RESEARCH ARTICLE

Effect of *Boerhavia diffusa* and *Bryophyllum calycinum* Extracts on Urine Profile in Male Wistar Rats having Adenine-induced Chronic Kidney Disease

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Abstract

The study was conducted on 62 adult healthy male Wistar rats (12-15 weeks of age) to examine the effect of herbal extracts on urine profile following adenine-induced chronic kidney disease (CKD). The rats were randomly divided into eight groups. Group I (n=6) was given only distilled water and served as healthy control. The rats of groups II to VIII (n=8 each) were given adenine @ 200 mg/kg body weight daily by oral gavage needle for 28 days to induce CKD. After 28th day, group II served as CKD positive group, while groups III and IV were given aqueous and alcoholic extract of *Boerhavia diffusa (BD)*, group-V and VI with aqueous and alcoholic extract of *Bryophyllum calycinum (BC)* @ 300 mg/kg body weight daily for 42 days. Group VII was given bihebral aqueous extract of BC plus 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. Urine parameters were studied on day 0, 28 and 70 of experiment to confirm the presence of CKD. Significantly ($p < 0.05$) increased levels of urine output, urine specific gravity, urine calcium, phosphorus, and total protein, with decreased levels of urine creatinine and urine pH were observed in all CKD groups as compared to normal control group by 28th day. These changes were significantly ($p < 0.05$) reverted to normal or near normal levels within next 42 days of daily administration of either single aqueous/alcoholic extract or a combination as biherbal extract (1:1.5), without statistical differences among formulations regarding their therapeutic/nephroprotective effect against CKD in terms of reducing the altered urine values.

Keywords: *Boerhavia diffusa, Bryophyllum calycinum*, Herbal extracts, Nephroprotective effect, Urine analysis, Wistar rats.

Ind J Vet Sci and Biotech (2022): 10.21887/ijvsbt.18.3.11

Introduction

Chronic kidney disease (CKD) is a worldwide health problem that primarily affects adults and is on the rise in both developed and developing nations. The condition worsens over time and can progress to end-stage kidney disease (ESKD), which requires dialysis or kidney transplantation to improve kidney function (Diwan *et al*., 2017). Chronic renal injury as initiated by CKD is characterized by a variety of clinical symptoms including loss of renal detoxification potential, disruption of water-electrolyte metabolism, and disturbance in acid-base equilibrium, and endocrine imbalance (Li *et al*., 2018).

Earlier, Singh *et al*., 2022 have investigated the effect of extracts of medicinal plants, *Bryophyllum calycinum* and *Boerhavia diffusa* on serum uromodulin and serum biochemistry of CKD induced Wistar rats. These plants contain a large number of phytochemical constituents with diuretic, purgative, laxative, anti-asthmatic, hepatoprotective, and anti-allergic properties (Karwasra *et al.,* 2016; Gehani *et al*., 2020; Patel *et al*., 2020). The present study was aimed to report the nephroprotective effect of herbal extracts of *B. calycinum* and *B. diffusa* through urine analysis following adenine induced chronic kidney disease in male Wistar rats.

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How to cite this article: Singh, M., Raval, S.K., Modi, R.J., Sadhu, D., Dave, K. (2022). Effect of *Boerhavia diffusa* and *Bryophyllum calycinum* Extracts on Urine Profile in Male Wistar Rats having Adenine-induced Chronic Kidney Disease. Ind J Vet Sci and Biotech. 18(3), 49-52.

Source of support: Nil

Conflict of interest: None.

Submitted: 11/01/2022 **Accepted:** 21/03/2022 **Published:** 10/07/2022

Materials and Methods

Animals and Experimental Design

The research work was carried out on 62 healthy mature (12-15 weeks old) male Wistar rats following approval of

Institutional Animal Ethics Committee of the College, dated 22/04/2021. The rats were divided randomly into eight groups (n=6, in group I and n=8 in other 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 30–70 % humidity and 22 ± 3°C temperature. Light/dark cycles for 12 hours 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 healthy (given only distilled water) and adenine induced CKD control groups, respectively. In group II to VIII (n=8 each), CKD was induced by administration of adenine @ 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 *B. calycinum* leaves (BC) @ 300 mg/kg b.wt, respectively, while groups V and VI received an aqueous and an alcoholic extract of *B. diffusa* roots (BD) @ 300 mg/kg b.wt., respectively, in 0.5% sodium bicarbonate using 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 of BC plus BD (1: 1.5), 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.

The aqueous and alcoholic extracts (solid residues) of BD root and BC leaves were prepared by drying the materials in a hot air oven and grinding with a mechanical grinder. 100 g of coarse powdered material from both the plants was then extracted with water and alcohol for 24 hours in the Soxhlet apparatus. The extracts were evaporated at a low pressure to obtain the aqueous and alcoholic extracts (solid residues), which were refrigerated at 4°C for further use.

Urine Collection and Analyses

Before and after induction of CKD, *i.e.,* on day 0 and 28, all the rats were kept in individual metabolic cages, and urine samples were collected over 24 hours for determining the daily urine output (volume of urine voided). During the urine collection period, the rats had free access to drinking water. After herbal treatment, *i.e.,* on day 70, again the urine samples were collected as above. Urine pH and specific gravity were determined in fresh samples by using Urine Analyzer (Uriscan Optima II) and URISCAN 10 SGL Strip (YD Diagnostics CORP, Korea). The urine samples were then stored at -20°C with a drop of concentrated hydrochloric acid. The stored samples were subjected to estimation for urine calcium, phosphorous, creatinine, uric acid, and total protein by using kits on standard auto-analyzer (CKK-300 Ark diagnostics, Bangalore, Karnata). The data were analyzed statistically using a one-way-analysis of variance (ANOVA), and Duncan's multiple range test to compare the effects of groups within the period and periods within the group using SPSS software (Version 20) (Snedecor and Cochran, 1994). The values at $p < 0.05$ were taken as statistically different.

Results and Discussion

The findings on renal function evaluated by measuring urine volume, urine pH, urine specific gravity, urine total protein, uric acid, creatinine, calcium, and phosphorus in rats of group I to VIII on day 0, 28 and 70 of the experiment are shown in Tables 1-3. In the present study, significant ($p < 0.05$) decrease in levels of urine pH and urine creatinine, and increased levels of urine volume, urine specific gravity, urine calcium, urine phosphorus and urine total protein were observed in all the CKD induced groups by day 28 of adenine treatment as compared to those of '0' day values and of normal control group; however, there was no any discernible change in the urinary uric acid levels among eight groups (Table 1-3). The increased specific gravity along with increased urine output observed in adenine induced CKD rats could be attributed to increased protein, calcium and phosphorus concentration in urine due to kidney damage. Further, the single extract or the co-treatment with aqueous or alcoholic extracts of both *B. calycinum* and *B. diffusa* for 42 days significantly reduced these changes, *i.e.,* by 70th day of experiment in most of the groups bringing the values almost normal or near normal, but no change was noticed in adenine control group (CKD, Gr-II) during that period. The combination of the extracts showed more or less equivalent influence to single extract in reducing the adenine effects (Tables 1-3). However, these biherbal alcoholic extracts had shown better efficacy in bringing the altered serum uromodulin and biochemical profiles to near normal levels over the aqueous biherbal extracts and even single aqueous or alcoholic extract in adenine induced CKD rats in our earlier study (Singh *et al*., 2022). Gehani *et al*. (2020) reported similar results in adenine induced CKD rats with *B. calycinum* and *Achyranthus aspera*. Rahman *et al*. (2018) observed a significant increase in urine output in adenine induced CKD rats due to impairment of kidney function with increased glomerular filtration rate. Jia *et al*. (2013) reported a non-significant change in urine calcium, however, a significant increase in urine phosphorus was recorded in adenine induced CKD mice. Similarly, Patel *et al.* (2018) observed significant increase in urine phosphorus level in adenine induced CKD rats, which decreased following 42 days of treatment with extracts of *B. calycinum* and *Solanum xanthocarpum* and similar were the results of Shanmugapriya *et al.* (2015).

In contrast, Abellan *et al.* (2019) reported significant decrease in mean value of urine phosphorus in adenine fed rats. In an earlier study, the adenine exposed mice did not show increased proteinuria compared to control (Jia *et al.,* 2013). This was likely explained by the known resistance of C57BL/6 strain of mice towards the development of

Table 1: Mean (± SE) values of urine calcium, phosphorus and creatinine levels in rats under different herbal CKD treatment and control group on day 0, 28th and 70th of experiment

		Urine Calcium (mg/dl)			*Urine Phosphorus (mg/dl)*			*Urine Creatinine (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	2.44 ± 0.22	2.21^{a} ± 0.24	2.68^{a} ± 0.14	2.74 ± 0.48	2.85^{a} ± 0.48	3.16^{ab} ± 0.67	9.94 ± 1.00	10.97^{b} ± 1.40	11.43^{c} ± 0.75
II	Adenine control	2.38_{x} ± 0.16	$4.88^{b}{}_{y}$ ± 0.23	$5.93^{c}{}_{z}$ ± 0.25	2.88_{x} ± 0.41	$4.49^{b}{}_{y}$ ± 0.50	$5.95^{c}{}_{z}$ ± 1.23	9.96_{y} ± 1.24	$04.62^{a}{}_{x}$ ± 0.52	$4.97^{a}{}_{x}$ ± 0.45
III	AQ. EX. B.C.	2.38_{x} ± 0.18	$4.86^{b}{}_{y}$ ± 0.52	$3.26^{ab}{}_{x}$ ± 0.45	2.81_{x} ± 0.53	$4.09^{b}{}_{y}$ ± 0.66	$2.77^{a}{}_{x}$ ± 0.77	10.01_{y} ± 0.82	$04.90^{a}{}_{x}$ ± 0.32	$8.72^{b}{}_{y}$ ± 0.42
IV	AL.EX. B.C.	2.68_{x} ± 0.20	$4.61^{b}{}_{z}$ ± 0.27	$3.09^{ab}{}_{x}$ ± 0.25	2.97_{x} ± 0.46	$4.19^{b}{}_{y}$ ± 0.66	$3.52^{ab}{}_{x}$ ± 1.08	10.08_{y} ± 1.04	$04.10^{a}{}_{x}$ ± 0.46	$8.74^{b}{}_{y}$ ± 0.64
V	AQ.EX. B.D.	2.71_{x} ± 0.29	$5.00^{b}{}_{y}$ ± 0.57	$3.06^{ab}{}_{x}$ ± 0.24	3.10 ± 0.49	$4.76^{bc}{}_{y}$ ± 0.51	$3.14^{ab}{}_{x}$ ± 0.89	10.48_{y} ± 0.93	$04.89^{a}{}_{x}$ ± 0.75	$8.75^{b}{}_{y}$ ± 0.59
VI	AL.EX. B.D.	2.77_{x} ± 0.35	$4.68^{b}{}_{y}$ ± 0.12	$2.79^{a}{}_{x}$ ± 0.13	3.27_{x} ± 0.71	$4.58^{b}{}_{y}$ ± 0.52	$3.11^{ab}{}_{x}$ ± 0.58	10.54_{y} ± 0.94	$04.59^{a}{}_{x}$ ± 0.47	$8.65^{b}{}_{y}$ ± 0.49
VII	BI AQ EX (BC+BD)	2.88_{x} ± 0.29	$4.96^{b}{}_{y}$ ± 0.32	$3.43^{ab}{}_{x}$ ± 0.28	3.26_{x} ± 0.71	$3.73^{b}{}_{y}$ ± 0.71	$3.31^{ab}{}_{x}$ ± 0.84	10.03_{y} ± 1.04	$04.45^{a}{}_{x}$ ± 0.51	$8.52^{b}{}_{y}$ ± 0.50
VIII	BI AL EX (BC+BD)	2.97_{x} ± 0.36	$4.88^{b}{}_{y}$ ± 0.27	$3.84^{b}{}_{x}$ ± 0.23	2.87_{x} ± 0.59	$3.89^{b}{}_{y}$ ± 0.70	$3.17^{ab}{}_{x}$ ± 0.84	9.67_{y} ± 1.14	$05.02^{a}{}_{x}$ ± 0.61	$8.11^{b}{}_{y}$ ± 0.58

B.C. =*B. calcynium*; B.D.= *B. 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 2: Mean (± SE) values of urine pH and urine uric acid and total protein levels in rats under different herbal CKD treatment and control group on day 0, 28th and 70th of experiment.

		Urine Uric Acid (mg/dl)			*Urine Total Protein (mg/dl)*			*Urine pH*		
Group	*Group name*	*'0' day*	*28th day*	*70th day*	*'0' day*	*28th day*	*70th day*	*'0' day*	*28th day*	*70th day*
I	Normal control	7.99 ± 0.26	8.13 ± 1.30	8.07 ± 1.30	6.85 ± 0.79	7.83^{a} ± 0.49	7.29^{a} ± 0.50	8.07 ± 0.63	8.93^{b} ± 0.22	9.28^{d} ± 0.23
II	Adenine control	7.98 ± 0.55	8.07 ± 0.77	8.08 ± 0.61	6.31_{x} ± 0.39	$25.08^{bc}{}_{y}$ ± 0.48	$25.29^{d}{}_{y}$ ± 0.56	8.19_{y} ± 0.33	$6.34^{a}{}_{x}$ ± 0.17	$6.20^{a}{}_{x}$ ± 0.16
III	AQ. EX. B.C.	7.92 ± 0.47	7.89 ± 0.39	7.97 ± 0.14	6.70_{x} ± 0.46	$26.63^{c}{}_{z}$ ± 0.36	$13.43^{c}{}_{y}$ ± 0.47	8.28_{z} ± 0.26	$6.38^{a}{}_{x}$ ± 0.19	$7.46^{b}{}_{y}$ ± 0.10
IV	AL. EX. B.C.	7.82 ± 0.35	7.88 ± 0.60	7.72 ± 0.62	6.79_{x} ± 0.44	$26.40^{c}{}_{z}$ ± 0.45	$11.46^{bc}{}_{y}$ ± 0.49	8.25_{y} ± 0.31	$6.53^{a}{}_{x}$ ± 0.12	$7.67^{bc}{}_{y}$ ± 0.19
V	AQ. EX. B.D.	7.84 ± 0.49	8.14 ± 0.61	7.83 ± 0.84	6.84_{x} ± 0.41	$24.52^{b}{}_{z}$ ± 0.38	$10.14^{b}{}_{y}$ ± 0.66	8.04_{y} ± 0.59	$6.42^{a}{}_{x}$ ± 0.95	$7.84^{bc}{}_{y}$ ± 0.30
VI	AL. EX. B.D.	7.71 ± 0.57	8.19 ± 0.34	8.26 ± 0.69	6.67_{x} ± 0.30	$25.60^{bc}{}_{z}$ ± 0.67	$12.04^{bc}{}_{y}$ ± 1.05	7.95_{y} ± 0.40	$6.18^{a}{}_{x}$ ± 0.25	$8.26^{c}{}_{y}$ ± 0.21
VII	BI AQ EX (BC + BD)	7.73 ± 0.33	8.01 ± .39	8.05 ± 0.67	6.52_{x} ± 0.38	$25.08^{bc}{}_{z}$ ± 0.81	$13.00^{c}{}_{y}$ ± 0.84	8.12_{y} ± 0.43	$6.77^{a}{}_{x}$ ± 0.15	$7.79^{bc}{}_{y}$ ± 0.20
VIII	BI AL EX (BC + BD)	7.81 ± 0.34	8.34 ± 0.69	8.14 ± 0.36	6.80_{x} ± 0.43	$25.19^{bc}{}_{z}$ ± 0.54	$11.84^{bc}{}_{y}$ ± 0.76	8.24_{y} ± 0.22	$6.34^{a}{}_{x}$ ± 0.21	$7.72^{bc}{}_{y}$ ± 0.23

B.C. =*B. calcynium*; B.D.= *B. 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 urine volume and specific gravity in rats under different herbal CKD treatment and control group on day 0, 28th and 70th of experiment.

		Urine Volume (ml/day)			*Urine Specific Gravity*		
Group	*Group name*	*'0' day*	*28th day*	*70th day*	*'0' day*	*28th day*	*70th day*
I	Normal control	6.28 ± 1.07	6.02^{a} ± 0.66	6.43^{a} ± 0.27	1.02 ± 0.26	1.01^{a} ± 0.27	1.02^{a} ± 0.15
II	Adenine control	6.15_{x} ± 0.66	15.16^{c}_{y} ± 1.84	15.21^{b}_{y} ± 0.69	1.03_{x} ± 0.25	1.33^{b}_{y} ± 0.87	1.32^{b}_{y} ± 0.01
III	AQ. EX. B.C.	6.45_{x} ± 0.62	14.77^{c}_{y} ± 0.85	6.65^{a}_{x} ± 1.10	1.04_{x} ± 0.14	1.34^{b}_{y} ± 0.82	1.01^{a}_{x} ± 0.12
IV	AL.EX. B.C.	6.16_{x} ± 0.35	9.63^{bc}_{y} ± 1.18	6.43^{a}_{x} ± 0.28	1.04_{x} ± 0.25	1.37^{b}_{y} ± 0.48	1.01^{a}_{x} ± 0.12
V	AQ.EX. B.D.	6.21_{x} ± 0.31	10.14^{bc}_{y} ± 1.57	6.69^{a}_{x} ± 0.29	1.42_{x} ± 0.32	1.22^{b}_{y} ± 0.23	1.01^{a}_{x} ± 0.12
VI	AL.EX. B.D.	6.08_{x} ± 0.80	11.41^{bc}_{y} ± 1.57	6.42^{a}_{x} ± 0.54	1.01_{x} ± 0.16	1.24^{b}_{y} ± 0.46	1.00^{a}_{x} ± 0.00
VII	BI AQ EX (BC+BD)	6.26_{x} ± 0.88	12.4^{bc}_{y} ± 1.04	6.91^{a}_{x} ± 0.77	1.03_{x} ± 0.56	1.24^{b}_{y} ± 0.87	1.03^{a}_{x} ± 1.15
VIII	BI AL EX (BC+BD)	6.09_{x} ± 0.51	13.10^{bc}_{y} ± 1.04	6.22^{a}_{x} ± 0.69	1.01_{x} ± 0.42	1.27^{b}_{y} ± 0.71	1.05^{a}_{x} ± 0.26

B.C. =*B. calcynium*; B.D.= *B. 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 ($p < 0.05$).

proteinuria in combination with the tubule interstitial nature of the renal damage in this model. From the study, it is concluded that the administration of aqueous or alcoholic extract of *B. calycinum* and *B. diffusa* at the dose rate of 300 mg/kg b.wt. either as single or biherbal formulation for 42 days orally reduced the changes in urine profile towards near normal in adenine induced CKD Wistar rats. Statistically, all formulations were equally efficacious in terms of reducing the altered urine profile.

Conclusions

The biherbal or co-treatment with aqueous and alcoholic extracts of both *B. calycinum* and *B. diffusa* plants for 42 days in CKD induced rats significantly restored the changes of increased urine volume, urine specific gravity, and urinary excretions of calcium, phosphorus, total protein and decreased urine pH and urine creatinine. The results showed some key inferences about the therapeutic values of the *B. calycinum* and *B. diffusa* extracts in curing adenine induced CKD in rats.

Acknowledgments

We are grateful to the Project Coordinator, Outreach Program of Ethno-Veterinary Medicine, IVRI, Izatnagar, UP, and ICAR, New Delhi, for providing financial support for this study.

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