RESEARCH ARTICLE

Prophylactic Effect of Biherbal Extracts of *Boerhavia diffusa* and *Tribulus terrestris* in Wistar Rats against Adenine Induced Chronic Kidney Disease: Haemato-Biochemical Profile

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Abstract

The experiment was carried out to study the prophylactic efficacy of extracts of roots of *Boerhavia diffusa* (Punarnava) and fruits of *Tribulus terrestris* (Gokhru) on adenine induced chronic kidney disease in male Wistar rats. Total 30 mature rats were randomly divided into 4 groups, group I (normal control, n=6), group II (adenine control group, in which adenine was given @ 200 mg/kg b.wt., daily orally for 28 days, n=8), while group III and IV served as biherbal prophylactic groups. In group III (n=8) biherbal aqueous extracts (ratio 0.5:1) and in group IV (n=8) biherbal alcoholic extracts (ratio 1:1) of *Boerhavia diffusa* and *Tribulus terrestris* were given in 0.5% sodium bicarbonate solution at the dose rate of 300 mg/kg b.wt. along with adenine solution @ 200 mg/kg b.wt. daily orally for 28 days. Haematological and serum biochemical parameters were studied on day 0 and 28 of experiment. The mean haemoglobin, TEC, and lymphocyte counts showed significant decrease and TLC and granulocyte counts showed significant increase in adenine control group as compared to normal control group after 28 days of treatment. Among serum biochemical parameters, ALT, AST, serum urea nitrogen, uric acid, creatinine, and phosphorus levels showed significant increase, whereas serum total protein showed significant decrease in adenine control group as compared to control group on day 28 over day 0. All the haemato-biochemical changes were significantly prevented in the biherbal prophylactic groups. The result revealed that aqueous and alcoholic biherbal extracts possess prophylactic and nephroprotective activities, which protected kidneys against adenine induced chronic kidney disease in rats.

Keywords: *Boerhavia diffusa, Tribulus terrestris,* Biherbal extract, Haemato-biochemical profile, Nephroprotective effect, Wistar rats.

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Introduction

Chronic kidney disease (CKD) is a health problem affecting many of the countries. The disease is very common in adults, increasing in both developed and developing countries and may develop as the end-stage kidney disease (ESKD) and require kidney function enhancement by dialysis or transplantation, which of course have poor outcome of recovery (Diwan *et al*., 2017). Ayurvedic literature shows that the *Boerhavia diffusa* (Punarnava) rejuvenate the urinary system in many of the kidney diseases and it is also claimed that *B. diffusa* can improve the function of impaired kidneys in many diseases and even in edematous conditions. It is also helpful and very effective in kidney diseases to expel the excess fluid out of the body. Many experimental studies have shown its diuretic and possible nephroprotective effects against acetaminophen-induced renal damage (Sawardekar and Patel, 2015). *Tribulus terrestris* (Gokhru) extracts are mainly used for kidney disorders. The fruits of plant help to remove gravel from the urine and urinary stone from urinary bladder and kidneys. It acts as cooling, tonic, diuretic and aphrodisiac agent and is also used in conditions like dysuria, stranguria, urolithiasis, urinary diseases and impotence (Kavitha and Jagadeesan, 2006). The literature on prophylactic effect of biherbal extracts of *Boerhavia diffusa* and *Tribulus terrestris* in human or animals is meagre. Hence, this experiment was carried out to study the prophylactic efficacy of plants *Boerhavia diffusa* and *Tribulus terrestris* as biherbal aqueous and alcoholic extracts on adenine induced chronic kidney disease in Wistar rats.

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Materials and Methods

The research work was carried out on 30 healthy mature (12-15 weeks) male Wistar rats after approval of the protocol by the Institutional Animals Ethics Committee of Veterinary College, AAU, Anand. The rats were divided randomly in to four groups and were kept in the separate cages. Group I and II served as normal healthy control and adenine control, respectively. Group II was given adenine solution at 200 mg/kg b.wt. daily orally by using syringe and rat gavage needle for 28 days for induction of CKD. Group III and IV were given biherbal aqueous (ratio 0.5:1) and biherbal alcoholic (ratio 1:1) extracts of *Boerhavia diffusa* and *Tribulus terrestris,* respectively @ 300 mg/kg b.wt. (total dose) daily orally along with adenine solution @ 200 mg/kg b.wt. for 28 days. All the rats under different groups were provided R.O. drinking water.

Preparation of Plant Extracts

Roots of *Boerhavia diffusa* and fruits of *Tribulus terrestris* were air dried and powdered. Coarse powdered material of both plants (100 gm each separately) was extracted with water and alcohol in Soxhlet apparatus for 24 hours. The extract was evaporated under reduced pressure to give solid residue. The residues of aqueous and alcoholic extracts were preserved in refrigerator at 4°C for subsequent experiments.

Blood Collection and Haemato-Biochemical Analysis

Blood samples were collected from all the rats twice during experiment, *i.e.,* on day 0 and day 28. The retro-orbital plexus was punctured by capillary tube for the collection of blood under mild anaesthesia using diethyl ether. Blood samples in quantity of 2 ml in K_3EDTA test tubes were collected for detailed haematological analysis using automatic whole blood analyzer (Abacus Junior Vet-5).

Blood samples (two ml) collected in centrifuge tubes without anticoagulant were used for separation of serum. Blood tubes were allowed to clot at room temperature in standing position. Serum was collected by centrifugation at 3000 rpm for 15 minutes at 10° C (Eppendorf 5804 R, Germany). The collected serum was stored at -20° C and used for serum biochemical analysis. Serum biochemical parameters like total protein, alanine amino transferase (ALT), aspartate aminotransferase (AST), urea nitrogen (SUN), uric acid, creatinine, calcium and phosphorus levels were determined by using standard assay kits on clinical serum biochemistry auto-analyser (CKK 300).

Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare the effects between groups within the period using SPSS software Version 20.0. The paired 't' test was used to compare parameters before and after treatment (Snedecor and Cochran, 1994).

Table 1: Haematological changes in adenine induced CKD rats under different control groups and biherbal prophylaxis for 28 days

		Treatment groups			
Blood parameter	*Day of treatment*	*I*	*II*	*III*	*IV*
Hb (g/dL)	0 day	14.55 ± 0.37	14.60 ± 0.36	14.63 ± 0.15	14.40 ± 0.21
	28th day	16.46 ± 0.54^{c}	10.98 ± 0.35^{a}	15.77 ± 0.46^{c}	14.23 ± 0.32^{b}
	't' value	0.02*	0.00**	0.05*	0.68^{NS}
TEC (10^6/µl)	0 day	8.04 ± 0.21	8.18 ± 0.19	8.12 ± 0.17	7.67 ± 0.32
	28th day	8.42 ± 0.25^{c}	6.55 ± 0.34^{a}	7.96 ± 0.12^{bc}	7.68 ± 0.19^{b}
	't' value	0.27^{NS}	0.014**	0.47$_{NS}$	0.987$_{NS}$
TLC (10^3/µl)	0 day	8.93 ± 0.64	9.98 ± 0.94	9.58 ± 1.41	10.75 ± 1.77
	28th day	8.91 ± 0.60^{a}	16.40 ± 1.17^{b}	10.39 ± 1.34^{a}	10.94 ± 1.49^{a}
	't' value	0.98^{NS}	0.00**	0.68^{NS}	0.93^{NS}
Granulocytes %	0 day	16.50 ± 1.54	20.16 ± 1.64	22.80 ± 2.07	17.86 ± 1.90
	28th day	16.20 ± 0.88^{a}	41.14 ± 2.75^{b}	20.32 ± 2.09^{a}	18.68 ± 1.44^{a}
	't' value	0.871^{NS}	0.000**	0.415^{NS}	0.73^{NS}
Lymphocytes %	0 day	76.25 ± 2.24	73.70 ± 2.23	70.08 ± 2.25	74.40 ± 1.32
	28th day	73.91 ± 2.47^{b}	51.88 ± 1.97^{a}	74.18 ± 1.55^{b}	78.75 ± 1.17^{b}
	't' value	0.50^{NS}	0.000**	0.93^{NS}	0.028*
Monocytes %	0 day	7.25 ± 1.87	6.13 ± 1.62	7.11 ± 1.80	7.73 ± 1.73
	28th day	5.75 ± 1.49	5.17 ± 1.19	5.31 ± 1.16	6.46 ± 1.41
	't' value	0.54^{NS}	0.64^{NS}	0.42^{NS}	0.58^{NS}

Gr-I Healthy control, Gr-II Adenine induced CKD, Gr-III Biherbal aqueous extracts, Gr-IV Biberbal acoholic extracts of *Boerhavia diffusa* and *Tribulus terrestris.*

Means with different superscripts (a,b,c..) within the row differ significantly ($p < 0.05$).

NS- Non-significant, *significant ($p < 0.05$), **Highly significant ($p < 0.01$).

Table 2: Serum biochemical alterations in adenine induced CKD rats under different control groups and biherbal prophylaxis for 28 days

		Treatment groups			
Serum biochemistry	*Days of treatment*	*I*	*II*	*III*	*IV*
	0 day	5.86 ± 0.28	6.23 ± 0.26	6.38 ± 0.33	6.25 ± 0.18
Total protein (g/dl)	28th day	5.78 ± 0.31^{ab}	4.76 ± 0.16^{a}	6.49 ± 0.24^{b}	6.35 ± 0.05^{b}
	't' value	0.86^{NS}	0 .00**	0.80^{NS}	0.61^{NS}
	0 day	23.66 ± 0.88	22.25 ± 0.67	22.62 ± 1.08	22.62 ± 0.77
ALT (IU/L)	28th day	24.83 ± 1.01^{a}	42.14 ± 2.29^{b}	26.00 ± 1.03^{a}	24.87 ± 1.80^{a}
	't' value	0.40^{NS}	0.00**	0.04*	0.28^{NS}
	0 day	54.16 ± 0.74	54.50 ± 0.73	54.00 ± 0.86	53.37 ± 0.98
AST (IU/L)	28th day	54.00 ± 0.89^{a}	62.71 ± 1.86^{b}	58.75 ± 2.20^{ab}	58.12 ± 0.95^{ab}
	't' value	0.88^{NS}	0.00**	0.07^{NS}	0.00**
	0 day	20.83 ± 1.88	20.25 ± 1.75	17.62 ± 1.17	16.87 ± 0.95
SUN (mg/dl)	28th day	18.00 ± 0.93^{a}	155.42 ± 5.86^{c}	53.87 ± 2.79^{b}	59.50 ± 3.56^{b}
	't' value	0.21^{NS}	0.00**	0.00**	0.00**
	0 day	2.49 ± 0.07	2.71 ± 0.21	2.68 ± 0.12	2.58 ± 0.08
Uric acid (mg/dl)	28th day	2.27 ± 0.11^{a}	3.56 ± 0.20^{b}	2.68 ± 0.22^{a}	2.44 ± 0.19^{a}
	't' value	0.13^{NS}	0 .01**	0.98^{NS}	0.52^{NS}
	0 day	0.40 ± 0.03	0.44 ± 0.03	0.41 ± 0.04	0.48 ± 0.05
Creatinine (mg/dl)	28th day	0.51 ± 0.06^{a}	4.30 ± 0.27^{c}	2.85 ± 0.26^{b}	2.39 ± 0.18^{b}
	't' value	0.15^{NS}	0.00**	0.00**	0.00**
	0 day	6.49 ± 0.05	6.51 ± 0.05	6.73 ± 0.06	6.57 ± 0.05
Calcium (mg/dl)	28th day	6.48 ± 0.03	6.55 ± 0.06	6.67 ± 0.04	6.66 ± 0.02
	't' value	0.95^{NS}	0.80^{NS}	0.87^{NS}	0.71^{NS}
	0 day	6.28 ± 0.22	6.31 ± 0.23	5.62 ± 0.21	5.49 ± 0.24
Phosphorus (mg/dl)	28th day	6.08 ± 0.17^{a}	8.12 ± 0.52^{b}	6.55 ± 0.22^{a}	6.71 ± 0.38^{a}
	't' value	0.20^{NS}	0.01**	0.02**	0.02*

Gr-I Healthy control, Gr-II Adenine induced CKD, Gr-III Biherbal aqueous extracts, Gr-IV Biberbal acoholic extracts of *Boerhavia diffusa* and *Tribulus terrestris*.

Means with different superscripts (a,b,c..) within the row differ significantly ($p < 0.05$).

NS- Non-significant, *significant ($p < 0.05$), **Highly significant ($p < 0.01$).

Results and Discussion

The findings on evaluation of renal function made through haematology and serum biochemical analysis are presented in Table 1 and 2, respectively. There was significant decrease in the levels of Hb and TEC and lymphocyte count, whereas significant increase in TLC and granulocyte count in adenine control (CKD) group-II on day 28 of experiment over day 0 values, however, there was no such change over period in normal healthy control group-I, except Hb which in fact increased during experimental period probably due to improved feed intake under controlled condition (Table 1).

These findings of group- I and II were in accordance with Sun *et al*. (2013) and Ali *et al*. (2014). Rahman *et al*. (2018), however, showed non-significant change in the haematocrit values during or after adenine administration at 200 mg/kg in rats and opined that the low TEC level in adenine treated rats might be due to insufficient production of erythropoietin hormone causing uremic anaemia. and impaired oxygen metabolism.

Both aqueous biberbal and alcoholic biherbal extracts of *Boerhavia diffusa* and *Tribulus terrestris*, significantly protected rats from severe haematological alterations induced by adenine as there was no significant change in the haemoglobin, TEC, TLC, granulocyte (%) and lymphocyte (%) between 0 and 28 days values (Table 1). Karwasra *et al*. (2016) recorded near normal Hb and TEC levels in ciplastin induced nephrotoxic rats treated with *Boerhavia diffusa* root extract as compared to ciplastin control rats. Similar were the findings of Gehani *et al*. (2019) for therapeutic effect of *Bryophyllum calcinum* and *Achyranthes aspera* on adenine induced chronic kidney disease in rats, where they found significantly decreased Hb and TEC in adenine treated rats and the values in biherbal extract treated rats were nearer to normal. These reports and present findings support that the biherbal extracts of *Boerhavia diffusa* and *Tribulus terrestris* have nephroprotective and antianemic role.

Among the serum biochemical profile, there was significant increase in the levels of serum ALT, AST, SUN, uric

acid, creatinine and phosphorus, while significant decrease in the level of serum total protein in the adenine control group-II (Table 2). This alteration in serum biochemical profile proved that the adenine dosing @ 200 mg/kg b.wt. daily along with drinking water for 28 days damaged kidney function by inducing CKD. Observations of serum biochemical analysis were in accordance with Jia *et al*. (2013), Sun *et al*. (2013) and Ali *et al*. (2017) for one or the other constituents.

The aqueous biherbal extract and alcoholic biherbal extract administered for 28 days along with adenine in group III and IV, respectively, significantly protected rats from severe serum biochemical alterations induced by adenine alone in group-II. Both extracts significantly prevented changes in serum total protein, serum ALT, serum AST, serum uric acid and serum calcium levels. However, there was highly significant rise in serum urea nitrogen, creatinine, and phosphorus levels by day 28 over day 0 in both the biherbal prophylactic groups (Table 2). Pareta *et al*. (2011) found pre-treatment of aqueous extract of *B. diffusa* root (200-400 mg/kg/day) in repeated dose in acetaminophen nephrotoxic rats for 14 days to significantly protect against changes in blood urea nitrogen and serum creatinine. Karwasra *et al*. (2016) found dose dependent reduction in the elevated levels of serum creatinine and serum urea nitrogen (SUN), serum uric acid following administration of *Boerhavia diffusa* root extract in cisplatin treated nephrotoxic rats. Padmini and Kumar (2013) also found same result in gentamicin induced nephrotoxic rats. Najafi *et al*. (2014) found that *Tribulus terrestris* extract significantly protected kidneys against changes in serum creatinine, SUN, uric acid in acute kidney injury induced rats. Serum biochemistry revealed that the biherbal extracts of *Boerhavia diffusa* and *Tribulus terrestris,* though reduced the severity of renal and hepatic damage induced by adenine, it could not completely safeguard the rats from CKD. However, in chronic natural kidney disease it might provide nephroprotective/prophylactic effect.

It was concluded that aqueous biherbal extracts (ratio 0.5:1) and alcoholic biherbal extracts (ratio 1:1) of *Boerhavia diffusa* and *Tribulus terrestris* have some nephroprotective effect as shown by significant protection against changes in haematological and serum biochemical profile in adenine induced kidney damaged rats, although it could not protect completely the renal and hepatic damage from adenine in rats.

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