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Effect of methanolic extract of tecomastans on metabolic parameters in adriamycin-induced renal impairment in rats

***Prof (Mrs.) A. Kavidha, Dr. N. Sriram, Prof Ebenezer David**

[1]*Associate Professor and Head, Department of Pharmacognosy and Phytochemistry, Cherranns College of Pharmacy, Coimbatore.*
[2]*Professor and Head Holy Marry College of Pharmacy (HITS) Telengana.*
[3]*Professor and Head Department of Pharmacology Cherranns College of Pharmacy, Coimbatore*
***Corresponding author: Prof (Mrs.) A. Kavidha**

ABSTRACT

This study is planned to investigate the effect of Tecomastans methanolic extract (METS) on proteinuria, glucosuria, and some other biochemical parameters in adriamycin-induced renal impairment in rats. Ether anesthetized rats received three intravenous injections (days 0, 14, and 28) of 2 mg/kg body weight of adriamycin. Repeated doses of the extract (0, 50, and 68 mg/kg b.w.) and losartan (10 mg/kg b.w.) were administered orally once daily, for 6 weeks, to these rats. Kidney functions were assessed through biochemical parameters. METS decreased proteinuria and also the urinary excretion of creatinine, glucose, and urea significantly in diseased rats. A decrease in serum levels of creatinine, urea, potassium, alkaline phosphatase, conjugate bilirubin, and alanine transaminase level was also recorded in nephropathic rats, but plasma levels of uric acid and glucose remained unchanged. Moreover, the plant extract markedly ($P < 0.05$) increased plasma sodium and decreased ($P < 0.01$) the urinary sodium and potassium levels. The results indicated that the treatment with the methanolic extract of T.stans may improve proteinuria and all other symptoms due to adriamycin-induced nephropathy and, more than losartan, could ameliorate kidney and liver functions. T.stans could be a potential source of new oral antinephropathic drug.

Keywords:Adriamycin, Antioxidant effect, Tecomastans, Methanolic extract, Nephropathy, Rat

INTRODUCTION

Regardless of etiology, glomerulosclerosis and tubule-interstitial fibrosis are the final common pathways of progression seen in most chronic renal diseases [1]. Nephropathy is characterized by specific renal alterations. Features of early renal changes are glomerular hyper filtration and hypertrophy and increased urinary albumin excretion. Advanced nephropathy is characterized by proteinuria, glycosuria, decline in renal function, increased blood creatinine or decreased creatinine clearance, glomerulosclerosis, and interstitial fibrosis.[1, 2] At present, diabetic kidney disease affects about 15-25% of Type 1 diabetic patients [3] 20-40% of patients with Type 2 diabetes,[4],[5] and 2% of patients with drug toxicity [6]. Thus, kidney diseases should be considered as a public health problem. Conventional treatment includes oral enzyme conversion inhibitors such as losartan. However, in places where safe

modern drugs and health centers are unavailable, the World Health Organization has suggested the use of indigenous plants as alternative medicine [7]. About 80% of rural African communities still use phytotherapy to control or treat many diseases.

Tecomastans (common name yellow bell) also known as yellow trumpet bush belongs to the family bignoniaceae. It is an ornamental plant. It is an erect, branched, sparingly hairy or nearly smooth shrub two to four meters in height. The leaves are opposite, odd-pinnate, Up to 20 centimeters in length with 5 to 7 leaflets. The leaflets are lanceolate to oblong-lanceolate, 6 to 13 centimeters long, pointed at both ends and toothed on the margins. Trumpet shaped flowers are yellow faintly scented and borne in short, dense, terminal clusters. The calys is green. 5 to 7 millimeters long and 5 toothed. Flowering can begin as early as April and continue in to fall. The flowers are followed by 6 inch long, tan pods that are filled with small, papery winged seeds. [8]

Leaves of Tecomastans contain the alkaloids tecomin and tecostamine are potent hypoglycaemic agent when given intravenously. Anthranilic acid is responsible for the anti-diabetic activity. Roots are powerful diuretic and vermifuge [9]. Tecoma is not a toxic because this plant is used in latine America as a remedy for diabetes and moreover for feeding cattle and goats in mexico [10]. The preliminary phytochemical screening of methanolic extract of flower extract of Tecoma stans showed the presence of flavaniods, phenol, alkaloids, tannins, steroids, triterpenes, anthraqunones and saponin0s etc.

MATERIALS AND METHODS

Plant extraction

The flowers of Tecoma Stan were collected in the month of May 2011 from Rasipuram (Namakkal District) Tamil Nadu. A herbarium specimen of the plant was deposited in the Department of Pharmacognosy. The plant was identified by Dr.G.V.S.Murthy, Joint Director of the Botanical Survey of India, Southern circle, TNAU Campus, Coimbatore, who authenticated the plant from information available in the literature. The flower petals were dried in the shade and then powdered and 100 g of the dried powder was extracted with methanol using a soxhlet apparatus. The solvent was removed under reduced pressure and controlled temperature using a rotary flash evaporator. Suspension METS in 2% (v/v) tween-80 was prepared for oral administration by gastric intubation method. The extract yielded 41.8 g (2.09%). Prior to the administration, the extract was dissolved in distilled water.

Preliminary Phytochemical Tests

Phytochemical constituents of the methanolic extract of T.stans were determined by standard methods using various reagents. [11] This included Mayer and Dragendoff's reagents for alkaloids, FeCl3 for tannin, frothing test for saponin, 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 METS was tested for its acute toxicity in mice. Five groups of six mice each were administered orally one of the different doses of the extract: 2, 4, 6, 8, and 10 g/kg body weight. Control group received only vehicle (water). 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, jerkiness, sensitivity to noise and touch, and respiratory rate. The lethal dose 50 (LD50) was determined with the following formulae. [12]

LD50 = Xs - d (Sp - ½)

Xs = Lethal dose 100; d = Interval between the doses

p = Death proportion per group; Sp = sum of death proportions

Induction of Renal Impairment

Male Wistar albino rats weighting 200-250 g, were used for this study. They were maintained under natural laboratory conditions (temperature and dark/light cycle) and allowed access to food and water ad libitum.

To induce the kidney disease, the rats anesthetized with ether received three intravenous (penile vein) injections (days 0, 14, and 28) of 2 mg/kg b.w. of adriamycin (2 mg/mL doxorubicin hydrochloride: Pharmacia Italia, S.P.A., Italy) in 9% NaCl. The control group received normal saline. Rats with proteinuria levels >3 g/L, serum creatinine >57 mmol/L, and creatinuria <10 mmol/L were used in the experiments as nephropathic rats.

Animal Treatment

The nephropathic rats were randomly divided into four groups of five animals each: one group of nephropathic control rats (NeC) received, as (five) normal control rats (NC), distilled water (5 mL/kg); two groups received 50 mg/kg b.w. (NeK50) and 68 mg/kg b.w. (NeK68) T.stansextract; the last group (standard reference: NeL10) received 10 mg/kg b.w. losartan (losartan + hydrochlorothiazide; HYZAAR, Merck Sharp and Dohmet-Chibret, MSD, Paris). The extract doses were obtained from the tradipractitioner method. The drugs were administered orally daily for 6 weeks.

Serum and Urine Samples

Urine and blood samples were obtained from each rat at day 0 and at 2 weeks interval thereafter until week 12. After 6 weeks of treatment, the rats were housed individually in the metabolic cages for 24 h for urine collection. Xylol was put in the collection container to prevent the evaporation. The rats were fasted for 24 h; spot urine samples were collected for protein, creatinine, urea, uric acid, sodium, and potassium level estimation. The biochemical assays were performed within 24 h of collection. The rats then were ether anesthetized, killed, and blood samples collected into normal tubes and were allowed to clot at room temperature. Serum was separated by centrifugation (3000 tr/min at 30°C, 10 min), aliquoted, and kept frozen at -20°C for the estimation of protein, creatinine, sodium, potassium, alkaline phosphatase, transaminase, urea, and uric acid levels within 3 months.

Serum and Urine Analysis

Glucose, total protein, creatinine, alkaline phosphatase, alanine aminotransferase (ALAT), urea, and uric acid levels were analyzed in serum using commercial diagnostic kits (Bio Direct Laboratories, La villeneuve-france and Elitect Laboratories, SEPPIMS A. France). Sodium and potassium were analyzed in blood and urine by a selective electrode ion autoanalyser (ILLYTE). Urinary creatinine, urea, uric acid, and glucose were estimated spectro-photometrically with commercially available kits (Biodirect and Elitech). The osmolality of plasma or urine was determined using the following formula [13].

Osmolality = [(Na+ + K+) × 2] (mEq/L) + [urea × 16] (g/L) + [glucose × 5.5] (g/L)

Statistical Analysis

The results were expressed as the mean ± SEM. These were analyzed statistically using one-way analysis of variance followed by Dunnett′s test using Graphpad Insad version 3.6 software. $P < 0.05$ was considered as statistically significant.

RESULTS

The preliminary phytochemical analysis revealed the presence of different classes of compounds such as tannins, phenols, sterol, anthraquinones, triterpens, phobotanins, and polyphenol in the METS.

In acute toxicity tests, METS (4, 6, 8, and 10 g/kg) reduced the sensitivity to noise and touch, jerkiness, lethargy, and caused soft feces and 66% mice died within 30 min of administration. The dose 10 g/kg caused 100% mortality and the LD50 was found to be 4.4 g/kg. There were no gross behavioral changes. Macroscopically, the organs (liver, kidney, heart) did not show any discoloration.

Animals with adriamycin-induced nephropathy showed a significant increase ($P < 0.01$) in the levels of creatinine, urea, and uric acid [Table - 1]. They also had increased urinary volume, protein, urea, uric acid, and glucose [Table - 2], while proteinuria and creatinuria decreased significantly ($P < 0.01$). The blood glucose level was not reduced significantly. The serum level of sodium and the urinary level of potassium also decreased significantly ($P < 0.01$), while blood potassium, urinary sodium and urinary Na+/K+ ratio [Figure - 1] increased significantly ($P < 0.01$). The blood osmolality was significantly decreased ($P < 0.05$) as compared to normal rats, while urine osmolality and urinary osmolality/blood osmolality ratio were significantly ($P < 0.01$) increased [Figure - 2]. The nephropathy also caused a significant increase ($P < 0.01$) of ALAT, alkaline phosphatase (ALP), and conjugate bilirubin levels in blood [Figure - 3] significantly ($P < 0.05$ and $P < 0.01$) reduced the blood creatinine, urea, uric acid, conjugate bilirubin, ALAT, ALP levels, and the urinary volume, protein, urea, uric acid, and glucose levels in urine of nephropathic rats. The proteinemia and the creatinuria were enhanced ($P < 0.01$). The extract and losartan have not changed the glycemia after 6 weeks treatment. In nephropathic rats the

METS and losartan have markedly (P < 0.01) increased the blood sodium and the urinary potassium level and decreased the blood potassium, urinary sodium level, as well as the Na+/K+ ratio was also decreased [Figure - 1].

Table 1: Effect of methanolic extract of T.stans on blood chemistry in adriamycin-induced nephropathic rats

Treatment	Protein (g/l)	Creatinine (□mol/l)	Urea (g/l)	Uric acid (mg/dl)	Glucose (mmol/l)
Normal control	48.64□1.71	55.98□0.22	1.5□0.1	2.87□0.31	4.14□0.18
Nephropathic control	31.84□0.07**	90.72□0.27**	4.53□0.1b*	6.49□0.12**	3.95□0.10
METS 50mg/kg	40.92□1.35[a*]	57.36□0.4[b]	2.59□0.06[b]	3.45□0.01[b]	4.10□0.13
METS 68mg/kg	48□0.54[b]	59.94□0.7[b*]	2.06□0.11[b]	3.26□0.21[b]	4.30□0.14
LOSARTAN 10mg/kg	41.65□0.68[b*]	62.67□0.44[b]	2.11□0.41[b]	3.85□0.26[b]	4.30□0.7

Data shown are mean □SEM (n=5), *P<0.05, **P<0.01 in comparison to normal control, [a]P<0.05 and [b]P<0.01in comparisons to nephropahtic control

Table 2: Effect of methanolic extract of T.stans on urinary chemistry in adriamycin-induced nephropathic rats.

Treatment	Urinary volume (ml/24h)	Protein (g/l)	Creatinine (□mol/l)	Urea (g/l)	Uric acid (mg/dl)	Glucosuria (mmol/l)
Normal control	3.04□0.11	1.84□ 0.04	11.2□ 0.71	20.10□ 0.23	211.01□ 4.63	0.30□ 0.1
Nephropahtic control	14.50□ 0.33**	4.99□ 0.11**	3.14□ 0.01**	360□ 1.94**	243.12□ 4.04**	3.45□ 0.21**
METS 50mg/kg	8.34□ 0.41b**	1.72□ 0.02b	8.21□ 0.50b*	23.50□ 0.15b	224.01□ 4.25b*	0.64□ 0.02b*
METS 68mg/kg	7.60□ 030b*	1.74□ 0.02b	8.32□ 0.98b*	22.20□ 0.27b	219.1□ 6.67b	0.80□ 0.04b*
LOSARTAN 10mg/kg	8.44□ 0.15b*	1.13□ 0.04b	9.65□ 0.25b	22.5□ 0.44b	230.1□ 4.07a*	0.73□ 0.04b*

Data shown are mean □SEM (n=5), *P<0.05, **P<0.01 in comparison to normal control, [a]P<0.05 and [b]P<0.01 in Comparisons to nephropahtic control

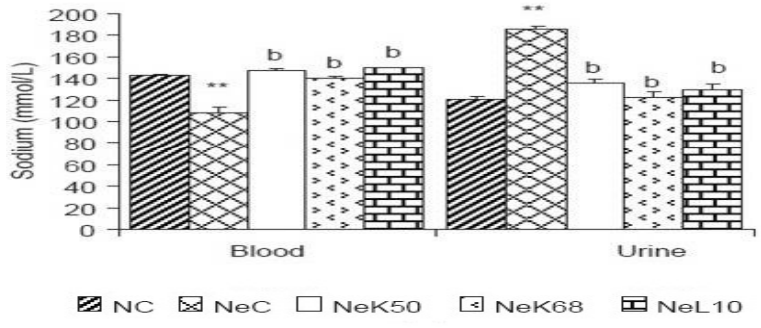

Figure 1a: Effect of methanolic extract of T.stans on sodium in adriamycin-induced nephropathy rats.

NC = normal control, NeC = nephropathic control, NeK50 = K crenata extract 50 mg/kg b.w., NeK68 = K. crenata extract 68 mg/kg b.w., NeL10 = losartan 10 mg/kg b.w. Data shown are mean ± SEM

(n = 5). *P < 0.05, **P < 0.01 in comparison to normal control, aP< 0.05 and bP< 0.01 in comparison to NeC

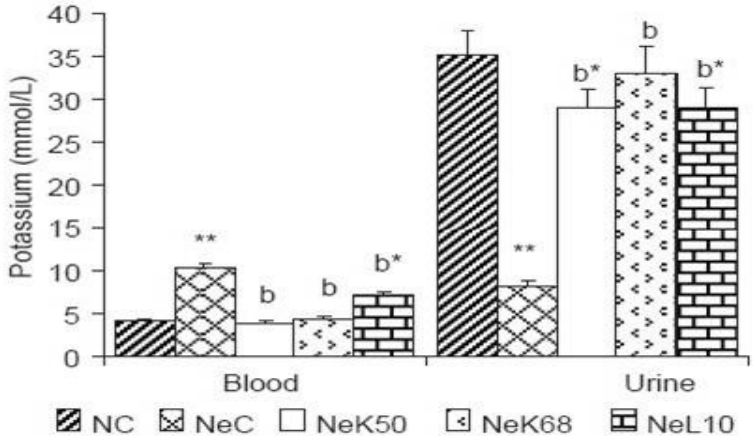

Figure 1b: Effect of methanolic extract of T.stans on potassium in adriamycin-induced nephropathy rats.

NC = normal control, NeC = nephropathic control, NeK50 = K crenata extract 50 mg/kg b.w., NeK68 = K. crenata extract 68 mg/kg b.w., NeL10 = losartan 10 mg/kg b.w. Data shown are mean ± SEM (n = 5). *P < 0.05, **P < 0.01 in comparison to normal control, aP< 0.05 and bP< 0.01 in comparison to NeC.

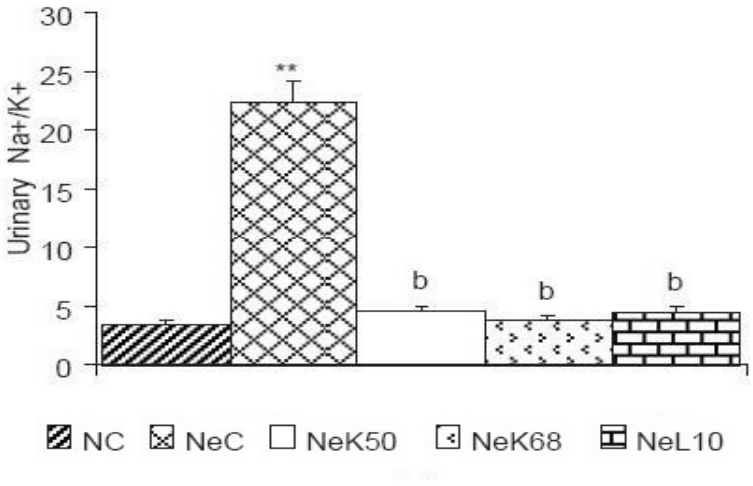

Figure 1c: Effect of methanolic extract of T.stans on urinary Na+/K+ ratio in adriamycin-induced nephropathy rats.

NC = normal control, NeC = nephropathic control, NeK50 = K crenata extract 50 mg/kg b.w., NeK68 = K. crenata extract 68 mg/kg b.w., NeL10 = losartan 10 mg/kg b.w. Data shown are mean ± SEM (n = 5). *P < 0.05, **P < 0.01 in comparison to normal control, aP< 0.05 and bP< 0.01 in comparison to NeC.

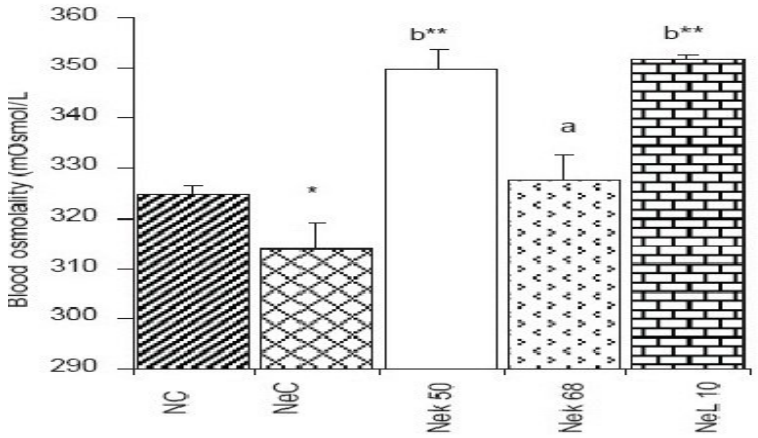

Figure 2a: Effect of methanolic extract of T.stans on blood osmolality in adriamycininduced nephropathic rats.

NC = normal control, NeC = nephropathic control, NeK50 = K crenata extract 50 mg/kg b.w., NeK68 = K crenata extract 68 mg/kg b.w., NeL10 = losartan 10 mg/kg b.w. Data shown are mean ± SEM (n = 5). *P < 0.05, **P < 0.01 in comparison to normal control, aP< 0.05 and bP< 0.01 in comparison to NeC.

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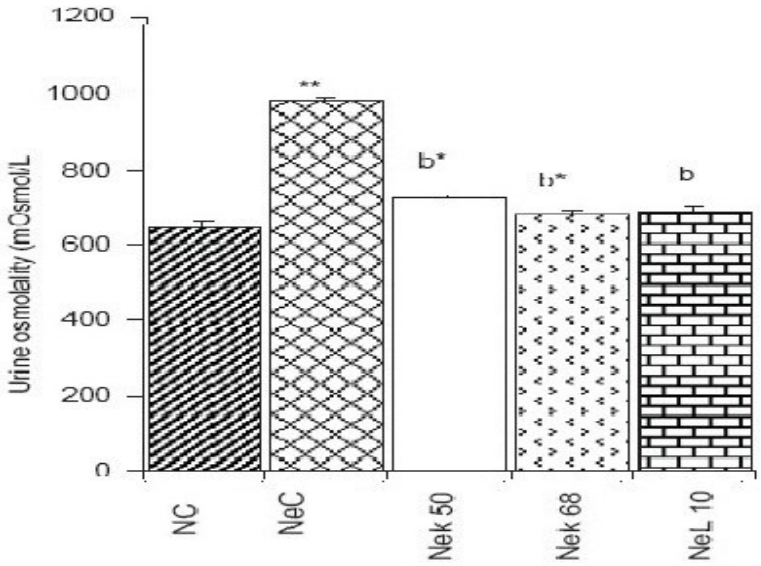

Figure 2b: Effect of methanolic extract of T.stanson urine osmolality in adriamycininduced nephropathic rats.

NC = normal control, NeC = nephropathic control, NeK50 = K crenata extract 50 mg/kg b.w., NeK68 = K crenata extract 68 mg/kg b.w., NeL10 = losartan 10 mg/kg b.w. Data shown are mean ± SEM (n = 5). *P < 0.05, **P < 0.01 in comparison to normal control, aP< 0.05 and bP< 0.01 in comparison to NeC.

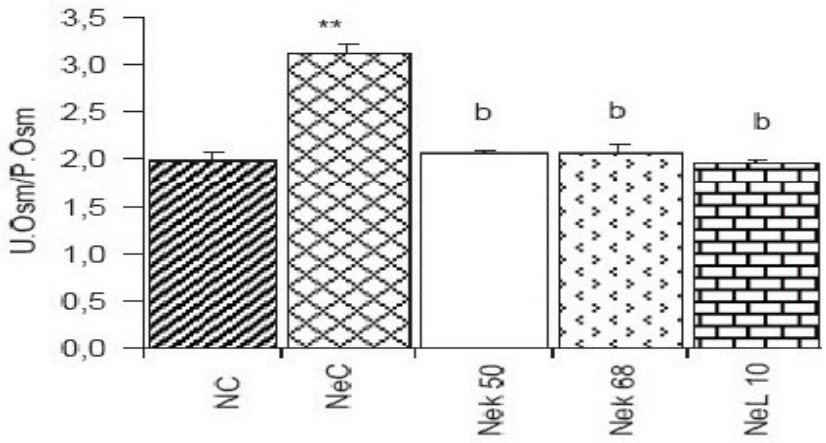

Figure 2c: Effect of methanolic extract of K crenata on urine/plasma osmolality ratio (c) in adriamycininduced nephropathic rats.

NC = normal control, NeC = nephropathic control, NeK50 = K crenata extract 50 mg/kg b.w., NeK68 = K crenata extract 68 mg/kg b.w., NeL10 = losartan 10 mg/kg b.w. Data shown are mean ± SEM (n = 5). *P < 0.05, **P < 0.01 in comparison to normal control, aP< 0.05 and bP< 0.01 in comparison to NeC.

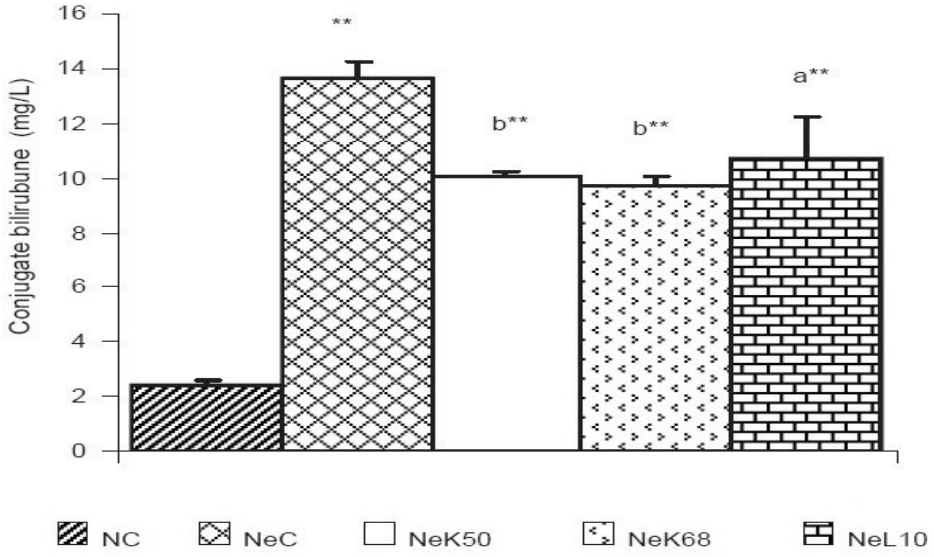

Figure 3a: Effect of methanolic extract of K crenata on conjugate bilirubin in adriamycin-induced nephropathic rats.

NC = normal control, NeC = nephropathic control, NeK50 = K crenata extract 50 mg/kg b.w., NeK68 = K crenata extract 68 mg/kg b.w., NeL10 = losartan 10 mg/kg b.w. Data shown are mean ± SEM (n = 5). *P < 0.05, **P < 0.01 in comparison to normal control, aP< 0.05 and bP< 0.01 in comparison to NeC.

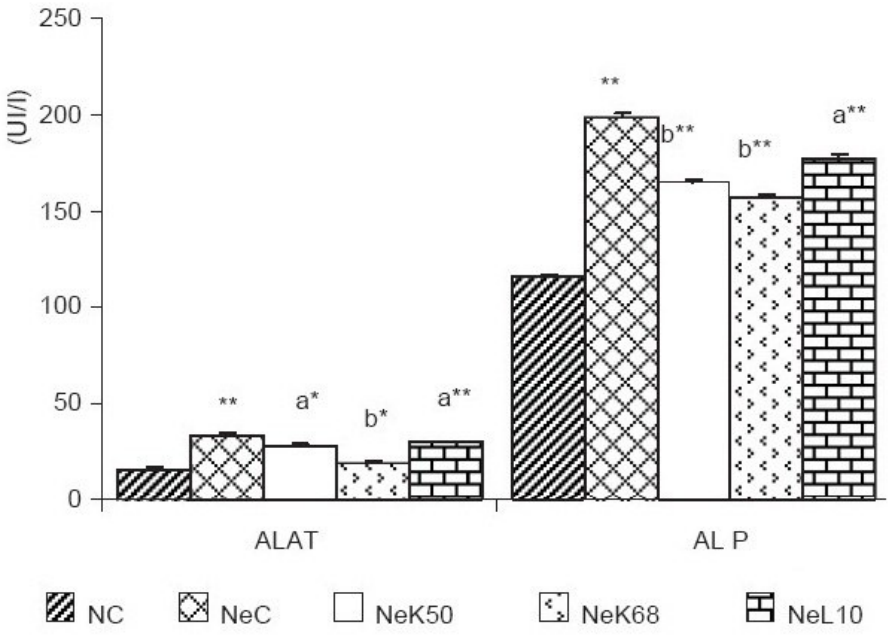

Figure 3c: Effect of methanolic extract of K crenata on alanine aminotransferase ALAT and alkaline phosphatase ALP in adriamycin-induced nephropathic rats.

NC = normal control, NeC = nephropathic control, NeK50 = K crenata extract 50 mg/kg b.w., NeK68 = K crenata extract 68 mg/kg b.w., NeL10 = losartan 10 mg/kg b.w. Data shown are mean ± SEM (n = 5). *P < 0.05, **P < 0.01 in comparison to normal control, aP< 0.05 and bP< 0.01 in comparison to NeC.

The METS 50 and 68 mg/kg b.w. and the 10 mg/kg b.w. losartan after 6 weeks of treatment have METS and losartan, after 6 weeks of administration; significantly (P < 0.01) reduced urine osmolality and urinary osmolality/blood osmolality ratio and enhanced the blood osmolality in nephropathic rats.

DISCUSSION

The objective of this work was to assess the effect of the METS on the renal and hepatic function in adriamycin-induced nephropathic rats. In the acute toxicity study, single oral dose of METS up to 2 g/kg was not lethal to both male and female mice. The apparent cause of death in mice at the higher doses may be due to respiratory depression or/and to methanol (solvent) poisoning, since earlier studies with aqueous methanol extract of T.stans did not show any overt sign or death in acute toxicity.8,14 These results suggest that METS possesses low toxicity since the LD50 is higher than 2 g/kg and inferior to 5 g/kg and represents 65 and 88 times, respectively, the assay doses. [15]

In adriamycin nephropathic rats, our results demonstrated hyperproteinuria (coupled with hypoproteinemia), hypercreatinemia, and increased urinary excretion of glucose, urea, and uric acid. Normally, the kidney excretes creatinine and only low amount of low-molecular weight protein passes through the glomerulus, whereas glucose, urea, and uric acid are reabsorbed by the proximal tubule. [15] Usually, hypercreatinemia (and hypocreatinuria) observed in nephropathic states are characteristics of glomerular hyperfiltration, [16, 17] and increased urinary glucose, urea, and uric acid levels indicate the alteration of tubular reabsorption. The METS showed a significant dose-dependent effect on protein excretion in nephropathic rats, similar to that of the losartan. The decrease of urinary level of proteins could be due, when compared with losartan effect, to a potential capability of the plant extract to restore the altered glomerular capillary function in

nephropathic rats and may be (like losartan) by the inhibition of angiotensin-converting enzyme or the blockage of angiotensin II receptors that reduce the capillary vessel contraction and hence decrease the retention of water and salt [18, 19] This could also explain the decreased level of urine volume and urinary sodium in nephropathic rats. As losartan, the extract also improved the filtration function and tubular reabsorption by normalizing glycosuria, urinary and blood protein, creatinine, urea, and uric acid level. The extract did not affect normal glycemia which confirmed that the plant has no hypoglycemic activity but an antihyperglycemic effect as described earlier in diabetic rats [8].

In nephropathic rats, the adriamycin caused a hydro electrolytic disorder due to the failure of tubular reabsorption causing sodium leak and potassium retention; this could explain the high ratio of Na+/K+.13 The blood decline and urinary increase of the osmolality (which is a consequence of ion movements) could mainly be related to the change in sodium levels in the two media. Thus, the increased urinary Na+ excretion, which may be due to the decrease of proximal reabsorption, also causes an increased urinary volume. [17] The increase of urinary osmolality might also be linked to the increase of the active osmotic substances: namely, Na+, glucose, and protein, which require important volume of water for their elimination. [17] In the treated rat, the decrease of urinary Na+, urinary osmolality, and urinary volume and the increase of blood potassium could then result from an improvement of the proximal tubular reabsorption by T.stans. The plant by normalizing the levels of sodium and potassium in the media also normalizes osmolality.

In the nephropathic rats, the enzymes (ALP and ALAT) and conjugate bilirubin level were increased. In normal case, the ALAT is found only in the cytoplasm and its blood level increases when the permeability of hepatic cell is affected. The blood proteins are mostly produced in the liver. In this study, decrease of proteinemia was observed in nephropathic control rat. The hypoproteinemia and the high level of blood enzymes may be the result of malfunctioning of liver cells20Low protein levels could also result due to dysfunctioning of the liver cells and the impaired renal function [21]. The increase of ALP results in the decrease or stoppage of the intestinal secretion of the bile, provoking the increase of blood conjugate bilirubin [13]. In nephropathic rats, the plant extract and losartan significantly increased blood prote in and decreased the levels of conjugate bilirubin, ALAT, and ALP. This indicates that T.stanscould prevent the alteration of the liver cell structure and/or function that could be induced by adriamycin.

In conclusion, methanolic extract of T.stans has improved the kidney and the liver function in nephropathic rats. T.stans extracts and hence shows a potential use in alternative medicine for the treatment or management of the kidney diseases.

REFERENCES

[1]. Fogo, AB., Kon, V. Pathophysiology of progressive renal diseases. An overview. In: Neilson EG, Couser WG (Eds.), Immunologic Renal Diseases. Philadelphia: Lippincott Williams and Wilkins, 2, 1987, 55-72.

[2]. Schnaper, HW. Focal segmental glomerulosclerosis. In: Neilson EG, Couser WG, (Eds.) Immunologic Renal Diseases. Philadelphia: Lippinott Williams and Wilkins, 2, 2004, 1001-27.

[3]. Hovind, P., Tarnow, L., Rossing, P., Eising, S., Larsen, N., Binder, C. Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes. Diabetes Care. 26, 2003, 1285-64.

[4]. Ritz E, Keller C, Bergis K, Strojek K. Pathogenesis and course of renal disease in IDDM/NIDDM: Differences and similarities. Am J hypertens, 10, 1997, 202S-7.

[5]. Yokoyama, H., Okudaira, M., Otani, T., Sato, A., Miura, J., Takaike, H. Higher incidence of diabetic nephropathy in type 2 than type 1 diabetes in early-onset diabetes in Japan. Kidney Int. 58, 2000, 302-11.

[6]. Winc, A,J., Brunner, FP., Geerlings, W., Broyer, M., Brynger, H., Fassbinder, W. Contribution of toxic nephropathies to end stage renal failure in Europe: A report from the EDTA-ERA registry. ToxicolLett. 46, 1986, 281-92.

[7]. WHO. Launches of the first global strategy on the traditional medicine, 38, 2002, WHO Press Release, 2.

[8]. Parrotta, J, A. Healing plants ofPennisularindia. CABI publishing, 2001, 701-02.

[9]. K.N.V Rao, Establishment of two varieties in Tecomastansofindian origin pharmacognostically and pharmacologically. J.Phytology. 2, 2010, 92-102.
[10]. Khare, CP. Indian medicinal plants and illustrated dictionary. Springer science publishers, new delhi, 2007.
[11]. Sofowora, A. Medicinal Plants and Traditional Medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd, 2, 1993, 1-153.
[12]. Molle, J. Limites de toléranceettoxicités de quelques amino-acides (formes L et D). In: Amino-peptides-protéines, Cahier N°4, 1986, 207-32.
[13]. Serge, B. Biochimieclinique. Instruments et technique de laboratoire. Diagnostics médico-chirurgicaux. Edition Maloine. 1985, 392.
[14]. Bruckner, JV, Kyle, GM., Luthra, R., Acosta, D., Mehta, SM., Sethuraman, S. Acute, short-term, and subchronic oral toxicity of 1, 1, 1-trichloroethane in rats. Toxicol Sci. 60, 2001, 363-72.
[15]. Viau, C., Tardif, R. Toxicologie. In: Gérin M, Gosselin P, Cordier S, Viau C, Quénel P, Dewailly E, (Eds). Environnement Santé publique. Fondementsetpratiques. Acton Vale, Vale: Edisem/Tec & Doc, 2003, 119-43.
[16]. Koshikawa, M., Mukoyama, M., Mori, K., Takyoshi, S., Kazutomo, S., Tetsuro, Y. Role of p38 Nitrogen-Activated protein kinase activation in podocyte. Injury and proteinuria in experimental nephritic syndrome. J Am Soc Nephrol, 16, 2005, 2690-701.
[17]. Frey, J., Daudon, M., Raby, N., Augereau, C., Dechaux, M., Diehl, JL.Valeurséméiologique des paramètresbiochimiquesurinaires. Ann BiolClin. 59, 2001, 13-25.
[18]. Kriz, W., LeHir, M. Pathways to nephron loss starting from glomerular diseases in sights from animal models. Kidney Int. 67, 2005, 404-19.
[19]. Harris, RC., Neilson, EG. Toward a unified theory of renal progression. Ann Rev Med, 57, 2006, 365-80.
[20]. Knuckles, M,E., Inyang, F., Ramesh, A. Acute and subchronic oral toxicity of benzo[a]pyrene in F-344 Rats. Toxico Sci. 61, 2001, 382-8.
[21]. Kaneko, J,J., Harvey, J,W., Bruss, M.. Clinical biochemistry of domestic animal. 5th ed. San Diego, USA, 5, 1997, 117-38.