Targeting the antiurolithic activity by entrapping the bioactive compounds of *Asparagus racemosus* in chitosan nanoparticles on ethylene glycol induced renal calculi in Wister albino rats

Kishore Bandarapalle*1, Prasanna Raju Yalavarthi2, Chandra Sekhar Kothapalli Bannoth3

1Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Anantapur, Ananthapuramu-515002, Andhra Pradesh, India
2Department of Pharmaceutics, Sri Padmavathi School of Pharmacy, Tiruchanoor-517503, Andhra Pradesh, India
3Krishna University, Machilipatnam-521001, Andhra Pradesh, India

**Article History:**
Received on: 01 Jul 2020
Revised on: 15 Jul 2020
Accepted on: 10 Aug 2020

**Keywords:**
Antiurolithic activity, *Asparagus racemosus*, Chitosan nanoparticle, *In vivo* antioxidant parameters, Nanotechnology, serum parameters, urine parameters

**ABSTRACT**

The objective of our research is to investigate the antiurolithic intervention of bioactive compounds of *Asparagus racemosus* loaded Chitosan nanoparticles (BACARNPs) on ethylene glycol engendered renal calculi in male Wister rats. The efficiency of bioactive compounds of *A. racemosus* (BACAR) at 800 mg/kg p.o and BACARNPs at 800 mg equivalent weight of BACAR/kg p.o was validated in ethylene glycol 0.75% (v/v) and ammonium chloride 1% (w/v) mediated renal calculi in rats. Cystone (750 mg/kg, p.o.) has been used as a standard drug. Urinary variables comprise calcium, magnesium, oxalate, phosphate, uric acid, creatinine, urine pH, urine volume, and Creatinine clearance; Serum parameters include creatinine, blood urea nitrogen (BUN), calcium and uric acid; calcium and oxalate deposition in the kidney were assessed. *In vivo* antioxidant parameters include lipid peroxidation, superoxide dismutase, catalase, and glutathione were determined and histopathological studies were also examined. In both control groups, a substantial increase in urinary excretion of calcium, oxalate, and their intensification in the kidney; enhanced amounts of phosphate, uric acid, and reduced magnesium levels in urine; elevated serum creatinine, BUN, calcium and uric acid; Creatinine clearance was declined were observed and normalized in treated groups. In *in vivo* antioxidant parameters and histopathological variations reinstated to conventional form. Chitosan serves as a ligand to renal epithelial cells leads to improved agglomeration of BACAR in kidney compared to BACAR administered solitarily results in increased antiurolithic activity.

*Corresponding Author
Name: Kishore Bandarapalle
Phone: +91 7729072650
Email: kishore.brr89@gmail.com

ISSN: 0975-7538
DOI: [https://doi.org/10.26452/ijrps.v11i4.3237](https://doi.org/10.26452/ijrps.v11i4.3237)

© International Journal of Research in Pharmaceutical Sciences 2020 | All rights reserved.
tary events from supersaturation of urine, crystallization of constituents, aggregation, and retaining in the urine. The majority of the calculi i.e., 80% of calculi are of calcium oxalate or in collaboration with the calcium phosphate and 15% of calculi are of infection or struvite calculi and 5-10% are of uric acid calculi (Vyas et al., 2011). Investigation have also evident that the renal cell damage serves as a crystal binding site and encourages the emergence and aggregation of CaOx crystal in the renal tubular structures.

Multiple treatment strategies are employed to monitor urinary calculi conditions depends on the calculi size, location, severity of obstruction, kidney function. Extracorporeal shock wave lithotripsy, ureteroscopy, and percutaneous nephrolithotomy have often used methods for the revocation of calculi based on size. These procedures are relatively painful, expensive, increased rate of retreatment and cause undesirable consequences, namely tubular necrosis, hypertension, hemorrhage, and associated renal fibrosis, culminating in renal cell damage, as well as recurrence of calculi.

Treatment with synthetic drugs is based on the calculi type associated with complications and recurrence of calculi (Bergsland et al., 2013). These concepts failed to treat the disorder at the root level responsible for recurrence of the calculi in the future. Therefore, cavity was developed to fill in the interventions of calculi illness, which should meet the demands such as influencing the cause at the preliminary stage, limiting complications, lowering the incidence of recurrence, and cost effective.

In the present scenario in order to meet out the multiple objectives in calculi treatment, the priority is oriented on herbal remedies. There is a growing interest in the herbal products in the field of health care because of having confidence and firmly documented in the minds because they are secure, inexpensive, multiple pharmacological activities, lack of risks, and readily accessible. In the case of urolithiasis, they focus on the prevention of early stage calculi initiation by retrieval of severely injured renal cells to normal, which were adversely affected by oxidation of reactive oxygen species (ROS) and behave as a crystal binding sites leading to formation of calculi.

The antioxidant property of bioactive compounds in the extract is effective in preventing the cell injury and diuretic effect hinders the urinary supersaturation which is the key determinant in calculi initiation. Such outstanding features of herbal medication mitigate both the development and recurrence of calculi.

*A*par*agus race*m*osus* is typically known as Shatavari belongs to family Asparagaceae. In English: Wild asparagus, Telugu: Pili gaddalu; Gujarati: Satavari; Hindi: Satav, Satavari, Satmul; Kannada: Shatavari; Malayalam: Satavari; Marathi: Asvel, Shatmuli, Satavari; Oriya: Chhotaru, Mohajolo, Sotabori; Punjabi: Bozidan, Satawar; Tamil: Tannirvittan, Nirmittan, Ammaikodi; Sanskrit: Satamuli, Satapadi, Shatavari. It comprises of different phytochemical components, namely alkaloids, amino acids, proteins, steroids, saponins, flavonoids, tannins, phenols, and carbohydrates (Singh and Sinha, 2014).

Roots are the therapeutic component of the plant that is extensively used for anti-infective, antioxidant, anti-rheumatic, diuretic, hepatoprotective, antimicrobial, analgesic, antipyretic, anticancer, antidiabetic, and anti-inflammatory effects. Out of diverse phytochemical constituents the steroidal saponins are proved of comprising antioxidant, diuretic, and alkalizing effects which are potential therapeutic agents in urolithiasis (Joshi et al., 2010).

Bioavailability and targeting of desired bioactive compounds in the extract to the respective site is a crucial move to enhance the pharmacological action to the maximum. Hence recently, nanotechnology has drawn the attention of researchers to augment the treating efficiency by fabricating green nanoparticles entrapping bioactive compounds of extract essential for pharmacological action. In the current research to reinforce the antiurolithiatic therapy, BACAR was entrapped in Chitosan nanoparticles using low molecular weight Chitosan (40 KDa) which serves as a ligand to the megalin receptors on renal cells resulting in substantial accumulation in the kidney (Yuan et al., 2009).

**MATERIALS AND METHODS**

**Chemicals**

Analytical grade chemicals (Sigma Aldrich, Merck India Ltd., and Hi-media) procured from Bros Scientifics, Tirupati, India, were utilized in the current investigation. Cystone, (Himalaya Drug Company, Bangalore, India) was procured from the Apollo pharmacy, Tirupati.

**Collection and Preparation of aqueous Extract of Asparagus racemosus**

Roots of *Asparagus racemosus* were accessed from the Sri Srinivasa ayurvedic pharmacy, Tirupati. It was identified and authenticated by Dr. K. Madhava chetty, Assistant professor, Department of botany, Sri Venkateswara University, Tirupati. Voucher specimen (voucher No: 0698) were submitted to the research centre. The roots were dried and coarsely...
Table 1: Consequence of BACARNPs on Urine biochemical parameters

| Groups | Treatment          | Calcium (mg/dl) | Magnesium (mg/dl) | Oxalate (mg/dl) | Phosphate (mg/dl) | Uric acid (mg/dl) |
|--------|--------------------|-----------------|-------------------|-----------------|-------------------|------------------|
| I      | Normal             |                 |                   |                 |                   |                  |
|        | On day 15          | 6.63±0.55       | 6.20±0.30         | 3.42±0.27       | 5.42±0.27         | 1.88±0.24        |
|        | On day 30          | 6.39±0.3        | 6.04±0.40         | 3.22±0.35       | 4.99±0.19         | 2.07±0.16        |
| II     | Preventive control | 12.07±0.50\*    | 3.31±0.28\#       | 7.86±0.44\#     | 8.64±0.25\#       | 4.39±0.18\#      |
| III    | Preventive standard| 7.95±0.35\c     | 5.45±0.13\c       | 4.39±0.21\c     | 5.90±0.22\c       | 2.54±0.33\c      |
| IV     | BACAR              | 9.00±1.10\b     | 5.11±0.30\c       | 5.36±0.73\b     | 6.17±0.85\b       | 3.32±0.14\b      |
| V      | BACARNPs           | 6.74±0.23\c     | 5.98±0.18\c       | 3.94±0.26\c     | 5.54±0.19\c       | 2.29±0.17\c      |
| VI     | Curative control   | 11.72±0.47\#    | 3.17±0.31\#       | 7.43±0.37\#     | 8.97±0.20\#       | 4.72±0.25\#      |
| VII    | Curative standard  | 7.67±0.19\f     | 5.38±0.19\f       | 4.02±0.10\f     | 5.82±0.20\e       | 3.14±0.14\f      |
| VIII   | BACAR              | 8.34±0.46\f     | 4.98±0.42\e       | 4.84±0.85\e     | 6.02±1.15\e       | 3.68±0.17\e      |
| IX     | BACARNPs           | 6.98±0.18\f     | 5.95±0.16\f       | 3.46±0.10\f     | 5.22±0.11\f       | 2.53±0.14\f      |

\*P<0.001 when compared to Group I; \*P<0.05, \#P<0.01, \fP<0.001 when compared to Group II; \*P<0.05, \#P<0.01, \fP<0.001 when compared to Group VI

Table 2: Consequence of BACARNPs on urine volume and creatinine clearance

| Groups | Treatment          | urine volume (ml) | Creatinine (ml/min) | Clearance (ml/min) |
|--------|--------------------|-------------------|---------------------|-------------------|
| I      | Normal             |                   |                     |                   |
|        | On day 15          | 12.66±0.34        | 0.085±0.002         |                   |
|        | On day 30          | 12.28±0.31        | 0.078±0.003         |                   |
| II     | Preventive control | 6.23±0.30\#       | 0.013±0.002\#       |                   |
| III    | Preventive standard| 10.02±0.44\c      | 0.061±0.004\c       |                   |
| IV     | BACAR              | 9.24±1.04\b       | 0.052±0.003\c       |                   |
| V      | BACARNPs           | 12.27±0.52\c      | 0.065±0.003\c       |                   |
| VI     | Curative control   | 6.52±0.36\#       | 0.015±0.002\#       |                   |
| VII    | Curative standard  | 11.39±0.65\f      | 0.065±0.003\f       |                   |
| VIII   | BACAR              | 9.87±0.79\d       | 0.051±0.003\f       |                   |
| IX     | BACARNPs           | 12.09±1.17\f      | 0.068±0.004\f       |                   |

\*P<0.001 when compared to Group I; \*P<0.05, \#P<0.01, \fP<0.001 when compared to Group II; \*P<0.05, \#P<0.01, \fP<0.001 when compared to Group VI

Preliminary Phytochemical Screening

BACAR was preliminary screened for the availability of alkaloids, glycosides, terpenes, anthraquinones, tannins, saponins, phenolic compounds, flavonoids, sterols, and carbohydrates using standard procedures.

Preparation of Bioactive compounds of Asparagus racemosus loaded chitosan nanoparticles (BACARNPs)

The BACAR was entrapped in Chitosan nanoparticles by employing the ionic gelation method. The optimization of BACARNPs is accomplished with the use of Box Behnken Design (BBD). BACARNPs were dispensed at a dose equivalent to 800 mg of BACAR.
Table 3: Consequence of BACARNPs on serum biochemical parameters

| Groups | Treatment          | Creatinine (mg/dl) | Blood urea Nitrogen (mg/dl) | Calcium (mg/dl) | Uric acid (mg/dl) |
|--------|--------------------|--------------------|-----------------------------|-----------------|-------------------|
| I      | Normal             |                    |                             |                 |                   |
|        | On day 15          | 0.58±0.04          | 21.43±0.95                  | 7.74±0.35       | 2.43±0.18         |
|        | On day 30          | 0.62±0.05          | 21.14±0.81                  | 7.65±0.27       | 2.63±0.38         |
| II     | Preventive control | 1.72±0.05          | 44.64±1.14                  | 11.6±0.54       | 7.17±0.38         |
| III    | Preventive standard| 0.89±0.22          | 27.50±1.23                  | 8.49±0.27       | 3.50±0.19         |
| IV     | BACAR              | 1.03±0.27          | 30.57±1.57                  | 8.95±0.84       | 4.14±0.16         |
| V      | BACARNPs           | 0.61±0.01          | 23.51±0.98                  | 7.97±0.076      | 2.88±0.23         |
| VI     | Curative control   | 1.57±0.19          | 39.60±0.99                  | 11.09±0.46      | 6.73±0.42         |
| VII    | Curative standard  | 0.79±0.04          | 25.44±1.13                  | 8.18±0.12       | 3.26±0.15         |
| VIII   | BACAR              | 0.98±0.19          | 30.21±1.23                  | 8.99±0.60       | 3.83±0.23         |
| IX     | BACARNPs           | 0.65±0.02          | 22.93±2.35                  | 7.89±0.12       | 2.8±0.29          |

*P<0.001 when compared to Group I; *P<0.05, #P<0.01, †P<0.001 when compared to Group II; *P<0.05, †P<0.01, ‡P<0.001 when compared to Group VI

Table 4: Consequence of BACARNPs on kidney weight and calcium and oxalate deposition in kidney

| Groups   | Treatment          | Wet kidney weight (g/100 g) body weight | Calcium (mg/g) of kidney | Oxalate (mg/g) of kidney |
|----------|--------------------|----------------------------------------|--------------------------|-------------------------|
| I        | Normal             | 0.43±0.02                              | 2.87±0.26                | 2.18±0.14               |
| II       | Preventive control | 0.81±0.04†                             | 6.41±0.30‡               | 5.42±0.22‡              |
| III      | Preventive standard| 0.52±0.04c                            | 3.90±0.23c               | 3.25±0.18c              |
| IV       | BACAR              | 0.55±0.08b                            | 4.57±0.55b               | 3.79±0.56b              |
| V        | BACARNPs           | 0.47±0.02c                            | 3.48±0.29c               | 2.79±0.14c              |
| VI       | Curative control   | 0.89±0.04f                            | 7.60±0.29f               | 6.00±0.29f              |
| VII      | Curative standard  | 0.57±0.03f                            | 4.83±0.18f               | 3.85±0.18f              |
| VIII     | BACAR              | 0.61±0.09c                            | 5.09±0.54f               | 4.24±0.50c              |
| IX       | BACARNPs           | 0.51±0.02f                            | 4.16±0.39f               | 3.32±0.20f              |

*P<0.001 when compared to Group I; *P<0.05, #P<0.01, †P<0.001 when compared to Group II; *P<0.05, †P<0.01, ‡P<0.001 when compared to Group VI

Experimental Animals

Male albino Wister healthy rats weighing about 200 to 225 g purchased from Sri Venkateswara Enterprises, Bengaluru. Animals were habituated to standard laboratory circumstances, supplemented with standard rat food pellets, and drinking water ad libitum. Animal surveillance and experimental protocols were following the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was authorized by the Institutional Animal Ethical Committee (IAEC) with the approval No. 1016/PO/Re/S/06/IAEC/2019/013.

Acute Toxicity Studies

Oral acute toxicity studies were accomplished in following the OECD guidelines 423.

Experimental Design

Rats were categorized into nine groups of six animals each (n = 6). In rats, CaOx calculi were instigated by administering the drinking water consisting of 0.75% (v/v) of ethylene glycol (EG) and by suspending in 2 ml of water by orally.
Table 5: Consequence of BACARNPs on in vivo antioxidant parameters

| Groups   | Treatment                  | LPO (µM/mg of tissue) | SOD (µM/mg of tissue) | CAT (µM/mg of tissue) | GSH (µM/mg of tissue) |
|----------|----------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| I        | Normal                     | 0.43±0.03             | 7.38±0.40             | 0.088±0.002           | 1.90±0.13             |
| II       | Preventive control         | 6.50±0.11*            | 3.41±0.14*            | 0.035±0.005*          | 0.68±0.03*            |
| III      | Preventive standard        | 1.42±0.22c            | 6.38±0.69b            | 0.073±0.005c          | 1.23±0.06c            |
| IV       | BACAR                      | 2.33±0.06c            | 5.39±0.64a            | 0.062±0.005b          | 1.06±0.06b            |
| V        | BACARNPs                   | 0.74±0.053c           | 6.96±0.5c             | 0.083±0.005c          | 1.60±0.07c            |
| VI       | Curative control           | 5.96±0.62a            | 3.06±0.25a            | 0.042±0.004b          | 0.63±0.03a            |
| VII      | Curative standard          | 1.35±0.20f            | 6.34±0.36f            | 0.077±0.003f          | 1.16±0.04f            |
| VIII     | BACAR                      | 2.03±0.25f            | 5.33±0.60e            | 0.060±0.008e          | 0.94±0.06e            |
| IX       | BACARNPs                   | 0.88±0.08f            | 6.88±0.24f            | 0.081±0.003f          | 1.65±0.06f            |

*P<0.001 when compared to Group I; *P<0.05, *P<0.01, *P<0.001 when compared to Group II; *P<0.05, *P<0.01, *P<0.001 when compared to Group VI

1 % (w/v) of ammonium chloride (AC) for 15 days. The treatment protocol was designed as follows, (Atmani et al., 2003).

**Group I**
Normal (untreated)

**Group II**
Preventive control (EG + AC + vehicle from day 1 to 15)

**Group III**
Preventive standard (EG + AC + cystone 750 mg/kg, orally from day 1 to 15)

**Group IV**
Preventive BACAR (EG + AC + BACAR 800 mg/kg, orally from day 1 to 15)

**Group V**
Preventive BACARNPs (EG + AC + BACARNPs Equivalent of 800 mg BACAR/kg, orally from day 1 to 15)

**Group VI**
Curative control (EG + AC from day 1 to 15, vehicle from day 16 to 30)

**Group VII**
Curative standard (EG + AC from day 1 to 15; cystone 750 mg/kg, orally from day 16 to 30)

**Group VIII**
Curative BACAR (EG + AC from day 1 to 15, BACAR 800 mg/kg, orally from day 16 to 30)

**Group IX**
Curative BACARNPs (EG + AC from day 1 to 15, BACARNPs Equivalent of 800 mg/kg, orally from day 16 to 30)

Biochemical examination in urine and serum

Urine and blood samples were extracted from all the groups of animals after termination of respective prescribed treatment schedules. Rats were hydrated orally with 6 ml of drinking water, placed in discrete metabolic cages and collected urine for 24 hours. The volume of urine was measured, and pH was explored. Urine was centrifuged at 2,500 rpm at 30 ± 2°C for 5 min, and the supernatant was estimated for calcium, magnesium, phosphate, uric acid, using ERBA diagnostic kits. Oxalate was quantified using the method of Hodgkinson and Williams, 1972 (Hodgkinson and Williams, 1972).

Blood samples from the retro-orbital venous plexus were retrieved and serum was isolated by centrifuging at 1500 rpm for 15 min and used to validate creatinine, blood urea nitrogen (BUN), calcium, and uric acid using ERBA diagnostic kits. Semi Autoanlyser (Mispa excel, version: 1.4) was used for estimation of parameters. Creatinine clearance was considered as a renal function test and was computed using the formula (Cockcroft and Gault, 1976).

Creatinine clearance (ml / min)

\[
\text{Creatinine clearance (ml / min)} = \frac{\text{(mg creatinine / dl urine)} \times (24 \text{ h urine output (ml)})}{\text{(mg creatinine / dl serum)} \times 1440}
\]

Kidney samples for in vivo antioxidant and histopathological studies
After the finished collection of urine and blood samples, rats were sacrificed by cervical decapitation; kidneys were attentively isolated and perfused with ice-cold saline. From each animal one kidney was meant for the determination of calcium and oxalate, in vivo antioxidant investigation includes lipid peroxidation represented as malondialdehyde (MDA) levels (Niehaus and Samuelsson, 1968), superoxide dismutase (SOD) (Misra and Fridovich, 1972), catalase (CAT) (Aebi and Catalase, 1984), and reduced glutathione (GSH) (Jollow et al., 1974). Another kidney was positioned in a 10% buffered formalin solution (pH 7.4) and polished with paraffin wax. The sections were processed employing a microtome and pigmented with hematoxylin and eosin and then inspected under a light microscope for renal impairment.

Statistical Analysis
The data acquired were analyzed by one-way ANOVA accompanied by Dunnettes multiple comparison test using Graph Pad Prism (version 5.0) p<0.05 was considered to be statistically significant. The values represented as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION
Phytochemical examination confirmed the existence of alkaloids, glycosides, terpenes, anthraquinones, tannins, saponins, phenolic compounds, flavonoids, sterols, and carbohydrates in the aqueous extract of Asparagus racemosus.

BACAR has been found to be safe in the acute oral toxicity studies, and has caused no mortality of up to 32000 mg/kg. The previous study manifests that BACAR exhibit antiurithiatic behaviour at a dose of 800 mg/kg (Jagannath et al., 2015). Therefore, 800 mg/kg of BACAR were adopted for the present research.

Urinary Biochemical Parameters
On the ingestion of EG and AC in both control groups (II and VI) animals a significant escalation of calcium, oxalate, phosphate, and uric acid levels and decline magnesium levels was reported when compared to the normal group (I). These results confirmed the formation of CaOx calculi triggered by EG and AC. An appreciable declined in calcium, oxalate, phosphate, uric acid levels, and improved magnesium levels were evidenced on treatment with cystone, BACAR, and BACARNPs, in the preventive (III, IV, and V) and curative (VII, VIII, and IX) treated groups demonstrated the preventive and curative effect on CaOx calculi (Karadi et al., 2006; Grases et al., 1989), (Table 1).

Standard urinary pH ranged approximately 6.0 to 7.0 in normal group (I) animals. Once CaOx calculi was installed, the pH in both control groups (II and VI) decreased dramatically to 5.0-6.0 relative to normal. The urine pH was significantly switched back normal for cystone, BACAR and BACARNPs treatment in both treated regimens owing to the alkalinizing effect of BACAR (King, 1967).

A significant decreased output of urine is discovered in both control groups (II and VI) is observed due to obstruction in urinary flow by the CaOx calculi as well as impairment of renal function unit responsible for the production of urine. Cystone, BACAR & BACARNPs therapy results in a substantial improvement in urinary ability in both treated group animals with the effect of preventing and curing of renal cell injury and the diuretic impact of BACAR (Table 2).

A significant decline in creatinine clearance was detected in the control groups (II and VI) and improved in the groups treated with cystone, BACAR & BACARNPs in both treated regimens (Table 2). It confirmed the increased effectiveness of BACAR loading in chitosan nanoparticles (BACARNPs) over urine biochemical studies, urine pH and urine volume in comparison with BACAR alone and standard cystone.

Serum Biochemical Parameters
A consequential increased levels of creatinine, BUN, calcium and uric acid were reported when EG and AC were administered in both control groups (II and VI) relative to the normal group (I). This was witnessed by a reduction in glomerular filtration rate in reaction to calculi blockade in bowman’s capsule which contributes to an accumulation of nitrogenous waste in the blood. Treatment with cystone, BACAR and BACARNPs in both treated regimens substantially inverted the concentrations of creatinine, BUN, calcium, and uric acid levels to usual in addition to enhanced glomerular filtration efficiency due to the BACAR antioxidant activity. The antioxidant activity safeguards the renal cell damage due to reactive oxygen species (ROS) supposed to act as a calculi established site (Rathod et al., 2012), (Table 3).

BACARNPs establish evidence of improving the serum parameters in comparison to BACAR administered alone and cystone due to increased deposition of BACAR in the kidneys.

Wet kidney weight and deposition of Calcium and Oxalate in the Kidney
A significant increase in the retention of calcium and oxalate levels in the kidney after consuming
drinking water comprising EG and AC for 15 days, in both control groups (II and VI) relative to the normal group (I). A remarkable drop in calcium and oxalate amounts was witnessed in both treated group animals administered with cystone, BACAR, and BACARNPs (Patel et al., 2012). In response to that significant increased wet kidney weight in control groups (II and VI) and significant decreased in treated groups was noticed (Table 4). Among the treated groups the BACARNPs extremely effective in declining the calcium and oxalate dumping due to the enhanced accumulation of BACAR in the kidneys with antioxidant and hypocalciuric effects.

**In Vivo Antioxidant Parameters**

In both control groups (II and VI) an extraordinary rise in MDA levels through an enhanced lipid peroxidation process was reported when EG and AC administered relative to the normal group (I). This is further evidenced by earlier studies that exposure to oxalate and CaOx crystals results in renal cell injury by intracellular ROS generation triggered by membrane lipid peroxidation (Namburu et al., 2017). Cystone, BACAR, and BACARNPs treatment effectively lowered MDA levels by obstructing the lipid peroxidation by antioxidant activity in both treated groups. A consequential depletion in the levels of enzymatic antioxidants SOD and CAT and non-enzymatic antioxidant GSH levels were noticed in both control groups (II and VI) when compared to the normal group (I). Such decreased levels weaken antioxidant defense against ROS, which may have also facilitated the aggregation and retention of oxalate and eventual dumping of CaOx calculi. The antioxidant activity of the cystone, BACAR, and BACARNPs in both treated regimens improved these.
enzyme levels to normal (Khan, 2014; Selvam, 2002), (Table 5).

A significant refinement of In vivo antioxidant parameters in BACARNPs was noticed among the treated regimens by increased deposition of BACAR due to appropriate renal targeting.

**Histopathological Studies**

Histological examination revealed glomerular atrophy, dilation of renal tubules, and necrotic changes in control groups (II and VI). These astonishing histological changes might be attributed to oxalate induced lipid peroxidation. Our results stated that treatment with cystone, BACAR & BACARNPs has both defensive and preventive effects on the intratubular crystal deposition in both treated groups leading to a substantial reduction in the renal tissue damage index (Figure 1). In figure A represents to histopathology of normal, B and F represents the preventive and curative control respectively, C, D, and E, and G, H, and I represents the preventive and curative cystone, BACAR, and BACARNPs respectively.

The BACARNPs treated group of animals recovered predominantly from histological changes over the rest of the treated groups.

**CONCLUSIONS**

In the present study, the findings, specially demonstrated the effectiveness of nanotechnology in enhancing the antiurolithiatic efficacy of BACAR loaded in Chitosan nanoparticles by targeting the kidneys. As a result of amplified deposition of BACAR in the kidneys, the antiurolithiatic activity of BACARNPs has been shown to be predominantly effective over both BACAR alone and as well as standard cystone.

**ACKNOWLEDGEMENT**

The first author desires to explicit utmost gratitude to the Management and Dr. D. Ranganayakulu, M. Pharm., Ph.D., Principal, Sri padmavathi school of pharmacy, Tiruchanoor, Andhra Pradesh, India, for presenting all the necessary laboratory demands of the research and constant support. The author is obliged to Dr. C. Sridhar, M. Pharm., Ph.D., Professor, Dept. of Pharmaceutical analysis, Sri padmavathi school of pharmacy, Tiruchanoor, Andhra Pradesh, India, for their valuable help and support.

**Funding Support**

The authors declare that they have no funding support for this study.

**Ethical approval**

Endorsed by the IAEC (Institutional Animal Ethics Committee).

**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

**REFERENCES**

Aebi, H., Catalase 1984. Methods of Enzymatic Analysis. pages 673–680, New York and London. Academic Press.

Atmani, F., Slimani, Y., Mimouni, M., Hacht, B. 2003. Prophylaxis of calcium oxalate stones by Herniaria hirsuta on experimentally induced nephrolithiasis in rats. *BJU International*, 92(1):137–140.

Bergsland, K. J., Worcester, E. M., Coe, F. L. 2013. Role of proximal tubule in the hypocalciuric response to thiazide of patients with idiopathic hypercalciuria. *American Journal of Physiology-Renal Physiology*, 305(4):F592–F599.

Cockcroft, D. W., Gault, H. 1976. Prediction of Creatinine Clearance from Serum Creatinine. *Nephron*, 16(1):31–41.

Ghosh, R. B., Sur, T. K., Maity, L. N., Chakraborty, S. C. 2000. Antiurolithiatic activity of coeleus Aromatarius Benth. In Rats. *Anc Sci Life*, 20(1-2):44–47.

Grases, F., Genestar, C., Conte, A., March, P., Costabauza, A. 1989. Inhibitory Effect of Pyrophosphate, Citrate, Magnesium and Chondroitin Sulphate in Calcium Oxalate Urolithiasis. *British Journal of Urology*, 64(3):235–237.

Hodgkinson, A., Williams, A. 1972. An improved colorimetric procedure for urine oxalate. *Clinica Chimica Acta*, 36(1):127–132.

Jagannath, N., Dass, A. P., Ahmed, K. 2015. A study Antiurolithiatic Activity of ethanolic extract of Asparagus racemosus in animal models. *Int J Pharm Res*, 5(11):316–335.

Jollow, D. J., Mitchell, J. R., Zampaglione, N., Gillette, J. R. 1974. Bromobenzene-Induced Liver Necrosis. Protective Role of Glutathione and Evidence for 3,4-Bromobenzene Oxide as the Hepatotoxic Metabolite. *Pharmacology*, 11(3):151–169.

Joshi, G. P., Rawat, M. S., Bisht, V. K., Negi, J. S., Singh, P. 2010. Chemical constituents of Asparagus. *Pharmacognosy Reviews*, 4(8):215–215.

Karadi, R. V., Gadge, N. B., Alagawadi, K. R., Savadi, R. V. 2006. Effect of Moringa oleifera Lam. root-wood on ethylene glycol induced urolithiasis in rats. *Journal of Ethnopharmacology*, 105(1-2):306–311.

Khan, S. R. 2014. Reactive oxygen species, inflam-
mation and calcium oxalate nephrolithiasis. *Transl Androl Urol*, 3(3):256–76.

King, J. S. 1967. Etiologic Factors Involved in Urolithiasis: A Review of Recent Research. *Journal of Urology*, 97(4):583–591.

Misra, H. P., Fridovich, I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*, 247(10):3170–3175.

Namburu, S. S. L., Dodoala, Koganti, K. V. S. R. G. B., Prasad 2017. Antiurolithiatic activity of Phaseolus vulgaris seeds against ethylene glycol-induced renal calculi in Wistar rats. *Int J Green Pharm*, 11(4):281–289.

Niehaus, W. G., Samuelsson, B. 1968. Formation of Malonaldehyde from Phospholipid Arachidonate during Microsomal Lipid Peroxidation. *European Journal of Biochemistry*, 6(1):126–130.

Patel, P. K., Patel, M. A., Vyas, B. A., Shah, D. R., Gandhi, T. R. 2012. Antiurolithiatic activity of saponin rich fraction from the fruits of Solanum xanthocarpum Schrad. & Wendl. (Solanaceae) against ethylene glycol induced urolithiasis in rats. *Journal of Ethnopharmacology*, 144(1):160–170.

Rathod, N. R., Biswas, D., Chitme, H. R., Ratna, S., Muchandi, I. S., Chandra, R. 2012. Anti-urolithiatic effects of Punica granatum in male rats. *Journal of Ethnopharmacology*, 140(2):234–238.

Selvam, R. 2002. Calcium oxalate stone disease: role of lipid peroxidation and antioxidants. *Urological Research*, 30(1):35–47.

Singh, A., Sinha, B. 2014. *Asparagus racemosus* and its phytoconstituents; an updated review. *Asian J. Biochem. Pharm. Res*, 4(4):230–240.

Vyas, B. A., Vyas, R. B., Joshi, S. V., Santani, D. D. 2011. Antiurolithiatic Activity of Whole-Plant Hydroalcoholic Extract of Pergularia daemia in Rats. *Journal of Young Pharmacists*, 3(1):36–40.

Yuan, Z., Zhang, Z., Zhu, D., Sun, X., Gong, T., Liu, J., Luan, C. 2009. Specific Renal Uptake of Randomly 50% N-Acetylated Low Molecular Weight Chitosan. *Molecular Pharmaceutics*, 6(1):305–314.