Indian Journal of Natural Products and Resources
Vol. 9(2), June 2018, pp. 117-125

Anti-nephrolithiatic potential and the protective role of saponin-rich extract of *Dianthus basuticus* against acetaminophen-induced damage in HEK293 cells

Mikhail Olugbemiro Nafiu, Anofi Omotayo Tom Ashafa* and Saheed Sabiu

Phytomedicine and Phytopharmacology Research Group, Department of Plant Sciences, University of the Free State, Qwaqwa Campus, Private bag x 13, Phuthaditjhaba 9866, South Africa

Received 2017; Revised 9 April 2018

Saponins have found ethnomedicinal applications against a number of diseases including oxidative stress, urinary and kidney ailments. This study investigated antioxidant, anti-nephrolithiatic and nephroprotective properties of saponin-rich extract of *Dianthus basuticus in vitro*. While nephroprotective activity was demonstrated against acetaminophen-mediated toxicity in HEK293 cells using the MTT method, its inhibition of calcium oxalate (CaOx) nucleation was evaluated in the anti-nephrolithiatic study. The antioxidant effect was demonstrated against OH and lipid peroxide radicals. The significant (p <0.05) acetaminophen-induced reduction of viable cells in the nephrotoxic cells following 24 hours incubation was reverted and cell mortality dropped from 81.98 to 13.15 % at 125 µg/mL dose of the extract. The extract significantly inhibited CaOx nucleation crystals in a dose-related manner with the highest dose being 51.11 % potent. The inhibitory effect of the extract had an overall IC_{50} of 1.03 µg/mL (R^2= 0.9698) which is corroborated by the degree of turbidity of the treated crystals solutions. The overall effects elicited by the extract may be attributed to its antioxidant activity which was concentration-related with the highest dose exhibiting the most potent effect (IC_{50}: 0.45–0.63 µg/mL) when compared with vitamin C (IC_{50}: 0.49–0.69 mg/mL). Hence, *D. basuticus* is potentially a new lead agent in the management of nephrolithiasis and renal disorders.

Keywords: Calcium oxalate, *Dianthus basuticus*, HEK293 cell, Hoechst 33342, MTT, Nephrolithiasis.

IPC code; Int. cl. (2015.01)– A61K 36/00, 36/36; A61P 13/00, 39/00

Introduction

The kidneys receive approximately 25 % of cardiac output and as key excretory and xenobiotic metabolizing organs, are naturally vulnerable to developing injuries resulting from exposure to circulating drugs and chemicals[1]. Renal toxicity has been reported for diverse agents ranging from microbial, heavy metals, chemicals to drugs (sulfonamides, aminoglycosides and non-steroidal

Although the most common manifestation of toxicity is renal failure, the cellular and subcellular targets of toxicity and molecular mechanisms of toxicity varies from agent to agent. For instance, acetaminophen (APAP) nephrotoxicity has been well studied and is characterized by morphologic and functional evidence of proximal tubular injury in humans and experimental animals[3]. Since proximal tubules are the most common site of injury by drugs, screening and understanding the proximal tubule toxicity effect of drugs is critical in drug evaluation. Information on mechanisms of toxicity will further guide structure-activity relationships and minimize risks of clinical renal damage. The use of cells derived from proximal tubules of kidneys like HEK293 is one of the *in vitro* approaches of screening in cytotoxicity assays.

Besides renal toxicity, nephrolithiasis (kidney stones) is another challenge consistent with the formation of small, hard, crystalline deposits of mineral and acid salts within the kidney. It is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical, malnutrition (hyperuricosuria), poor diet and genetic risk factors[4]. Essentially, varying degrees of calcification (in the form of either calcium oxalate (CaOx) or calcium phosphate) in the kidney, bladder, or urethra are its common features[5]. It afflicts both genders but the risk is three times higher in men than women, because of the enhancing capacity of testosterone and the inhibiting capacity of estrogen in stone formation[6].

*Correspondent author
Email: ashafaAOT@ufs.ac.za

Reports have also estimated 1 in every 20 individuals as being affected by kidney stones at some point in their life[5,7]. Globally, dietary modification coupled with surgical methods, extracorporeal lithotripsy, and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are expensive and recurrence is also common[8]. Furthermore, the recurrence rate without preventive treatment ranges from 15-50 % within 1 to 10 yrs, respectively[9]. Various therapies are being used to prevent recurrence, however scientific evidence for their efficacy, expected to be nearly 50 %, is still less convincing[7,10].

In the traditional systems of medicine, saponin-rich plants are of overall pharmacological importance. Additionally, they are very potent in stemming the recurrence rate of renal calculi with minimal side effects[11]. *Dianthus basuticus* is one of the saponin-rich botanicals that have been well advocated in this regard.

D. basuticus, commonly called Lesotho carnation, are evergreen soft-wooded perennials with bright pink flowers and deeply fringed. It is native to the Drakensberg Mountains in South Africa and also found in KwaZulu Natal, Lesotho, Cape Province, Free State and Northern Provinces (grassland and rocks)[12]. The decoction from its root is used as a blood cleanser and to boost fertility in bulls[13]. The root is chewed as a charm to retain a loved one's affection, and it is rich in alkaloids, tannins, cardiac glycosides and saponins[14]. The plant has found pharmacological significance as antioxidant, antidiabetic, cytotoxic and antimicrobial agent[14,15]. Extracts of *D. basuticus* have also been scientifically documented to be non-toxic and practically safe for consumption[16].

In view of the saponin-rich nature of *D. basuticus* and coupled with its ethnomedicinal uses in the management of renal dysfunctions by the Basotho of the Free State Province, this study investigated the antioxidant and anti-nephrolithiatic activity of its saponin-rich extract via *in vitro* approaches. The cytotoxicity and ameliorative properties of the extract on APAP-induced toxicity in HEK293 cells were also evaluated.

Materials and Methods

Chemicals, drugs, and reagents

Sodium dodecyl sulfate (SDS), calcium chloride ($CaCl_2$), sodium oxalate ($Na_2C_2O_4$), L-ascorbic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 3-(4, 5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), phosphate buffered saline (PBS), dimethyl sulfoxide (DMSO), Modified Eagle's Medium (MEM) and trypsin were obtained from Sigma Aldrich Co, USA. Propanol was a product of Merck (Pty) Ltd., South Africa. Antibiotics and fetal bovine serum (FBS) were procured from Biochrom, GmbH, Germany. The water used was glass-distilled and all other chemicals and reagents used were of analytical grade.

Cell lines and culture medium

HEK293 cell line (from a human embryonic kidney) was a gift from the University of the Free State, Bloemfontein, South Africa. Stock cells of HEK293 were cultured in MEM supplemented with 10 % inactivated FBS, and antibiotics in an incubator with a humidified atmosphere of 5 % CO_2 at 37 °C until confluent. The cells were dissociated with TPVG solution (0.2 % trypsin, 1.0 % L-glutamate, PSA, NEA, 1.0 % glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and experiments were carried out in 96-well microtiter plates. All subsequent experiments with cell cultures were carried out at 37 °C in a 5 % CO_2 atmosphere.

Plant material

The plant material (the aerial parts and the roots) were collected in January 2013 from multiple populations in the field around Qwaqwa within the Golden Gate Mountains (28º28/ 111/ S and 28º 48/ 314/ E; altitude 11950 m). The species abundance was taken into consideration and collections were made in such a way that the existence of the species was not threatened. Proper identification and authentication was done by Dr C.J. Potgieter at the Bews Herbarium of the University of Kwazulu Natal, Pietermaritzburg Campus, where a voucher sample (LamMed/01/2013/Qhb) was deposited.

Saponin extraction and confirmation

The extraction process was carried out with slight modification as previously reported by Lakshmi *et al*[17]. Exactly 30 g of the dried plant sample was suspended in 200 mL of 95 % ethanol overnight. Subsequently, the sample was successively extracted with petroleum ether, ethyl acetate, chloroform, methanol and acetone. Petroleum ether

was used for delipidization and chloroform for deproteinization of the dried mixture. For extraction of crude saponin, methanol was used to mellow the developing mixture, followed by dropwise addition of acetone solution leading to precipitation. The precipitated material was extracted and dried in hot air oven leading to the formation of whitish brown crystals.

For the confirmation, frothing test was carried out. Briefly, a small quantity of the extract was dissolved in distilled water and the test tube was shaken vigorously for about 30 seconds. It was allowed to stand in a vertical position and observed over a 30 minutes period of time. The thick persistent froth was observed on the surface of the liquid indicating the presence of saponin. The saponin-rich extract (SEDB) so obtained was kept air-tight and refrigerated (4 °C) prior to use.

Antioxidant assays

Hydroxyl radical scavenging assay

The OH radical scavenging capability of the extract was determined as previously described by Smirnoff and Cumbes[18]. Briefly, 200 µL of either SEDB or vitamin C (concentrations 200-1000 µg/mL) were mixed with 600 µL of $FeSO_4$ (8 mM), 500 µL of H_2O_2 (20 mM) and 2 mL of salicylic acid (3 mM) in a test tube. Following a 30 minute incubation period at 37 ^oC, distilled water (900 µL) was added and the resulting mixture centrifuged (4472x *g*, 10 minutes). The absorbance was subsequently read at 510 nm and the IC_{50} value was calculated from the calibration curve following estimation of percentage OH* radical scavenging capacity of the extract as per the expression:

% Hydroxyl radical scavenged = $A_{Control} - (AS_{ample} - A_{Extract})/A_{Control}] \times 100$. $A_{Control}$, A_{Sample} and $A_{Extract}$ represent the absorbance of the mixture without extract, mixture with the extract and that of the extract alone, respectively.

Lipid peroxidation evaluation

The assay was performed adopting the method of Oyedemi *et al.*[19]. In brief, 500 µL 10 % v/v egg-yolk homogenate in sterile distilled water and 100 µL of varying concentrations (200-1000 µg/mL) of the extract were mixed in different test tubes and each made up to 1 mL with distilled water. To the resulting mixtures, 50 µL of $FeSO_4$ (0.07 M) was added prior to incubation (25 ^oC, 30 minutes) to induce lipid peroxidation. Subsequently, the working solution (1.5 mL of 20 % acetic acid (pH 3.5), 1.5 mL of 0.8 % w/v TBA prepared in 1.1 % w/v SDS and 0.05 mL of 20 % w/v TCA) was added, vortexed and heated in a boiling water for 1 hour. After cooling, n-butanol (5 mL) was added to each tube, centrifuged (3000 rpm, 10 min), and the absorbance of the resulting supernatant was read at 532 nm. For the blank and the standard, 100 µL each of distilled water and vitamin C was used instead of the extract and the IC_{50} values were thereafter estimated.

Cytotoxicity studies

Preparation of test solutions

For the cytotoxicity studies, SEDB was dissolved in sterile distilled water and the volume made up with MEM supplemented with 10 % inactivated FBS to obtain a stock solution of 1 mg/mL. This was sterilized by filtration (0.2 µm pore diameter) and varying test concentrations were subsequently prepared and used for the cytotoxicity studies.

Cell viability assay by the MTT method

In this experiment, the procedure of Pedraza-Chaverri *et al.*[20] was adopted. Briefly, the prepared monolayer cell culture was trypsinized and the cell count adjusted to 1.0 x 10^5 cells/mL using MEM containing 10 % FBS. To each well of the 96 well microtitre plate, 100 µL of the diluted cell suspension was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off and the monolayer washed once with medium. Subsequently, 100 µL of varying concentrations (62.5-1000 µg/mL) of SEDB were added on to the partial monolayer in microtitre plates. The plates were then incubated (37 ^oC, 5 % CO_2 atmosphere) for 72 hours, and microscopic examination (Nikon Eclipse TS100, USA) was carried out and observations noted once daily.

Following the 72 hours of incubation, the extract solutions in the wells were discarded and 20 µL of MTT (5 mg/mL) in PBS was added. The plates were gently shaken and further incubated (37 ^oC, 5 % CO_2 atmosphere) for 3 hours. The supernatant from each well was thereafter removed, 100 µL of DMSO was added and the plates were gently shaken to solubilize the resulting formazan. The absorbance was subsequently taken using a microplate fluorescence reader Spectra-Max M2 (Molecular Devices, USA) with excitation and emission at 540 and 620 nm, respectively. The percentage growth inhibition was calculated using the expression:

% Growth inhibition= 100 – [(As/Ac) x 100]

Where A_S and A_C represents the mean absorbance of the Sample and Control respectively. Using a standard dose-response curve, the concentration of SEDB needed to inhibit cell growth by 50 % (CC_{50} value) was determined.

Nephroprotective activity

This assay was achieved by adopting the method of Mosmann[21]. A preformed monolayer cell culture was trypsinized and adjusted as above (2.6.2). To each well of the microtitre plate, 100 µL of the diluted cell suspension was added. After 24 hours (when a partial monolayer had been formed), the supernatant was flicked off and the monolayer washed once with MEM containing 10 % FBS. Following this, 50 µL each of the supplemented medium and 1000 µg/mL of APAP in warm (37 ^{o}C) PBS[22] and 50 µL of different therapeutic concentrations of the extract and vitamin C (0.25 mM) were added. The plates were thereafter incubated (37 ^{o}C, 5 % CO_2) for 24 hours. Following incubation, the cell supernatants were discarded and 50 µL of MTT solution was added. The resulting mixture in the plates was gently shaken and incubated (37 ^{o}C, 5 % CO_2) for a further 3 hours. The cells in the plates were then swiftly microscopically examined (Nikon Eclipse TS100, USA) for probable alterations prior to flicking off of the new supernatant and addition of DMSO (100 µL) to solubilize the formed formazan. The absorbance was subsequently read (microplate fluorescence reader Spectra-Max M2) with excitation and emission at 540 and 620 nm, respectively. From the % cell viability estimated, the % protection offered by SEDB and vitamin C against APAP toxicity was calculated.

Morphological examination using Hoechst 33342

This was performed as reported by Elumalai *et al*.[23] In brief, following the treatment of the cells with APAP and with or without SEDB for 24 hours, the cells were stained with Hoechst 33342 (10 µg/mL) and the changes in the morphology were microscopically viewed (Nikon Eclipse TS100, USA).

Anti-nephrolithiasis study

Nucleation and time course crystallization assays

The method of Hennequin *et al.*[24] with slight modification was adopted for this experiment. Briefly, sterile solutions of $CaCl_2$ and $Na_2C_2O_4$ were prepared at a final concentration of 3.0 mM and 0.5 mM respectively, in a buffer (0.05 M Tris and 0.15 M NaCl (pH 6.5)) and filtered (0.2 µm pore diameter) three times. Exactly, 0.95 mL of $CaCl_2$ solution was mixed with 0.1 mL of different concentrations (0.1-1.0 mg/mL) of the extract. Crystallization was then initiated following the addition of 0.95 mL $Na_2C_2O_4$ solution. The control was prepared without the extract solution. The resulting solution in each case was thoroughly but diligently stirred and the working temperature maintained at 37 °C. The experiment was performed in triplicate and the absorbance of the solution was subsequently taken at 620 nm. The rate of nucleation (as a function of absorbance) was estimated by comparing the induction time (time of appearance of crystals that reached a critical size and thus became optically detectable) in the presence of the extract with that of the control (without extract). For each evaluation, the absorbance obtained with the extract was subtracted from that of the control.

For the time course measurement of turbidity changes, the anti-crystallization potential of the extract was conducted over a 5 minute period of observation and the rate of crystallization at each interval recorded.

Statistical analyses

Except where stated otherwise, results are presented as mean±standard error of the mean (SEM) of replicate experiments. One way analysis of variance (ANOVA) coupled with the Duncan Multiple Range Test using SPSS 16.0 (SPSS Inc., USA) was used to determine significant differences in all the parameters. Values were considered statistically significant at $p < 0.05$.

Results

Antioxidant and cytotoxicity

The *in vitro* radical scavenging effects of SEDB on OH* and lipid peroxidized products are shown in Table 1. The extract dose-dependently inhibited and scavenged the radicals formed with the highest dose exhibiting the most potent effect in the two assays. The tendency of SEBD to scavenge OH* radical revealed a significant effect judging by the IC_{50} value (0.63 mg/mL) when compared with vitamin C

Table 1 — Antioxidant effects of saponin extract of *Dianthus basuticus*

	OH*		LPO*	
Treatments	IC_{50} (mg/mL)	R^2	IC_{50} (mg/mL)	R^2
SEDB	0.63	0.9142	0.45	0.9082
Vitamin C	0.69	0.8976	0.49	0.9382

SEDB= Saponin extract of *Dianthus basuticus*

(0.69 mg/mL). For the lipid peroxidized radicals, the extract also displayed significant radical scavenging effect (with IC_{50} values of 0.45 mg/mL) and competed favourably with vitamin C (0.49 mg/mL). Their coefficients of determination (R^2) values are also presented (Table 1).

The degrees of cell viability and cytotoxicity of SEDB treatment on HEK293 cell lines revealed a concentration-dependent effect with the highest dose-treated cells showing a 15.17 % survival tendency compared with 89.72 % for those on the lowest dose of the extract (Fig. 1). The 72-h CC_{50} value for the extract in HEK293 cells was 301.56 µg/mL.

Data obtained with respect to the nephroprotective study showed significant (p <0.05) reduction in the number of viable cells in the nephrotoxic groups (APAP-treated cells alone) compared with control (only MEM-treated cells) over the 24 hours incubation period (Fig. 2). In contrast, when compared with the nephrotoxic cells, the extract-treated groups had significantly (p <0.05) higher number of viable and metabolically active cells with the effect elicited by the extract administered at 62.5 µg/mL competing favorably with that of vitamin C. It is also noteworthy that the observed 81.98 % cell mortality in the APAP-treated cells was significantly reduced (13.15 %) following treatment with the 125 µg/mL dose of the extract (Fig. 2).

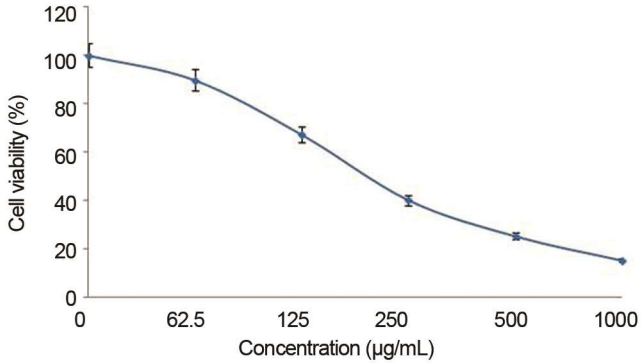

Fig. 1 — Cytotoxicity attributed to saponin extract of *Dianthus basuticus* in HEK293 cells. Results are expressed as percent cell survival compared to control (MEM containing 10 % FBS only supplemented cells). Values are mean±SEM of three determinations.

Microscopic examination of cells from the control group revealed essentially normal morphological features (Fig. 3a). However, while cells treated with MTT following APAP treatment for 24 hours showed architectural alterations consistent with apoptotic morphological changes like cyto-constriction, detachment and nuclear condensation (Fig. 3b), those treated along with SEDB and vitamin C had significantly lesser morphological infiltrations compared with the nephrotoxic cells (Fig. 3c-e). These apoptotic morphological changes were confirmed by Hoechst staining.

Anti-nephrolithiasis

The effect of treatments with SEDB on the nucleation of CaOx crystals is presented in Fig. 4. There was a consistent decrease in the absorbance with increasing concentration of the extract with the

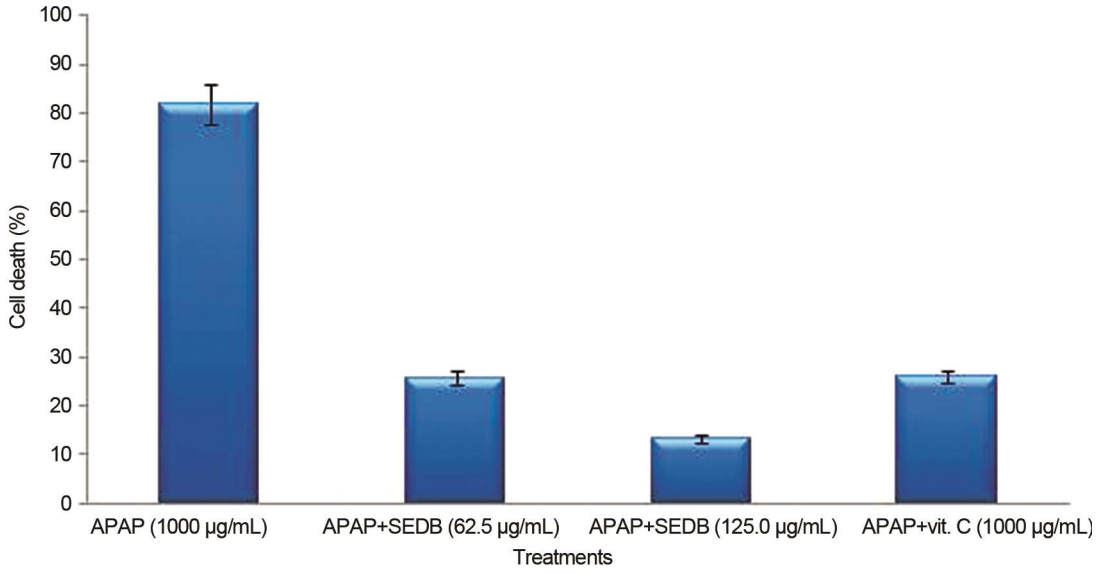

Fig. 2 — Effect of treatments with saponin extract of *Dianthus basuticus* and vitamin C on APAP-induced cell death in HEK293 cells. Cells were simultaneously treated with APAP and the drugs for 24 hours and the cell viability was determined by the ability to reduce MTT[abc] bars with different superscript for the parameter are significantly different (p <0.05).

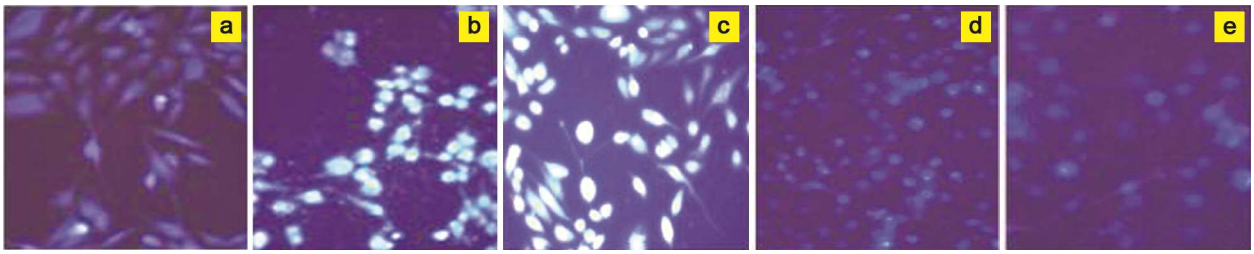

Fig. 3 — Respective phase contrast image of cells in the (a) Control, (b) APAP-treated, (c) APAP + 62.5 µg/mL SEDB, (d) APAP + 125 µg/mL SEDB, and (e) APAP + 0.25 mM vit C groups following 24 hour incubation and nuclear staining using Hoechst 33342.

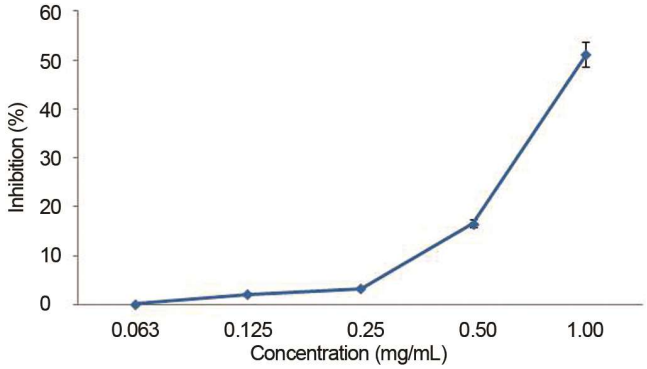

Fig. 4 — Inhibitory effect of saponin extract of *Dianthus basuticus* on the nucleation of CaOx crystallization.

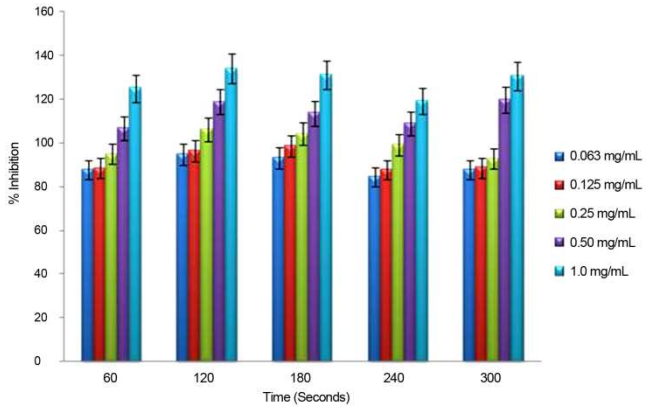

Fig. 5 — Time course effects of saponin extract of *Dianthus basuticus* on the crystallization of CaOx.

corresponding inhibitory effect of the extract ranging between 0.11-51.11 %. The concentration-related increase in % inhibition of nucleation of CaOx crystals by SEDB was significant with an IC_{50} of 1.03 mg/mL (R^2= 0.9698). In addition, the degree of turbidity was generally lower in the extract-treated solutions than in the control and the inhibitory potential increased with increasing time over the 300 s monitoring period (Fig. 5).

Discussion

Under the concerted influence of glutathione peroxidase and catalase in the presence of iron or copper ion, H_2O_2 is reduced to OH* which is capable of damaging important macromolecules (membrane lipids, proteins, and DNA base pairs) in biological systems[25]. In this study, SEDB had very strong potential to annihilate the deleterious effect of OH* judging by its IC_{50} values that competed well with that of vitamin C. This observation may indicate the capability of the extract to halt cascade of reactions involving this radical by sacrificing either electron or hydrogen atom thereby making it relatively stable. Consequently, this could prevent probable cellular and macromolecular damage. Our submission is in agreement with the report of Rahmat *et al.*[26] on the reactive oxygen species property of extracts of *Sonchus asper* (L.) Hill. Another event consistent with OH* oxidative damage is auto-oxidation of membrane-bound lipids (lipid peroxidation). This peroxidative event could disrupt membrane fluidity and inflict physiological alterations on other metabolically important biomolecules (proteins and DNA). The present study employed egg-yolk homogenate as lipid-rich source and the inhibitory effect of SEDB on the lipid peroxidized products was significant and suggestive of its tendency to detoxify OH and other reactive metabolites, which could have initiated and propagated peroxidation of membrane-bound polyunsaturated lipids in biological systems. Furthermore, the tending towards 1.0 of R^2 values of the antioxidant assays evaluated in this study is another predictive and supporting fact for the significant antioxidative capability of SEDB. Mehdi *et al.*[27] have also given similar reports on the antioxidant activity of saponin-rich extracts of *Chlorophytum borivilianum*.

Following the ethical issues on the use of animals, many *in vitro* protocols for animal toxicity and pharmacological testing have been developed, validated and gained global regulatory acceptance as suitable alternatives to whole animal tests. These protocols have been developed and validated using the 4Rs (Replacement, Reduction, Refinement and

Responsibility) approach. One of the most routinely adopted approaches for *in vitro* cytotoxicity evaluation of chemicals or pharmacological agents utilizes various continuous cell lines[28,29]. In this study, the HEK293 cells were sensitive to SEDB treatments and the degree of sensitivity varied with varying concentrations. Judging by the CC_{50} value (301.56 µg/mL), it may be logically inferred that the extract was well tolerated by the cells and holds promising pharmacological potentials. However, the lower concentrations (62.5 and 125 µg/mL) of SEDB where the least cytotoxic effects were observed on HEK293 cells with corresponding 89.72 and 67.19 % viability were used for the subsequent nephroprotective assay.

APAP-induced renal tubular damage has been attributed either to translocation of GADD153 (growth arrest- and DNA damage-inducible gene 153) and subsequent proteolysis of caspase-12[30] or involvement of N-acetyl-*p*-benzoquinone imine (NAPQI)[31]. NAPQI arylates selenium-binding protein and glutamine synthetase in the S3 segment of the tubule with consequential depletion of reduced glutathione[32]. This subsequently results in auto-oxidation of renal macromolecules (lipids, proteins and DNA) with associated tubular cell necrosis. Since APAP accumulates in the kidney, HEK293 cell line established from human kidney cells was used in this study to mimic renal-like features. The decreased number of viable cells following 24 hours exposure to APAP could suggest epithelia injury and cell necrosis resulting from free radicals generation. This observation is in agreement with the previous study by Lijuan *et al.*[22], where APAP treatment proved lethal to established cell lines. However, the significant and concentration-dependent increase in the number of viable and metabolically active cells in the extract-treated groups may be suggestive of the ability of SEDB to ameliorate the toxic influence of APAP on the cells. This assertion may also indicate that SEDB at its pharmacological doses may preserve renal functions and protect against renal pathological conditions. In addition to complementing the cell viability assessment, microscopic examination of the MTT and Hoechst treated cells could provide information on how pharmacologically potent an agent is against cell necrosis. The significant alterations in the morphological features of the APAP-treated cells could have impaired and facilitated their mortality. However, the apparently annulled onslaughts by APAP on the architectural features of cells in the SEDB-treated groups could mean that the extract offered significant protection and stabilized the morphological integrity of the cells. The effect elicited by the extract compared favourably with that of vitamin C and is consistent with the results of the viability tests. Kalaivani *et al.*[33] also gave similar assertions on the nephroprotective effect of ethanolic root extract of *Boerhaavia diffusa* against cisplatin-induced nephrotoxicity in LLC-PK1 cells. Consequent upon the foregoing, the overall effects elicited by SEDB as a nephroprotective agent against APAP toxicity on HEK293 cells may be adduced to its antioxidant properties which were significantly elicited in effectively scavenging OH and lipid peroxidized radicals in this study. This may also be attributed to its membrane stabilization potential which might have prevented the release of lactate dehydrogenase from the mitochondrial epithelia of the SEDB-treated cells into the medium and consequently preventing necrosis.

The crystallization of CaOx begins with increased urinary supersaturation, with the subsequent formation of solid crystalline particles within the urinary tract. This is followed by nucleation via which stone-forming salts in supersaturated urinary solution coalesce into crystals that consequently increase in size by the accumulation of new constituents[9]. These crystals then grow and aggregate with other crystals in solution, and are ultimately retained and accumulated in the kidney[7]. Renal injury encourages crystal retention and the development of a stone nidus on the renal papillary surface that further worsens crystal nucleation at lower supersaturation levels[34,35]. Therefore, levels of urinary supersaturation correlate with the type of stone formed, and reducing supersaturation is effective in preventing stone recurrence.

In the present study, CaOx crystals were formed by the incubation of $CaCl_2$ and $Na_2C_2O_4$ solutions and their presence were confirmed by the turbid nature of the resulting solutions. Plants or plant constituents with good antioxidative and anti-crystallization potentials have been found to be highly anti-nephrolithiatic by disaggregating the mucoproteins which are major promoters of crystallisation in the renal tubules[36-38]. This was evidently shown in this study with the extract at the highest investigated dose conferring a nucleation inhibitory potential of 51.11 % against crystals formation. Furthermore, the apparently decreased degree of turbidity in the SEDB-

treated solutions relative to the control was another probable indication that the amount of crystals formed in the presence of the extract was significantly reduced. Aggarwal *et al.*[39] also gave similar assertions on the *in vitro* CaOx crystals inhibitory effects of *Tribulus terrestris*. In addition to the *in vitro* CaOx crystals inhibitory effects of plants, the anti-nephrolithiatic activity of saponin-rich extracts have also been well demonstrated *in vivo*[11,40]. The saponins are known to either disaggregate suspension of mucoproteins or optimize urinary excretion which subsequently aids decrease in the concentration of urinary salts that prevents supersaturation of the crystallizing salts[7,37,41]. In view of these, it may be appropriate to infer that SEDB is capable of inhibiting reactions or processes leading to CaOx crystals formation.

Conclusion

Consequent upon the available results in this study, it may be logically inferred that SEDB elicited significant radical scavenging activity. This might have potentiated its ability to inhibit CaOx nucleation and facilitated vitality of metabolically active HEK293 cells following APAP treatment. Hence, further purification and characterization of this saponin-rich extract are imperative. In this direction, efforts are ongoing.

Acknowledgement

Many thanks to the Directorate of Research Development of the University of the Free State, Bloemfontein, South Africa for postdoctorol fellowship granted to Dr. M.O. Nafiu.

References

1 Schnellmann R G, Kelly K J, Pathophysiology of nephrotoxic acute renal failure, in Berl T, Bonventre JV, eds, (Blackwell Science, Philadelphia, PA), 1999.

2 Sabiu S, O'Neill F H and Ashafa A O T, Membrane stabilization and detoxification of acetaminophen-mediated oxidative onslaughts in the kidneys of Wistar rats by standardized fraction of *Zea mays* L. (Poaceae), *Stigma maydis*, *Evid based Complement Alternat Med,* 2016, **2016**, 1-14.

3 Cekmen M, Ilbey Y O, Ozbek E, Simsek A, Somay A, *et al*., Curcumin prevents oxidative renal damage induced by acetaminophen in rats, *Food Chem Toxicol,* 2009, **47**, 1480-1484

4 Rodgers A, The riddle of kidney stone disease: lessons from Africa, *Urol Res,* 2006, **34**, 92-95.

5 Tyagi S, Patel C, Patel J and Tarun P S, Review on kidney stones, *J Biomed Pharm Res,* 2012, **1**(3), 06-09.

6 Selvam P, Kalaiselvi P, Govindaraj A, Murugan V B and Sathishkumar A S, Effect of *A. lanata* leaf extract and vediuppu chunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria, *Pharmacol Res,* 2001, **43**, 89–93.

7 Bashir S and Gilani A H, Antiurolithiatic effect of *Bergenia ligulata* rhizome: An explanation of the underlying mechanisms, *J Ethnopharmacol,* 2009, **122**, 106–116.

8 Prasad K, Sujatha D and Bharathi K, Herbal drugs in urolithiasis – A review, *Pharmacogn Rev,* 2007, **1**, 175–179.

9 Basavaraj D R, Biyani C S, Browning A J and Cartledge J J, The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones, *EAU-EBU Update Series*, 2007, **5,** 126–36.

10 Knoll T, Stone disease, *Eur Urol Suppl*, 2007; **6**, 717–722

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

12 Raimondo D, Staden L V, Foden W, Victor J E, Helme N A, *et al*., Red list of South African plants, South African National Biodiversity Institute, 2009.

13 Moteetee A and Van Wyk B E, The medical ethnobotany of Lesotho: A review, *Bothalia*, 2011, **41**, 209-228.

14 Lamula S Q N and Ashafa A O T, Antimicrobial and cytotoxic potential of *Dianthus basuticus* used in Basotho traditional practice, *Bangladesh J Pharmacol*, 2014, **9**, 105–111.

15 Kazeem M I and Ashafa A O T, *In-vitro* antioxidant and antidiabetic potentials of *Dianthus basuticus* Burtt Davy whole plant extracts, *J Herb Med,* 2015, **5**, 158–164.

16 Ashafa A O T and Kazeem M I, Toxicopathological evaluation of hydroethanol extract of *Dianthus basuticus* in Wistar rats, *Evid Based Complement Alternat Med*, 2015, 1–10.

17 Lakshmi V, Mahdi A A, Agarwal S K and Khanna A K, Sterodal saponin of *Chlorophytum nimonii* (Grah) with lipid lowering and antioxidant activity, *Chronicles of young scientists*, 2012, **3**(3), 227–232.

18 Smirnoff N and Cumbes O J, Hydroxyl radical scavenging activity of compatible solutes, *Phytochem,* 1996, **28**, 1057-1060.

19 Oyedemi S O, Oyedemi B O, Arowosegbe S and Afolayan A J, Phytochemicals analysis and medicinal potentials of hydroalcoholic extract from *Curtisia dentata* (Burm.f) C.A. Sm Stem Bark, *Int J Mol Sci,* 2012, **13**, 6189-6203.

20 Pedraza-Chaverri J, Yam-Canul P, Chirino Y I, Sánchez-González D J, Martínez-Martínez C M, *et al*., Protective effects of garlic powder against potassium dichromate-induced oxidative stress and nephrotoxicity, *Food Chem Toxicol,* 2008, **46**(2), 619-627.

21 Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J Immunol Methods,* 1983, **65**(1-2), 55-63.

22 Lijuan Z, Xiaoqun M, Juanling F and Zongcan Z, *In vitro* cytotoxicity assay with selected chemicals using human cells to predict target-organ toxicity of liver and kidney, *Toxicol in Vitro,* 2007, **21**, 734–740.

23 Elumalai P, Gunadharini D N, Senthilkumar K, Banudevi S, Arunkumar R, *et al*., Induction of apoptosis in human breast

cancer cells by nimbolide through extrinsic and intrinsic pathway, *Toxicol Lett*, 2012, **215**, 131-42.

24 Hennequin C, Lalanne V, Daudon M, Lacour B and Drueke T, A new approach to studying inhibitors of calcium oxalate crystal growth, *Urol Res,* 1993, **21**, 101–108.

25 Sabiu S, Sunmonu T O, Ajani E O and Ajiboye T O, "Combined administration of silymarin and vitamin C stalls acetaminophen-mediated hepatic oxidative insults in Wistar rats," *Revista Brasileira de Farmacognosia*, 2015, **25,** 29-34.

26 Rahmat A K, Muhammad R K, Sumaira S and Mushtaq A, Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill., *Chem Cent J,* 2012, **6**, 12.

27 Mehdi F A, Maheran A, Johnson S, Ismanizan I and Mihdzar A, Assessment of antioxidant and cytotoxicity activities of saponin and crude extracts of *Chlorophytum borivilianum*, *Sci World J*, 2013, **2013**, 1-7.

28 Eun-Kee P, Sally K M, Dietmar K and Bruce D, Evaluation of cytotoxicity attributed to thimerosal on murine and human kidney cells, *J Toxicol Environ Health,* Part A, 2007,**70**, 2092–2095

29 Soumen R, Sandip P and Abhay C, Evaluation of *in vitro* cytotoxic and antioxidant activity of *Datura metel* Linn. and *Cynodon dactylon* Linn. extracts, *Pharmacogn Res,* 2016, **8**(2), 123–127.

30 Lorz C and Justo P A, Paracetamol nephrotoxicity: A role for ER stress, *J Am Soc Nephrol,* 2004, **15**, 380-389.

31 Parameshappa B, Sultan A B, Saikat S, Raja C, Vinod G K, *et al*., Acetaminophen-induced nephrotoxicity in rats: Protective role of *Cardiospermum halicacabum*, *Pharm Biol,* 2012, **50**(2), 247-253.

32 Adeneye A A and Benebo A S, Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin and acetaminophen-induced nephrotoxic rats., *J Ethnopharmacol,* 2008, **118**, 318-323.

33 Kalaivani M K, Soundararaja P, Vasanthi H R and Arociaswamy S, *In viro* nephroprotective effect of ethanolic root extract of *Bo*erhaavia *diffusa* against cisplatin-induced nephrotoxicity, *Int J Phytomed,* 2016, **7,** 388-395.

34 Parmar M S, Kidney stones, *Br Med J*, **328**, 1420-1424.

35 Worcester E M and Coe F L, Nephrolithiasis, *Primary care: Clinics in Office Practice,* 2008, **35**, 369–391.

36 Atmani F and Khan S R, Effects of an extract from *Herniaria hirsute* on calcium oxalate crystallization *in vitro, Br J Urol Int,* 2000, **85**, 621-625.

37 Fouada A, Yamina S, Nait M A, Mohammed B and Abdlekrim R, *In vitro* and *in vivo* antilithiatic effect of saponin rich fraction isolated from *Herniaria hirsute*, *J Bras Nefrol,* 2006, **28**, 199–203.

38 Rathod V D, Fitwe P, Sarnaik D and Kshirsagar S N, *In vitro* anti-urolithiatic activity of corn silk of *Zea Mays*, *Int J Pharm Sci Rev Res,* 2013, **21(**2), 16-19.

39 Aggarwal A, Tandon S, Singla S K and Tandon C, Diminution of oxalate induced renal tubular epithelial cell injury and inhibition of calcium oxalate crystallization *in vitro* by aqueous extract of *Tribulus terrestris, Int Braz J Urol,* 2010, **36**(4), 480-489.

40 Gurocak S and Kupeli B, Consumption of historical and current phytotherapeutic agents for urolithiasis: A critical review, *J Urol,* 2006, **176**, 450–455.

41 Kapilraj P K, Prophylactic and curative activity of '*Barleria buxifolia*. Linn' on experimentally induced calcium oxalate nephrolithiasis in animal model, *Int J Pharmacol Clin Sci,* 2015, **4**(4), 68-75.