ABSTRACT

Objectives: The effect of methanolic extract of Kalanchoe crenata (MEKC) was investigated on renal morphology and function in adriamycin-induced kidney impairment in rats. 

Methods: Ether anesthetized rats received three intravenous injections (days 0, 14, 28) of 2 mg/kg body weight of adriamycin. Repeated doses of the extract (0, 50 and 68 mg/kg bw) and losartan (10 mg/kg bw) were administered orally once daily, for 6 weeks, to adriamycin-nephropathic rats. Kidney functions were assessed through proteinuria, creatinine and creatininuria, renal malondialdehyde (MDA) level, superoxide dismutase (SOD) activity and morphology analyses.

Results: The 50 and 68 mg/kg MEKC, as the losartan, decreased proteinuria: -63.74 % and -64.94 % respectively, significantly (P<0.01) increased the creatininuria and creatininuria/creatinemia ratio, and also decreased the creatininemia in diseased rats. The plant extracts markedly (P<0.05) increased plasma sodium, and decreased (P<0.01) the urinary sodium and potassium levels. The MEKC has remarkably (P<0.01) decreased the level of the thio-barbituric acid reactive substances and increased the SOD level in nephropathic rats. The extract has improved the damage of kidney induced by adriamycin.

Conclusion: The results indicate that the treatment with the K. crenata methanolic extract may improve proteinuria and all the symptoms that breed from nephropathy, and could improve kidney morphology. Therefore, K. crenata could be promising for the development of a standardized phytomedicine for the treatment of kidney disease.

Keywords: Adriamycin, Kalanchoe crenata, Nephropathy, Antioxidant, Rat, Methanol extract.

INTRODUCTION

Chronic kidney disease is a worldwide global public health problem, and is characterized by glomerulosclerosis and tubulointerstitial fibrosis which are the final common pathways of progression [1,2]. Its prevalence is increasing as the result of an increased prevalence of diabetes, arterial hypertension and drugs toxicity [3,4]. Most chronic renal diseases are characterized by increased glomerular matrix accumulation and collapse of the capillary lumina. The structural alterations in the glomeruli are accompanied by sustained proteinuria, often of nephrotic range. Tubular atrophy and dilatation, interstitial inflammatory infiltrates, accumulation of interstitial fibroblasts, and increased matrix deposition are associated with progressive glomerulosclerosis which ultimately lead to interstitial fibrosis and loss of renal function [1,5].

The interstitial recruitment of macrophages and lymphocytes as a major source of inflammatory and profibrotic mediators plays an important role in chronic interstitial inflammation and fibrosis [2]. Therapeutic strategies to prevent or delay loss of organ function in chronic renal disease therefore target pathways in the fibrotic tissue remodeling. Conventional treatment of kidney diseases includes oral Enzyme conversion inhibitors (ECI) like losartan, n-hexane to remove its hydro insoluble compounds. The final residue (not soluble in hexane) obtained after drying constituted the methanol extract of K. crenata (MEKC). The yield of the extract was 41.8 g (2.09 %). Prior to the administration to animals, the extract was solubilized in distilled water, with the volume of administration < 1 mL for each experimental animal.

Preliminary phytochemical tests

Phytochemical properties of the methanolic fraction of Kalanchoe crenata were tested by the standard methods described by Sofowora, using various reagents [12]: Mayer and Dragendorff’s reagents for alkaloids; FeCl3 for tannins; frothing test for saponins; magnesium turning and HCl for flavonoids; NaCl and Fehling’s solutions for glycoside; diethyl ether, sulphuric acid and anhydride acetic for steroids; ether-chloroform and NaOH for anthraquinones and FeCl3 and K3Fe(CN)6 for phenols and polyphenols.
Acute toxicity evaluation

The MEKC was tested for its acute toxicity in mice. Five groups of six mice each were orally administered one of the different doses of the extract: 0 (control group), 2, 4, 6, 8, 10 g/kg body weight. The animals were observed continuously for initial 2 h, intermittently for the next 6 h and then at 24 h and 48 h following drug administration for death and overt behavior: lethargy, jerksiness, sensitivity to noise and touch, and respiratory rate. The lethal dose 50 (LD50) was determined with the formulae [13].

\[ DL_{50} = X_s - d(\Sigma p - \Sigma) \]

\( X_s = \text{lethal dose for 100\% of mice; } d = \text{interval between the doses} \)
\( p = \text{proportion of death per group; } \Sigma p = \text{sum of death proportions} \)

Induction of kidney disease

Male wistar albino rats (200-250 g) raised in the animal house of the Faculty of Science of the University of Yaoundé I, were maintained under natural laboratory conditions (temperature, and dark/light cycle) and allowed access to food and water ad libitum. Animal housing and experiments in vivo were done according to the guidelines of the European Union directive on Ethical Evaluation of Animal Experiments (CEE Council 86/609)[14] and approved by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon.

To induce renal impairment, the rats anesthetized with ether received three intravenous (penile vein) injections (days 0, 14, 28) of 2 mg/kg body weight Adriamycin (2 mg/mL doxorubicin hydrochloride: Pharmacia Italia, S. P. A., Italy) in 9 % NaCl. The specific activity of SOD was expressed as number of units/mg protein. A unit is the quantity of SOD that inhibits 50 % adrenaline oxidation per min [17].

The pathological assessment exhibited a significant change and expansion of the mesangium was observed, with disappearance of inflammation (fig. 3B).

Serum and urine analysis

Creatinine level was analyzed in serum using commercial diagnostic kits (Biodirect Laboratories, SEPPEMS, A. France). Sodium and potassium were analysed in blood and urine by a selective electrode ion auto-analysers (ILLYTE). Urinary creatinine was estimated spectrophotometrically with commercially available kits (Biodirect and Elitetch). Assays with kits were carried on according to the manufacturers’ recommendations. Urinary protein was quantified by precipitation turbidimetric test of protein in trichloroacetic acid (TCA 12 %) or sulfoalicylic acid [13].

Homogenate analysis

TBARS level (MDA activity)

To estimate TBARS, 0.4 mL of homogenate was added to 2 mL of glacial acetic in a test tube. To this mixture was added 2 mL of 1 % thiobarbituric acid in 0.5 M NaOH. The loosely stopped tubes were immersed in boiling water bath for 1 h. The tubes were then cooled under running tap water and absorption measured at 532 nm (JENWAY Spectrophotometer, Barloworld Scientific U. K) against MDA reactive [16].

SOD activity

To assay the SOD activity, 0.2 mL of homogenate was added to 2.5 mL of sodium carbonate 0.05 M pH10.2. The reaction began when 0.3 mL adrenaline was added. The reaction mixture was stirred vigorously and absorption measured at 480 nm against the blank (Sodium carbonate + adrenaline + distilled water). The serum level of sodium and the urinary level of potassium also significantly (P<0.01) decreased (Table 1). The lethal dose 50 (LD50) was 4.4 g/kg. There were no gross behavioural changes. Macroscopically, the organs (liver, kidney, heart) did not show any discoloration.

During the experiment, 6 weeks after the treatment of the rats, the adriamycin induced significant increase (P<0.01) of creatinemia, urinary volume, proteinuria, when the creatinuria/creatinemia ratio and creatinuria significantly (P<0.01) decreased (Table 1).

The pathological assessment exhibited a significant change and damage of kidney in nephropathic control rat. In most glomeruli the expansion of the mesangium was observed, with disappearance of urinay space. Glomerular pathology was accompanied by prominent tubule interstitial changes, including tubular inflammation (fig. 3B).
Table 1: Blood and urinary biochemical parameters of adriamycin-induced kidney dysfunction after 6 weeks daily treatment with *Kalanchoe crenata* methanolic fraction or losartan

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proteinuria (g/L)</th>
<th>Creatinuria (µmol/L)</th>
<th>Creatinemia (µmol/L)</th>
<th>Creatinuria/creatinemia</th>
<th>Urinary volume (mL/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>1.86 ± 0.09</td>
<td>11.3 ± 0.81</td>
<td>56.99 ± 1.23</td>
<td>0.19 ± 0.65</td>
<td>13.05± 0.13</td>
</tr>
<tr>
<td>NeC</td>
<td>5.02 ± 0.24**</td>
<td>3.28 ± 0.02**</td>
<td>91.82 ± 1.28**</td>
<td>0.03±0.02**</td>
<td>15.50±0.43**</td>
</tr>
<tr>
<td>NeK50</td>
<td>1.82 ± 0.14**</td>
<td>8.31 ± 0.60**</td>
<td>58.37 ± 1.50**</td>
<td>0.99±0.38**</td>
<td>8.44±0.51**</td>
</tr>
<tr>
<td>NeK68</td>
<td>1.76 ± 0.01b</td>
<td>8.42 ± 1.04**</td>
<td>60.95 ± 1.89**</td>
<td>0.95±0.57**</td>
<td>8.70±0.40**</td>
</tr>
<tr>
<td>NeL10</td>
<td>1.23 ± 0.16b</td>
<td>9.75 ± 0.35b</td>
<td>63.68 ± 1.45b</td>
<td>1.25±0.24b</td>
<td>9.45±0.25**</td>
</tr>
</tbody>
</table>

Normal (NC) and nephropathy (NeC) control rats. Nephropathy rats treated with 50 mg/kg bw (NeK50), 68 mg/kg bw (NeK68) and with losartan 10 mg/kg bw (NeL10). Data are means ± SEM, n = 5. Significant difference: *P< 0.05 and **P< 0.01 compared with NC values; aP< 0.05 and bP< 0.01 compared with NeC values.

The *Kalanchoe crenata* methanolic extract (MEKC) 50 and 68 mg/kg bw, and the 10 mg/kg bw losartan, after 6 weeks treatment, significantly (P< 0.01) reduced the blood creatinine and the urinary volume and proteinuria in nephropathy rats. The Creatinuria/creatinemia ratio and the creatinuria were enhanced (P< 0.01) by these different drugs (table 1). The MEKC and the losartan have markedly (P< 0.01) raised the blood sodium, the urinary potassium and decreased the blood potassium and the urinary sodium levels in nephropathic rats (fig. 1).

*K. crenata* extract and losartan, after 6 weeks daily administration, markedly (P< 0.01) reduced the renal MDA level and significantly (P<0.01) raised the SOD activities. The extract and losartan improved the morphology of kidney in nephropathic rats (fig. 3). The treatment reduced the tubular inflammation, the expansion of the mesangium and improved the urinary space in nephropathic rats.

**DISCUSSION**

The main aim of this work was to assess the effect of *Kalanchoe crenata* methanol extract (MEKC), an anti-inflammatory and antihyperglycemic [7] plant on the renal function through the hydroelectrolytic balance (sodium, potassium), the proteinuria, creatinuria, creatinemia and on the renal morphology through the activities of MDA, SOD and the histology in Adriamycin-induced nephropathy in rats.
In the acute toxicity study, single oral dose of the MEKC up to 2 g/kg was not lethal to both male and female mice. The apparent cause of death of mice in the current study at the higher doses might be due to respiratory depression or/to methanol (solvent) poisoning; it is noteworthy that the aqueous-ethanol extract of K. crenata did not show lethality in a previous acute toxicity [8]. The results suggest that MEKC possesses low toxicity since its LD_{50} (inferior to 5 g/kg b.w.) is 65 and 88 times respectively the assay doses [18].

Adriamycin-induced nephropathy resulted in hyperproteinuria. Parallel to the increase of proteinuria, there was hypercreatinemia and hypocreatinuria, along with the decrease of creatininur/creatine ratio. Normally, the kidneys excrete creatinine and only a slight amount of low molecular weight protein passes through the glomerular [19]. Usually hypercreatinemia and hypocreatinuria observed in nephropathic states are characteristic of glomerular hyperfiltration[20]. The alteration of the glomerular filtration may explain the hyperproteinuria and the decrease of creatininur/creatine ratio [21].

The MEKC showed significant dose-dependent effect on protein excretion in nephropathic rat, similar to that of the losartan. When compared with losartan effect, the decrease of urinary protein level could be due to a potential capability of the plant extract to restore the altered glomerular capillary function in nephropathic rats, and by the inhibition of enzyme-converting angiotensin (ECA) or the blockage of angiotensin II receptors, that reduces capillary vessel contraction and then decreases the retention of water and salt [19,22-24]. This could explain the decreased level of urine volume and urinary sodium in treated nephropathic rats.

Normally, the tubular reabsorbs water and Na+, to form the hypotonic urine. In nephropathic rats, Adriamycin caused an electrolyte disorder due to the failure of tubular reabsorption causing sodium leak and potassium retention. Thus, the raise of urinary Na+ excretion, which may be due to the decrease of proximal reabsorption, also causes an increased urinary volume [25]. In the treated rat, the decrease of urinary Na+, urinary volume, and the increase of blood potassium, could then result from an improvement of the proximal tubular reabsorption function by K. crenata. The plant by normalizing the levels of sodium and potassium in the media also normalizes urinary volume.

In the nephropathic rats, the adriamycin provoked the lipid peroxidation products (MDA) increase and the SOD activities decrease in the kidneys. SOD is the major scavenging enzyme that removes toxic free radicals in vivo and protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical (O_2^-) which damages the cell membrane and biological structure in kidney tissues. Reduced activities of SOD associated with adriamycin nephropathy in rats, may lead to a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide [26]. Increased in lipid peroxidation (MDA) in adriamycin nephropathy may cause peroxidative tissue damage and inflammation [2,10]. Otherwise, the proteinuria increase could lead to the accumulation of the protein in the proximal tubular cells cytoplasm, which with associated interstitial inflammatory reaction, may cause glomerular and kidney tubule damage [24].

In addition, glomerular damage, mesangium expansion and the tubular inflammation in nephropathic rats could result from the oxidant stress markers (SOD and MDA) increase by inflammatory mechanisms in the nephropathy progression [27,28]. In the treated rats, plant extract and losartan exhibited antioxidative properties by normalizing SOD activity and MDA level, and also improved the renal morphology. Restoration to the normal level SOD activity, MDA level in tissues, and kidney morphology could be attributed to the presence of polyphenols, triterpenes and tannins in the extract, as they are known to possess antioxidant properties[29]. This indicates that K. crenata could prevent the alteration of the renal cells structure and/or function that could be induced by adriamycin.

CONCLUSION

Methanolic fraction of K. crenata could improve glomerular and renal tubular, and the expansion of the mesangium, could lower the urinary protein level, prevent the increase of creatininemia, MDA level and the decrease of SOD activities in nephropathy rats. Thus, this extract shows a potential to produce an alternative medicine for the treatment or management of the kidney disease.
CONFLICTS OF INTEREST

The authors have none to declare.

REFERENCES


