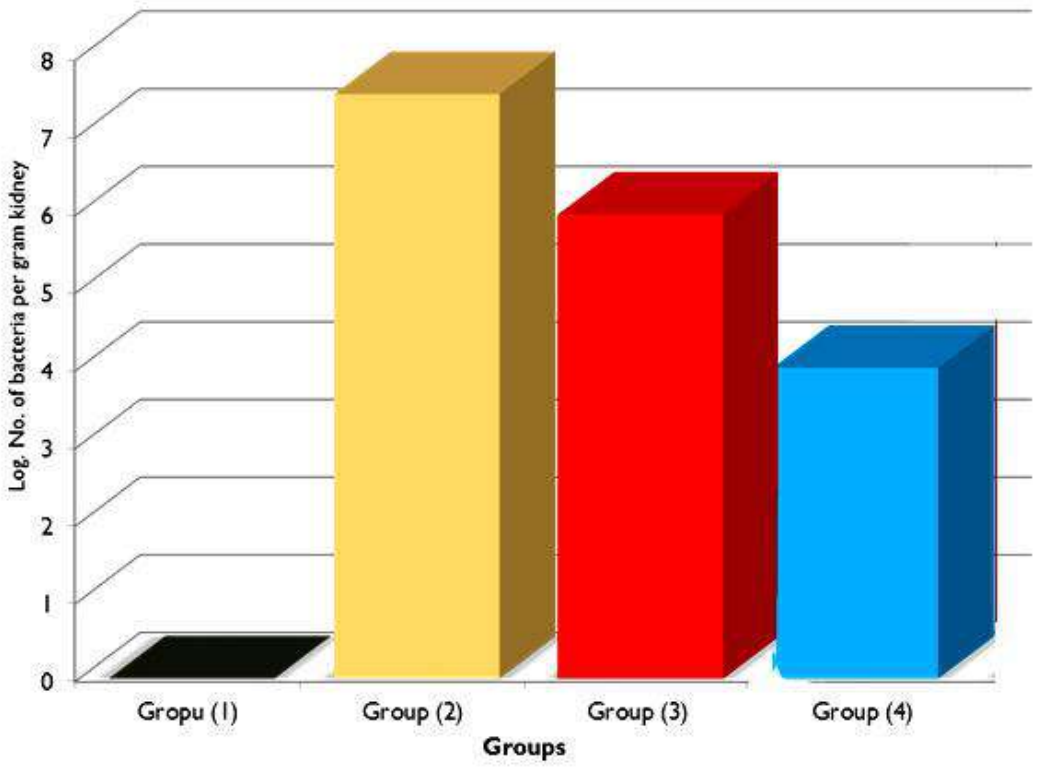

Fig.1: Bacterial counts in rats' kidneys after 7 days of infection, Group (1): control zero, Group (2): negative control (bacteria only without treatment), Group (3): *Boswellia carterii* gum aqueous extract, Group (4): positive control (Ciprofloxacin).

Kidney Functions:

The outcome of our nephrotoxicity studies revealed that the mean serum levels of urea and alkaline phosphatase were reduced after treatment with *Boswellia carterii* gum aqueous extract 500mg/kg/bid for 5 days (from 43.3 to 33.53 µmol/ L; and from 285.5 to 248.66 µmol/L respectively); while the mean serum levels of creatinine was decreased (from 0.30 to 0.263 µ mol/ L) while acid phosphatase was increased from 33.5 to 53.50 µ mol/ L), although the mean serum levels of acid phosphatase were increased in group 2 (bacteria only) from 33.5 to 42.533 µ mol/ L, in group 4 (Ciprofloxacin 7.5 mg/kg/bid for 5 day) from 33.5 to 37.566 µmol/ L.

Table.2: Blood Urea (Bu), Serum creatinine (Sc), Alkaline Phosphatase (ALP), and acid phosphatase (ACP) levels in rats after 5 days of treatment

Groups	**Blood Urea**	**Serum Creatinine**	**Serum Alkaline Phosphates**	**Serum Acid Phosphatase**
Group 1 control zero	43.3	0.30	285.5	33.5
Group 2 negative control (only bacteria)	43	0.3066	255	42.533
Group 3 *Boswellia carterii* gum aqueous extract	33.53	0.263	248.66	53.50
Group 4 positive control (Ciprofloxacin)	41.6	0.28	277.6	37.566

Histopathological Examination:

Group (1) Kidney

Kidney showed normal architecture of renal tissue characterized by glomeruli, proximal renal tubes, and distal renal tubules (figure 2).

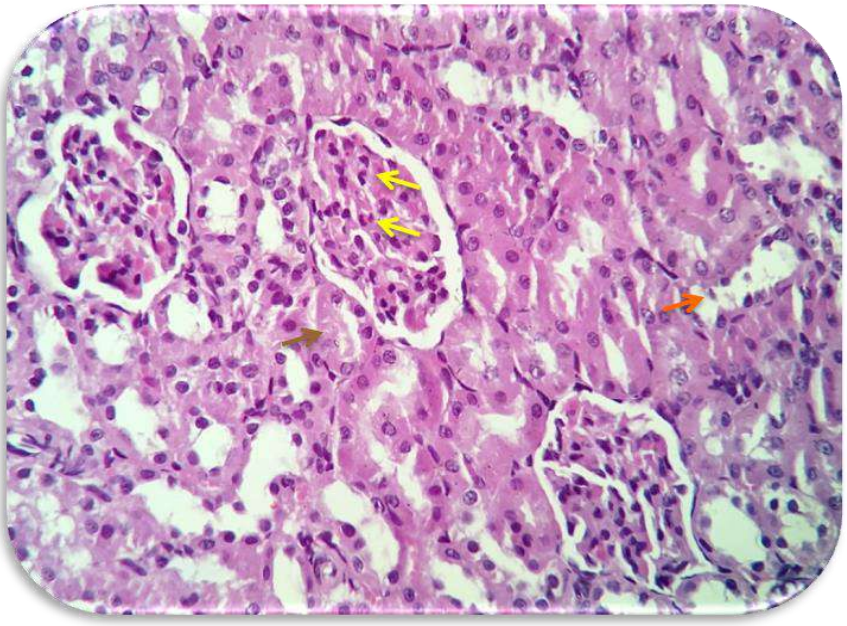

Fig.2: Photomicrograph of Kidney of Group (1) Show Normal Architecture of Renal Tissue Characterized by Glomeruli (Arrow Yellow), Proximal Renal Tubes (Arrow Brown), and Distal Renal Tubules (Arrow Orange). H & E Stain, 400X.

Group (2) kidney

The kidney showed inflammatory cell infiltration (fig. 3).

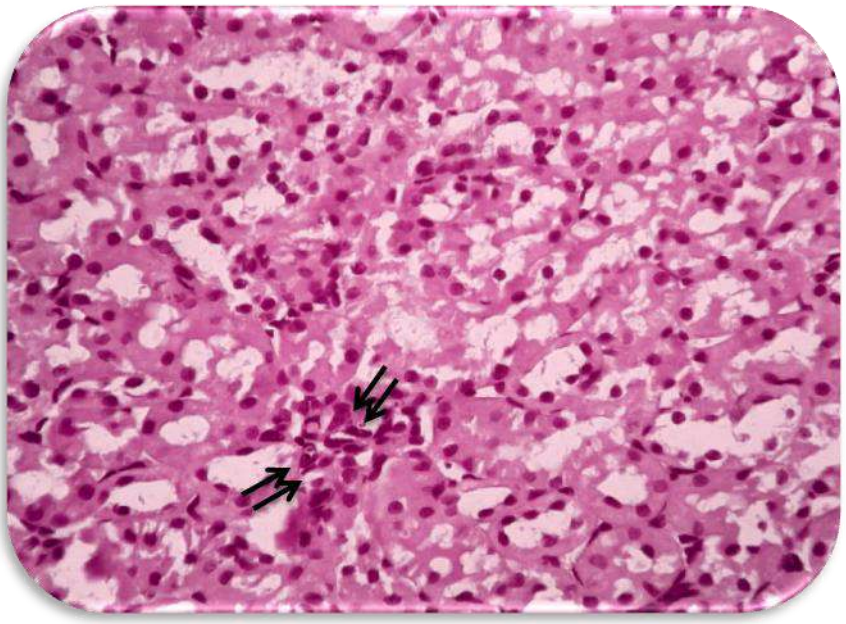

Fig.3 : Photomicrograph of Kidney of Group (2) shows Inflammatory cell infiltration in renal tubules (Black Arrow). H & E Stain, X 400.

Group (3) kidney

Renal structure with regular glomerular architecture, no apparent pathological alterations except for a few infiltrations of inflammatory cells (fig.4).

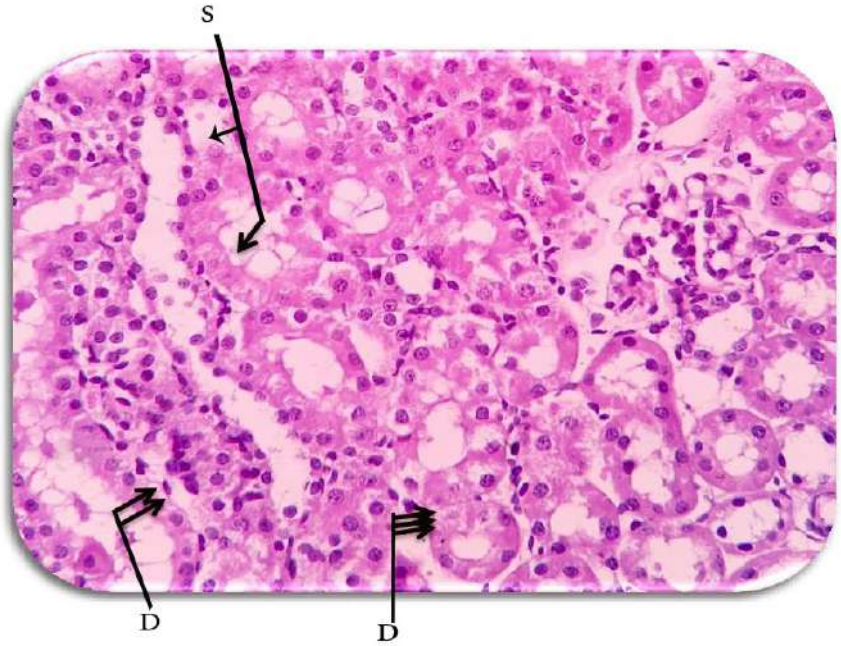

Fig. 4: Photomicrograph of Kidney of Group (3) shows swollen renal tubular epithelial cells (S) and degeneration (D) . H & E Stain, 400X.

Group (4) kidney

Kidney showed degeneration or cloud cell swelling of epithelium lining renal tubules (fig 5).

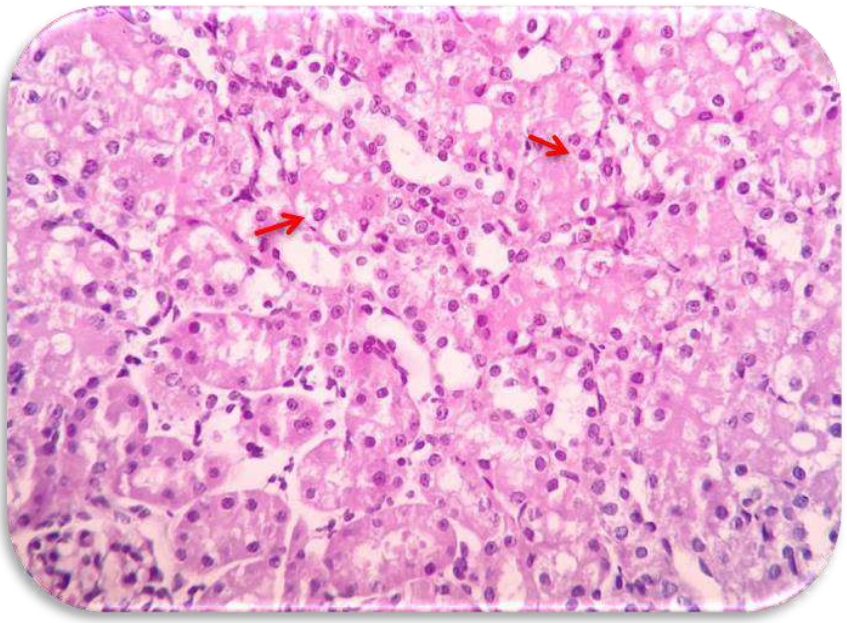

Fig.5: Photomicrograph of Kidney of Group (4) shows Cloudy swelling of renal tubular epithelial cells (Red Arrow). H & E Stain, 400X.

4. Discussion

A model for ascending UTI in rats was used to study the pathogenicity mechanisms of urinary bacteria without any mechanical stimulation or injury to the urinary system. Bacterial attachment is essential for the survival of bacterium, as seen in an accompanying document (18). Isolates that cause human pyelonephritis also contain certain forms of lipopolysaccharide and capsular polysaccharides at the same time that are immune to serum bactericide and attached to the uroepithelial cells (19).

As far as we know, this is the first study that employs *Boswellia carterii* gum aqueous extract at ascending UTI in rats model for treatment of antibacterial effect. Many studies found that *Boswellia carterii* gum extract is using Gram positive (*Bacillus subtilis, Staphylococcus aureus* and *Streptococcus pneumonia)* and Gram negative (*E.coli*, *Klebsiella pneumonia*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) microbes, beside there are studies found that *Boswellia carterii* gum aqueous extract has inhibitory activity against *Escherichia coli* in vitro (20).

There were many studies on nephrotoxicity had revealed a significant decrease in urea, creatinine, and alkaline phosphatase enzyme in their results, such as (21) study which found a significant decrease in urea and creatinine levels when giving 1 g/rabbet dose orally of *Boswellia carterii* gum for 10 days, act as prohibit calcium oxalate precipitation in kidney similar to diuretic drugs. Additionally, (22) explains how a variety of natural substances i.e. Oilfish, *boswellia carterii* grass, and curcumin powder, contributed in activating the kidney with renal failure. It was found that treatment with *boswellia carterii* grass had no significant impact on blood levels of creatinine, indicating that natural substances or extracts are ineffective in treating renal failure.

In addition, our findings are compatible with (23) argument that *Boswellia carterii* gum mechanism is similar to Zileuton drug when it is dosed to rats since it acts as an inhibitor of (Lox-S) lipoxygenase. Raise in acid phosphatase levels in rats treated with *Boswellia carterii* gum aqueous extract.

According to Buchele's findings that *Boswellia carterii* gum contains phenolic compounds as well as *boswellic* acids in its biochemical structure, the results demonstrate that *Boswellia carterii* gum possesses antibacterial activity materials (24). According to Lindequist and Muthana, the components of monoterpenes, diterpenes, and sesquiterpenes are given medicinal properties in *Boswellia carterii* oil (25). Another study proposed that *Boswellia carterii* gum include 24-cembrane A, incenole acetate, norusa-3, 12-dien-9 with antibacterial activity, since these materials are a part of volatile oils and this explain the use of *Boswellia carterii oil* in evaporation to sterilize contaminated areas. (26)

The study (27) show that frankincense and ginger were useful in treating severe kidney and renal failure, as the results of their study showed that frankincense caused a slight improvement in kidney function through its ability to reduce urea levels as well as the death of renal tube cells as there was no change in the levels of the enzyme LDH Lactate Dehydrogenase, an enzyme involved in metabolic processes. This enzyme is found in a high concentration in the kidneys, frankincense had an anti-infective effect by reducing the levels of C-reactive protein in the blood, these results indicated that *B. carterii* has a slightly beneficial effect in protecting against kidney failure, Also, according to the results of (27) the antiinflammation effect of frankincense may be attributed to the active compound 3-O-acetyl-11-keto-β-boswellic acid, which plays as a potent inhibitor of 5-Lipoxygenase (5-LOX), the major element in the biosynthesis of leukotrienes.

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