RESEARCH ARTICLE

Effect of Extracts of *Boerhavia diffusa* and *Bryophyllum calycinum* on Serum Uromodulin and Biochemical Profile of Rats Having Adenine Induced Chronic Kidney Disease

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Abstract

The present instigation was carried out on 62 adult healthy male Wistar rats (12-15 weeks of age) to study the therapeutic efficacy of *Boerhavia diffusa* (BD, roots) and *Bryophyllum calycinum* (BC, leaves) on adenine induced chronic kidney disease (CKD) through evaluation of serum uromodulin and biochemical changes after therapy. The rats were randomly divided into 8 groups. Group I (n = 6) was given only distilled water and served as healthy control. Groups II to VIII (n = 8 each) were given adenine chemical @ 200 mg/kg body weight daily for 28 days by oral ravage needle to induce CKD. After 28th day, groups III and IV were given an aqueous and alcoholic extract of BD, group-V, and VI with aqueous and alcoholic extract of BC @ 300 mg/kg body weight daily for 42 days. Group VII was given bihebral aqueous extract of BC and BD (1:1.5), while group VIII received bihebral alcoholic extract of BC and BD (1:1.5) @ 300 mg/kg body weight daily for 42 days. Results of serum uromodulin and biochemistry revealed that aqueous and alcoholic extracts of both the plants possess good therapeutic/nephroprotective efficacy against CKD as reflected by a significant increase in the serum levels of uromodulin, ALT, BUN, uric acid, creatinine, and phosphorus, with a decreased level of total protein in the CKD induced groups as compared to the normal control group on 28th day followed by reversal towards normal values after 42 days of herbal therapy, *i.e.* on 70th day of an experiment, in all groups, and particularly in groups VII and VIII co-treated with aqueous and alcoholic extracts of both plants. The effect of biherbal alcoholic extract of the plants was much better in restoring the serum uromodulin and biochemical values of CKD-induced rats by 42 days of administration.

Keywords: *Boerhavia diffusa, Bryophyllum calycinum*, Herbal extracts, Nephroprotective effect, Serum biochemistry, Uromodulin, Wistar rats.

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Introduction

Chronic kidney disease (CKD) is a global health issue that affects mostly adults and is increasing in both developed and developing countries. The condition develops slowly over time and can lead to end-stage kidney disease (ESKD), which necessitates dialysis or transplantation to improve kidney functions. Cardiovascular disease is a major cause of increased morbidity and mortality in patients with CKD, but the correlation between CKD or Chronic Renal Failure (CRF), and cardiovascular disease is poorly understood (Diwan *et al*., 2017). Chronic renal injury as initiated by CKD is characterized by a variety of clinical symptoms, including renal detoxification potential loss, water-electrolyte metabolism disruption, acid-base equilibrium disturbance, and endocrine imbalance (Li *et al*., 2018). Uromodulin is a kidney specific protein that is released into the bloodstream by ascending loop of tubular cells. Uromodulin in serum/plasma/urine is used as a biomarker to assess kidney function and detect CKD at an early stage (Steubl *et al.,* 2016).

The plant *Boerhavia diffusa* (BD, family; Nyctaginaceae), also known as *Raktapunarnava*, is considered therapeutically effective for the treatment of inflammatory renal disorders and nephrotic syndrome. Pharmacological studies have shown that root of *B. diffusa* contains punarnavocide, which has diuretic, antihypertensive, anti-inflammatory, and antibacterial activities. It has also been documented that

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BD has regenerative effects on the kidneys (Oburai *et al*., 2015). Many experimental studies have shown its diuretic and neuroprotective effect against acetaminophen-induced renal damage (Sawardekar and Patel, 2015). The plant, *Bryophyllum calycinum* (BC, family; Crassulaceae), also known as air plant, love plant, miracle weed, life plant, panfutti, and ghayamari, has been used as a herbal remedy for the treatment of kidney/bladder stones in almost all part of the world. It grows widely and is used as indigenous medicine. The antioxidant and oxidative radical scavenging activities of BC leaf juice protect rats from gentamicin-induced nephrotoxicity. It is also used to cure kidney stones in India, where it is known as Pather Chat or Panfutti. In male Wistar rats, an aqueous and alcoholic extract of the leaves of the plant BC demonstrated diuretic and antiurolithiatic function (Yadav *et al*., 2019). Despite a large amount of work done in CKD using a wide range of herbal extracts, there is still a lot to understand the effects systematically. The current work was done to understand the effect of aqueous and alcoholic extracts of BD and BC on serum uromodulin and the biochemical profile of Wistar rats having adenine-induced CKD.

Materials and Methods

Preparation of Plant Extracts

BD root and BC leaves were dried in a hot air oven and grinded with a mechanical grinder. In the Soxhlet apparatus, 100 g of coarse powdered material from both the plants was extracted with water and alcohol (at 80°C and 85°C respectively for 24 hours. To obtain a solid residue, the extracts were evaporated under reduced pressure. The aqueous and alcoholic extracts (solid residues) were preserved in a refrigerator at 4°C for further use.

Animals and Experimental Design

The project was approved by the Institutional Animal Ethics Committee (IAEC) of the College (Approval No: AAU/GVC/CPCSEA/IAEC/341/2021, dated 22/04/2021). The work was carried out on 62 healthy mature (12 to 15 weeks old) male Wistar rats. The rats were divided randomly into eight groups (n = 6, in group I and n = 8 in others groups) and were kept in separate cages from one week prior to the experiment at the small animal house facility, which was environmentally controlled by maintaining 30 to 70% humidity and 22 ± 3°C temperature. Light/dark cycles for 12 hrs were provided throughout the experiment. Standard pellet diet procured from Keval Sales Corporation, Laboratory Animal Feed Suppliers, Vadodara, Gujarat, was given to rats regularly. Rats were provided drinking water *ad libitum* throughout the study.

The rats of group I and II served as Normal and Adenine control, respectively. Group I (n = 6) was given only distilled water and served as healthy control. In group II to VIII (n = 8 each), CKD was induced by adenine administration @ 200 mg/kg, b.wt. along with drinking water daily for 28 days. After the 28th day, the rats of CKD induced groups III and IV received an aqueous and an alcoholic extract of BC @ 300 mg/kg b.wt, respectively. In comparison, groups V and VI received an aqueous and an alcoholic extract of BD @ 300 mg/kg b.wt., respectively, in 0.5% sodium bicarbonate using a syringe and rat gavage needle for the next 42 days. The groups VII and VIII received a combination of bi-herbal aqueous and bi-herbal alcoholic extracts (1: 1.5) of two plants, respectively, @300 mg/kg b.wt., in 0.5% sodium bicarbonate as above for the next 42 days, *i.e*., till day 70th of the experiment.

Blood Sampling and Serum Uromodulin and Biochemical Assays

Blood samples were collected from all of the rats through retro-orbital plexuses punctured under mild isoflurane anesthesia using a capillary hose three times during the experimental period, *i.e.,* on the first day ('0' day), on the 28th day of CKD induction, and on 70th day (*i.e.,* 42nd day after treatment). Blood samples (2 mL) were collected without anticoagulant and were allowed to clot at room temperature (26 ± 2°C). Serum was harvested by centrifugation at 3000 rpm for 15 minutes at 10°C (Eppendorf 5804 R, Germany) and stored at –40°C until analyzed for total protein (g/dL), albumin (g/dL), alanine amino transferase (ALT, U/L), aspartate aminotransferase (AST, U/L), blood urea nitrogen (BUN, mg/dL), uric acid (mg/dL), creatinine (mg/dL), calcium (mg/dL) and phosphorus (mg/dL) using standard procedures and assay kits on the Clinical Serum Biochemistry Analyzer (CKK 300).

Serum uromodulin concentration was estimated using uromodulin ELISA, MBS2024146, 96 tests Kit as per the manufacturer's instructions (Patel *et al*., 2020). It is a sandwich enzyme immunoassay for *in vitro* quantitative measurement of uromodulin in biological fluids. Uromodulin in plasma is used as a biomarker to assess kidney function and detect CKD at an early stage (Steubl *et al.,* 2016).

Statistical Analysis

The data was analyzed using one-way analysis of variance (ANOVA) for between-group and between period within the group effects employing software SPSS version-20 (Snedecor and Cochran, 1994). The values at $p < 0.05$ were taken to indicate statistically significant differences.

Results and Discussion

The findings on renal function evaluated by measuring the serum uromodulin and biochemical profile in group I to VIII of experimental rats are presented in Tables 1 to 4.

There was no change in serum uromodulin concentration mean values in normal control group I between days 0, 28 and 70 (Table 1). The mean uromodulin concentrations in adenine control group II and therapeutic Groups III to VIII were significantly increased ($p < 0.05$) on the 28th day of adenine

Table 1: Comparison of mean (± SE) serum concentrations of uromodulin in rats under different CKD treatment and control groups

		Serum Uromodulin (ng/mL)		
Group	*Group name*	*'0' day*	*28th day*	*70th day*
I	Normal control	5.34 ± 0.23	5.40^{a} ± 0.21	5.35^{a} ± 0.28
II	Adenine control	4.97_{x} ± 0.33	$10.71^{b}{}_{y}$ ± 0.56	$10.45^{c}{}_{y}$ ± 0.24
III	AQ. EX. BC.	5.14_{x} ± 0.28	$11.16^{b}{}_{z}$ ± 0.41	$7.63^{b}{}_{y}$ ± 0.24
IV	AL.EX. BC.	5.21_{x} ± 0.24	11.33^{b} z ± 0.60	7.61^{by} ± 0.28
V	AQ.EX. BD.	5.39_{x} ± 0.15	$11.46^{b}{}_{z}$ ± 0.68	$7.20^{b}{}_{y}$ ± 0.32
VI	AL.EX. BD.	5.32_{x} ± 0.41	$11.35^{b}{}_{y}$ ± 0.14	$6.56^{b}{}_{x}$ ± 0.15
VII	BI AQ EX (BC+BD)	5.10_{x} ± 0.37	$11.09^{b}{}_{y}$ ± 0.04	$5.36^{a}{}_{x}$ ± 0.19
VIII	BI AL EX (BC+BD)	5.29_{x} ± 0.39	$11.10^{b}{}_{y}$ ± 0.90	$5.39^{a}{}_{x}$ ± 0.82

B.C. = *Bryophyllum calcynium*; B.D.= *B. diffusa.* Means with different superscripts (a, b, c, d) within the *column* and subscripts (x, y, z) within the *row* differ significantly ($p < 0.05$).

treatment compared to '0' day values. It then declined slightly on 70th day in adenine control group II, where no subsequent prophylactic treatment was given. However, the rats of groups III to VIII showed a significant ($p < 0.05$) decrease in values on 70th day of treatment in comparison to 28th day. These observations indicated the effect of alcoholic and aqueous extracts of *Bryophyllum calcynium* and *B. diffusa* on adenine induced rats. Further on 70th day, the value of uromodulin was significantly higher in adenine control group II as compared to normal control group I and the values among different herbal treatment groups were intermediate of group I and II, and did not differ significantly among each other, indicating that both the herbas either single or in combination had nephroprotective and curing effect of CKD. These finding concurred well with the earlier reports of Gehani *et al.* (2019). Skowron *et al*. (2018) studied various biomarker concentrations in acute kidney injury induced by pyelonephritis in a rat model and reported that uromodulin rapidLy increased at 21 days post-infection in pyelonephritis induced rats. Uromodulin in plasma is used as a biomarker to assess kidney function and detect CKD at an early stage (Steubl *et al.,* 2016). Patel *et al*. (2020) reported significant increase in the mean values of granulocytes in adenine to induce rats, while normal values were found in prophylactic groups treated with adenine along with biherbal alcoholic and aqueous extract of *Boerhavia diffusa* and *Tribulus terrestris*.

Furthermore, significantly ($p < 0.05$) increased levels of serum ALT, BUN, creatinine, phosphorus, and uric acid, and decreased level of serum total protein were observed in all the CKD induced groups by day 28 of adenine treatment as compared to day 0 values of respective groups, and those of normal healthy control group I (Table 2 to 4). This alteration in serum biochemical profile proved that the adenine dosing @ 200 mg/kg b.wt. along with drinking water for 28 days damaged kidney function by inducing CKD in Wistar rats. These observations were in accordance with the reports of Mashiyava *et al.* (2016) Tani *et al.* (2017), Rahman *et al*. (2018), Patel *et al*. (2018 and 2020), and Gehani *et al*. (2019 and 2020) for one or the other constituents. The increased serum ALT might be due to leakage into the general circulation from the collateral circulation (Ali *et al.,* 2016; Gehani *et al*. (2019). The present findings of non-significant alterations in AST were in line with reports of Chang *et al*. (2017) and Thakur *et al*. (2018) in adenine induced rats.

Administration of either single extract or the co-treatment subsequently with aqueous and alcoholic extracts of BC and BD for 42 days significantly ($p < 0.05$) restored the biochemical changes, *i.e.,* by 70th day of the experiment in most of the groups. Shanmugapriya *et al*. (2015), Sawardekar and Patel (2015), and Karwasra *et al*. (2016) also found marked detritions of renal function, characterized by a significant increase in BUN and creatinine in acetaminophen induced CKD rats, that were significantly ($p < 0.01$) attenuated by pre-treatment with *B. diffusa* extract. Adenine increases BUN, uric acid and creatinine, and causes azotemia. A possible pathophysiological mechanism may be that excretion of nitrogen compounds is suppressed by renal tubular occlusion due to 2, 8-dihydroxyadenine (DHOA), a constituent of the stone in adenine induced rats. This leads to accumulation of urea nitrogen and creatinine in blood. Decreased level in treatment groups than in adenine control group might be attributed to the anti-inflammatory, antioxidant, antilithiatric and diuretic effects of these plants for their compounds such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins.

Bi-herbal alcoholic extract compared to mono-herbal extract of the said plants was better in restoring the values of serum total protein, ALT, BUN, creatinine, phosphorus, and uric acid. The serum ALT and total protein values came down nearer to the vehicle control group by the 70th day (Table 2-4). Tani *et al.* (2017) and Rahman *et al*. (2018) recorded a significantly elevated level of serum creatinine in the adenine model rats. Tani *et al.* (2017) and Gehani *et al.* (2019) also reported a significant increase in serum phosphorus level in CKD induced rats that decreased after treatment with extracts of BC and BD, however, they did not observe a significant change in serum calcium level. It was concluded

Table 2: Mean (± SE) values of serum total protein, albumin and alanine aminotransferase levels in rats under different herbal CKD treatment and control groups on day 0, 28th and 70th of experiment.

		Serum Total Protein (g/dL)			*Serum Albumin (g/dL)*			*Serum ALT (U/L)*		
Group	*Group name*	*'0' day*	*28th day*	*70th day*	*'0' day*	*28th day*	*70th day*	*'0' day*	*28th day*	*70th day*
I	Normal control	$06.76^{c} \pm 0.34$	$6.49^{c} \pm 0.25$	$6.98^{c} \pm 0.62$	4.84 ± 0.18	4.73 ± 0.31	4.98 ± 0.19	$26.33^{a} \pm 0.46$	$26.16^{a} \pm 0.81$	$25.98^{a} \pm 4.23$
II	Adenine control	$6.07^{a}_{y} \pm 0.21$	$4.86^{a}_{x} \pm 0.14$	$4.65^{a}_{x} \pm 0.42$	4.62 ± 0.16	4.76 ± 0.14	4.32 ± 0.22	$26.16^{a}_{x} \pm 0.52$	$51.05^{b}_{y} \pm 4.00$	$52.23^{c}_{y} \pm 7.43$
III	AQ. EX. BC.	$6.58^{ab}_{z} \pm 0.14$	$4.84^{a}_{x} \pm 0.17$	$5.71^{ab}_{y} \pm 0.55$	4.71 ± 0.21	4.18 ± 0.17	4.20 ± 0.40	$27.50^{ab}_{x} \pm 0.66$	$52.50^{b}_{z} \pm 6.33$	$38.54^{bc}_{y} \pm 1.71$
IV	AL.EX. BC.	$6.59^{c}_{z} \pm 0.11$	$4.85^{a}_{x} \pm 0.16$	$5.72^{ab}_{y} \pm 0.40$	4.50 ± 0.14	4.74 ± 0.21	4.59 ± 0.28	$32.83^{a}_{x} \pm 2.64$	$50.16^{b}_{y} \pm 3.84$	$35.26^{bc}_{x} \pm 4.79$
V	AQ.EX. BD.	$6.45^{bc}_{z} \pm 0.04$	$5.45^{bc}_{x} \pm 0.19$	$6.26^{bc}_{y} \pm 0.42$	4.64 ± 0.17	4.27 ± 0.17	4.94 ± 0.31	$31.39^{ab}_{x} \pm 1.56$	$52.61^{b}_{y} \pm 4.21$	$31.26^{bc}_{x} \pm 3.54$
VI	AL.EX. BD.	$6.99^{d}_{z} \pm 0.03$	$5.53^{abc}_{x} \pm 0.20$	$6.05^{abc}_{y} \pm 0.30$	4.50 ± 0.15	4.68 ± 0.16	4.31 ± 0.16	$24.94^{a}_{x} \pm 0.74$	$52.39^{b}_{y} \pm 3.34$	$29.54^{bc}_{x} \pm 4.46$
VII	BI AQ EX (BC+BD)	$6.53^{bc}_{z} \pm 0.06$	$4.68^{a}_{x} \pm 0.12$	$5.67^{ab}_{y} \pm 0.34$	5.07 ± 0.20	4.95 ± 0.21	4.69 ± 0.23	$31.39^{a}_{x} \pm 1.52$	$50.61^{b}_{y} \pm 3.28$	$32.83^{bc}_{x} \pm 2.70$
VIII	BI AL EX (BC+BD)	$6.43^{bc}_{z} \pm 0.14$	$5.24^{ab}_{x} \pm 0.10$	$5.84^{ab}_{y} \pm 0.37$	4.82 ± 0.15	5.01 ± 0.18	4.83 ± 0.13	$25.50^{a}_{x} \pm 1.88$	$51.72^{b}_{z} \pm 2.76$	$32.50^{bc}_{y} \pm 1.73$

B.C. =*Bryophyllum calcynium*; B.D.= *Boerhavia diffusa.*
Means of a trait with different superscripts (a, b, c, d) within the column and subscripts (x, y, z) within the row differ significantly ($p < 0.05$).

Table 3: Mean (± SE) values of serum aspartate aminotransferase, blood urea nitrogen and uric acid levels in rats under different herbal CKD treatment and control groups on day 0, 28th and 70th of experiment.

		Serum AST (U/L)			*BUN (mg/dL)*			*Serum uric acid (mg/dL)*		
Group	*Group name*	*'0' day*	*28th day*	*70th day*	*'0' day*	*28th day*	*70th day*	*'0' day*	*28th day*	*70th day*
I	Normal control	53.83 ± 1.62	53.92 ± 1.41	52.83 ± 1.45	23.87 ± 1.01	$24.83^{a} \pm 0.60$	$24.33^{a} \pm 0.71$	1.61 ± 0.33	$1.73^{a} \pm 0.22$	$1.93^{b} \pm 0.19$
II	Adenine control	52.86 ± 0.67	53.12 ± 0.67	53.22 ± 0.43	$21.89_{x} \pm 0.56$	$145.11^{b}_{y} \pm 6.59$	$142.00^{f}_{y} \pm 1.90$	$1.48_{x} \pm 0.25$	$3.10^{d}_{y} \pm 0.58$	$3.14^{c}_{y} \pm 0.52$
III	AQ. EX. BC.	52.12 ± 1.68	52.90 ± 2.80	53.28 ± 1.8	$22.00_{x} \pm 1.09$	$148.44^{b}_{z} \pm 9.39$	$67.29^{e}_{y} \pm 3.48$	$1.22_{x} \pm 0.55$	$2.50^{bc}_{y} \pm 0.58$	$1.68^{b}_{x} \pm 0.13$
IV	AL.EX. BC.	52.79 ± 0.56	51.67 ± 0.60	52.04 ± 2.10	$20.11_{x} \pm 0.95$	$135.33^{b}_{z} \pm 6.72$	$57.00^{cde}_{y} \pm 7.58$	$1.38_{x} \pm 0.18$	$2.60^{bcd}_{z} \pm 0.62$	$1.93^{b}_{y} \pm 0.46$
V	AQ.EX. BD.	52.93 ± 1.40	52.07 ± 1.06	52.75 ± 0.13	$21.33_{x} \pm 0.57$	$148.33^{b}_{z} \pm 7.39$	$44.50^{bc}_{y} \pm 2.74$	$1.51_{x} \pm 0.30$	$3.17^{d}_{y} \pm 0.58$	$1.55^{ab}_{x} \pm 0.19$
VI	AL.EX. BD.	53.87 ± 0.45	52.11 ± 2.30	52.65 ± 1.76	$20.22_{x} \pm 0.54$	$125.67^{b}_{z} \pm 10.98$	$49.14^{bcd}_{y} \pm 5.28$	$1.69_{x} \pm 0.77$	$3.00^{cd}_{y} \pm 0.54$	$1.54^{ab}_{x} \pm 0.20$
VII	BI AQ EX (BC+BD)	52.74 ± 2.63	52.77 ± 2.52	52.96 ± 0.06	$22.78_{x} \pm 0.70$	$142.78^{b}_{z} \pm 10.17$	$62.42^{de}_{y} \pm 8.20$	$1.45_{x} \pm 0.21$	$2.14^{ab}_{y} \pm 0.52$	$1.73^{b}_{x} \pm 0.43$
VIII	BI AL EX (BC+BD)	53.66 ± 1.75	53.89 ± 2.37	53.09 ± 0.63	$21.56_{x} \pm 0.82$	$139.44^{b}_{z} \pm 5.36$	$37.57^{ab}_{y} \pm 1.76$	$1.37_{x} \pm 0.34$	$2.39^{b}_{y} \pm 0.56$	$1.19^{a}_{x} \pm 0.42$

B.C. =*Bryophyllum calcynium*; B.D.= *Boerhavia diffusa.*
Means of a trait with different superscripts (a, b, c, d) within the column and subscripts (x, y, z) within the row differ significantly ($p < 0.05$).

Table 4: Mean (± SE) values of serum creatinine, calcium and phosphorus levels in rats under different herbal CKD treatment and control groups on day 0, 28th and 70th of experiment.

Group	Group name	Serum Creatinine (mg/dL)			Serum Calcium (mg/dL)			Serum Phosphorus (mg/dL)		
		'0' day	*28th day*	*70th day*	*'0' day*	*28th day*	*70th day*	*'0' day*	*28th day*	*70th day*
I	Normal control	0.44 ± 0.08	$0.53^{a} \pm 0.06$	$0.57^{a} \pm 0.10$	6.22 ± 0.90	6.02 ± 0.78	6.30 ± 0.69	6.82 ± 0.26	$6.86^{a} \pm 0.27$	$6.52^{ab} \pm 1.76$
II	Adenine control	$0.51_{x} \pm 0.56$	$4.18^{b}{}_{y} \pm 0.49$	$4.71^{c}{}_{y} \pm 0.81$	6.21 ± 0.47	6.62 ± 0.44	5.80 ± 0.38	$6.39_{x} \pm 0.25$	$7.99^{b}{}_{y} \pm 0.87$	$7.73^{c}{}_{y} \pm 0.47$
III	AQ. EX. BC.	$0.49_{x} \pm 0.94$	$4.06^{b}{}_{z} \pm 0.36$	$1.30^{b}{}_{y} \pm 0.21$	5.90 ± 0.26	6.54 ± 0.23	6.04 ± 0.27	$5.97_{x} \pm 0.14$	$10.06^{c}{}_{y} \pm 0.82$	$6.03^{a}{}_{x} \pm 0.91$
IV	AL.EX. BC.	$0.60_{x} \pm 0.36$	$3.58^{b}z \pm 0.38$	$1.25^{b}{}_{y} \pm 0.20$	5.89 ± 0.41	5.82 ± 0.37	5.76 ± 0.35	$6.07_{x} \pm 0.25$	$8.49^{b}{}_{y} \pm 0.48$	$6.78^{ab}{}_{x} \pm 0.18$
V	AQ.EX. BD.	$0.51_{x} \pm 0.98$	$3.34^{b}{}_{y} \pm 0.37$	$0.94^{ab}{}_{x} \pm 0.16$	6.05 ± 0.46	5.68 ± 0.89	6.05 ± 0.25	$6.05_{x} \pm 0.32$	$9.14^{bc}{}_{y} \pm 0.23$	$6.79^{ab}{}_{x} \pm 0.71$
VI	AL.EX. BD.	$0.59_{x} \pm 0.61$	$3.37^{b}{}_{z} \pm 0.31$	$1.18^{b}{}_{y} \pm 0.16$	6.20 ± 0.59	5.98 ± 0.79	5.45 ± 0.09	$6.18_{x} \pm 0.16$	$7.98^{b}{}_{y} \pm 0.46$	$6.35^{ab}{}_{x} \pm 0.39$
VII	BI AQ EX (BC+BD)	$0.58_{x} \pm 0.26$	$3.91^{b}{}_{z} \pm 0.49$	$1.33^{b}{}_{y} \pm 0.34$	5.89 ± 0.20	5.90 ± 0.16	6.26 ± 0.28	$6.15_{x} \pm 0.56$	$7.97^{b}{}_{y} \pm 0.87$	$6.09^{a}{}_{x} \pm 1.06$
VIII	BI AL EX (BC+BD)	$0.47_{x} \pm 0.07$	$3.88^{b}{}_{y} \pm 0.39$	$0.78^{ab}{}_{x} \pm 0.13$	6.01 ± 0.52	5.76 ± 0.44	6.20 ± 0.52	$6.67_{x} \pm 0.42$	$9.29^{bc}{}_{y} \pm 0.71$	$6.81^{ab}{}_{x} \pm 0.42$

B.C. =*Bryophyllum calcynium*; B.D.= *Boerhavia diffusa.*
Means of a trait with different superscripts (a, b, c, d) within the column and subscripts (x, y, z) within the row differ significantly ($p < 0.05$).

that alcoholic extract of BD and BC at the dose rate of 300 mg/kg b.wt., compared to aqueous extracts and single herbal aqueous and alcoholic extract of the said plants for 42 days orally was much better in restoring the changes in biochemical parameters in adenine induced CKD Wistar rats.

Conclusions

The serum uromodulin and biochemical analysis showed that aqueous and alcoholic extracts of *Bryophyllum calycinum* (BC leaves) and *Boerhavia diffusa* (BD roots) possess good therapeutic efficacy against CKD. The effect of bi-herbal alcoholic extract of the selected plants, *i.e.,* BD and BC was much better in restoring the serum uromodulin and biochemical values of CKD induced rats by 42 days of administration. The results showed some key inferences about the therapeutic values of the BD and BC in curing adenine induced CKD in rats.

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