Ethanolic extract of *Boerhavia diffusa* attenuates renal fibrosis in chronic kidney disease rats through downregulation of TGF-β

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Abstract

Introduction: Renal failure is a common and catastrophic complication of chronic kidney disease (CKD),which is characterised by gradual kidney function loss and degradation of the kidney parenchyma. *Boerhaviadiffusa* (BD) is said to have rejuvenating effects in Ayurvedic literature, particularly in relation to the urinarysystem.To evaluate effect of ethanolic extract of BD in adenine induced chronic kidney disease rats and whether it may prevent chronic renal disease in wistar male rats that was brought on by adenine. **Materials andmethods:** Wistar albino rats were used in the study, which had 5 groups.Parameter measured in kidney fuction test,complete blood count,kidney histopathology,immunohistochemistry,westen blot and RT-PCR **Results:** The BD treatment group significantly decreased urea,serumcreatinine, and significantly reduced TGF-β. Expression and kidney histological damage as compared to the disease control group ($P < 0.05$). Adenine-induced nephrotoxicity caused structural and functional damage,which caused BD to guard against and downregulate the expression of TGF-β. based on the results of the current investigation. **Conclusion:** BDE appears to have a beneficial effect on TGF- β down regulation, which may help to reduce renal fibrosis. For the

prevention and treatment of CKD and other related disorders, BD plant that is widely eaten as a vegetable, can be used as a nutraceutical or therapeutic food.

Key words: *Boerhavia diffusa*, Chronic Kidney Disease, Transforming Growth Factor (TGF)-β, Adenine, Pirfenidone

Introduction

Being a major excretory organ, the kidney is invariably exposed to high levels of both endogenous and external toxins. When the kidneys are damaged, they are unable to filter blood as effectively as they should. This condition is known as chronic kidney disease. A gradual decrease in glomerular filtration rate is a hallmark of chronic kidney disease, which initially exhibits no symptoms (1). Tubular atrophy, tubulointerstitial fibrosis, and proteneuria are symptoms of chronic kidney disease. Additionally glumerulosclerosis the end-stage renal disease that occurs from unchecked disease development is still difficult to treat. Triggering mechanisms that are particular to the underlying etiology, such as genitical abnormalities, toxin exposure, immune complex deposition, and inflammation. Hyperinfiltration and hypertrophy of the remaining functional nephrons are progressive mechanisms. Hemodynamic, immunologic, or metabolic stress damages the glomerulus' endothelial cells, causing them to develop pro-coagulant and inflammatory characteristics. This causes glomerular micro-inflammation, platelet attraction and activation, and micro thrombus development in glomerular capillaries, as well as the attraction, adhesion, and infiltration of glomerular tufts by inflammatory cells. Protein leakage into the tubular fluid is caused by hyperinfiltration, which is caused by renal mass loss. A proteinuric filtrate that has been damaged by glomerulosclerosis also contains active mediators. Because albumin is present in large quantities in the filtrate, it interacts with the activated mediators to cause inflammation in the tubules. Proteins build up in the tubular interstitial space due to the

proteniuric infiltration, which causes tubulointerstitial damage and encourages inflammation and fibrosis. Additionally, glomerular damage can decrease peritubular perfusion and start local ischemia and hypoxia that results in tubular atrophy and interstitial fibrosis (2, 3, and 4). Myofibroblasts that are positive for smooth muscle actin (-SMA) are the main cause of kidney fibrosis because they create an excessive amount of extracellular matrix (ECM), which causes scarring. According to studies, interstitial fibroblasts become activated and transform into myofibroblasts, which produce the extracellular matrix (ECM), where TGF- β plays a crucial role. TGF-β recruits TGF-β receptor type 2 (TGF-β R2) to carry out its signalling, when it activates Smad1/2 and Smad2/3. TGF-β also stimulates a number of mitogen-activated protein kinases (MAPK), including as c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase-1 and -2 (ERK1/2), and p38 MAPK. TGF-β regulates the expression of a wide variety of fibrosis-related genes through both Smad-dependent and -independent pathways. In this situation, limiting TGF-β signalling is crucial for creating potent anti-kidney fibrosis defences (5, 6, 7, and 8). The direct TGF-β actions in fibrotic kidney illnesses have been shown by a variety of evidences, including up-regulation of TGF-β signalling in glomeruli or tubulointerstitium in fibrotic kidney, kidney fibrosis-induced by increased TGF-β, and amelioration of kidney fibrosis by anti-TGF-β therapy (9, 10, 11 and 12) Normalization of insulin, glucose, and blood pressure are the only treatment strategies available today to halt its progression. Drugs that reduce blood pressure, blood sugar, and cholesterol levels may be used as first therapies. Edema can be managed with the help of loop diuretics known as angiotensin converting enzyme inhibitors (ACEIs). The proper amount of protein should be consumed, as well as maintaining an active lifestyle and making certain dietary changes like cutting back on salt. Treatments for bone disorders and anaemia might also be necessary. Hemodialysis, peritoneal dialysis, or a kidney transplant is necessary for patients with severe illness to survive. It is imperative that new treatments be created in order

to either stop or slow down the decline in kidney function. Herbal medications with demonstrated safety profiles are particularly intriguing in this regard. Boerhavia diffusa (BD) is commonly known as punarnava. BD is a reliable source of dietary supplements. Together with is palmitate acetate, behenic acid, arachidic acid (6.3%), saturated fatty acids (38%), vitamins C, B3, and B2 (44.80, 97.00 mg, and 22.00 mg), calcium (174.09 mg), and other nutrients, the plant includes 15 amino acids in total and 14 amino acids in the roots. BD includes a variety of secondary metabolites, such as alkaloids, flavonoid glycosides, isoflavonoids (rotenoids), steroids (ecdysteroid), phenolic and lignan glycosides, and isoflavonoids. The rotenoid family includes the molecular marker for BD, boeravinone B. The plant has grown significantly in significance in the field of phytochemistry due to its wide range of pharmacological and biological effects, including immunomodulatory effects, immunosuppressive activity, antimetastatic activity, antioxidant activity, antidiabetic activity, antiproliferative and antiestrogenic activity, analgesic and anti-inflammatory activity, antibacterial activity, antistress and apoptogenic activity, anti-lymphoproliferative activity, and nitric oxide scavenging activity (13,14,15,16,17,18,and 19). BD has been primarily used to treat diuretic and nephroprotective effects in a number of nephrotoxic models. It is a plant that has been used to treat a variety of diseases. Although some preliminary research on BD in adenine-induced nephrotoxicity discovered protection against kidney damage, the precise molecular mechanism behind this nephroprotection is still not fully understood. To the best of our knowledge, determining that BD therapy would reduce nephrotoxicity brought on by adenine was our main hypothesis for the current study

Materials and Methods

Experimental animals

The animals were housed in standard laboratory conditions at a constant temperature (25 ± 2˚ C) with a relative humidity of (60 ± 5%) under a 12 h light/dark cycle. They had free access to standard diet and tap water.

Test drugs

The plant material was extracted using the previously reported procedure, with a few minor adjustments (6). In a nutshell, fresh entire plants were air-dried and then extracted with ethanol at room temperature (27 ±1˚C) while being stirred for 6 hours. The extraction process was repeated until the solvent became colourless. The supernatant was filtered using Whatman No. 1 filter paper, condensed under decreased pressure in a rotavapor and then lyophilized. The lyophilized BD extract was kept at 4˚C until usage. The extract's yield was determined to be 4.33% w/w. All of the tests were conducted using the same extract sample.

TGF- β was purchased from Abcam (Cambridge, UK). Purchases of adenine were made from Sisco Research Laboratories private limited India.

Animals

1week of 1 acclimatization rats will be divided in to 5 groups with 6 rats in group 1 and 2 group 3.4 and 5 consisted 8 rats based on the G power analysis. Animals in Group 3 - 5 will receive 600 mg/kg/body weight of adenine orally once day for 10 days, along with 0.5% carboxyl methylcellulose (CMC) (20). The levels of blood glucose, urea, and serum creatinine were used to confirm the induction of CKD. And then two weeks of treatment 400mg/kg/body weight of BD extract for group 4 (21) and 500mg/kg/body weight of pirfenidone (PFD) for group 5 (22) animals. group 1(control) given only CMC solution through orally for throughout the experiment group 2 (BD extract only) given BD extract only for 14 days group 3 (CKD induced) given 600mg/kg/body weight adenine for 10 days group 4 (CKD +BD) 400mg/kg/bodyweight of BD extract for 14 days group 5 (CKD+PFD)

500mg/kg/bodyweight for 14 days. Animals were anaesthetized with overdose of anesthesia (Halothane by inhalation) venous blood will be obtained from retro-orbital plexus (2 ml) in EDTA containing tube and it was centrifuged at 3000rpm for 15 min. The separated serum stored at -80°C for biochemical analysis.

All of the animals were housed in separate metabolic cages, and daily 24-hour urine samples were taken. Water was available to animals at all times when pee was being collected.

Kidney were dissected from euthanized animals and fixed in 4% formaldehyde for histological studies.

Histopathology

Sections of kidney tissue were fixed in formaldehyde fixative for 24 hours. Following fixation, the fixed tissue was rinsed in tap water, dehydrated using a graduated sequence of ethanol (30%e100%), and then embedded in paraffin wax at 60˚ C. The paraffin blocks were made and serially sectioned at 5 mm. The cut sections were stretched out on albumin-coated slides and allowed to air dry before staining. The sections were next dewaxed with xylene and serially hydrated and dehydrated with graduated series of ethanol before being stained with hematoxylin and eosin and special stain. The sections were mounted and examined under a microscope after dehydration and alcohol differentiating.

Immunohistochemistry

Immunohistochemical analysis (IHC) was done for tissue localization of TGF-β. The kidney tissues were fixed in 4% paraformaldehyde for 24 hours, cleaned, and cryoprotected in 15%–30% sucrose before being cut into 6 mm sections with a microtome. To halt any natural peroxidase activity in the tissues, hydrogen peroxide (30% H2O2 in methanol) was

administered. Sections were then treated with a primary polyclonal antibody (pAb) against TGF-β for 1 hour at room temperature following a 1 hour bovine serum albumin blockade. The next step was to incubate the sections for 2 hours with a secondary antibody that has been HRP-conjugated. A colorimetric immunological response was created using 3, 3'-diaminobenzidine (DAB) tetrahydrochloride. Then, sections were mounted with DPX to see TGF-β activity under a light microscope after being dried in ethanol.

Western blotting

In order to determine the protein concentrations, frozen kidney tissue samples were homogenised in RIPA lysis buffer. For 10 minutes, the whole proteins were incubated in boiling water. To transfer equal amounts of total protein electrophoretically to polyvinylidene difluoride membrane, 12% SDS-PAGE was used to separate the proteins. The transfer solution also contained methanol to preactivate the membranes. The membranes were treated with specific primary antibodies against TGF-β (PA1-29032) overnight at 4°C after being blocked with 5% skimmed milk for 2 hours. The membranes were then cleaned, and a secondary antibody specific for rabbit IgG was added for an additional 60 minutes of incubation at room temperature. Using an improved chemiluminescence kit, the immunoreactive proteins were found. The Phototope HRP Western Blot Detection was used to see the immunoreactive bands.

RT-PCR

Gene expression was evaluated using qPCR. Total RNA was collected from individual rat kidneys in order to evaluate the mRNA expression of the genes encoding TGF-β. Using HiScript II QRT SuperMix, cDNA was produced in 20-L reactions for qPCR and stored at 20 °C until analysis. On the ABI Step One Plus equipment, real-time PCR amplification was carried out using the SYBR-Green reaction kit. The primers for RT-qPCR are listed in **Table**

1. To analyse the gathered data, comparative cycle threshold (CT) analysis was used. The normalisation and the fold change in each gene's expression were calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 1: Primers

S.NO	**Gene**	**Forward primer (5′ ⟶ 3′)**	**Reverse primer (5′ ⟶ 3′)**	**Length**
1	TGF-β (NM_021578.2)	CCCCTGGAAAGGGCTCAACAC	TCAACCCAGGTCCTTCCTAAA	21 21
2	β-Actin (NM_031144.3)	GCAGATGTGGATCAGCAAGC	GGTGTAAAACGCAGCTCAGTAA	20 22

Statistical analysis

The findings were presented as Mean Standard Deviation (SD). The Kolmogorov-Smirnov test was used to determine the normality of the data. Student's paired t-test was employed to compare parametric data among members of the same group. One-way analysis of variance (ANOVA) was used to compare parametric data from several groups; if ANOVA revealed any significance, a post-hoc Tukey's test was employed. Histopathology scores were represented as medians, and the median scores between different groups were compared using the Kruskal-Wallis test. A "P" value of 0.05 or less was deemed significant for all parameters.

RESULTS

1. Effect on the body weight

Impact on body weight at days 11 and 33, the body weight of the rats in different groups is shown in **Table 2.** On day 11, the body weights of all rats in all groups were comparable. In comparison to day 11, the rats in group 1 significantly gained weight over the course of 33 days ($P < 0.05$). However, the rats in this group 3 exhibit a marked decrease in body weight from the 11. After 33 days, there was a noticeable increase in body weight in post-treatment groups 2, 4, and 5.

Table 2: Effect on the body weight

Groups	Day11(gram)	Day 33 (gram)
1 (control)	245.5±4.991	275.2±9.959
2 (BDextract only)	216.6±15.105	254.1±19.545
3 (CKD)	210.5±18.214	162.7±33.77*
4 (CKD+BD)	210.2±23.535	244.34±17.64**
5 (CKD+PFD)	190.7±23.33	194±22.51*

(Values are expressed as mean standard deviation, with paired t-test significance levels of * $p < 0.05$, ** $p<0.01$ as compared with respective day 11)

2. Effect of water and food intake levels

Table 3 shows the food and water intake of the rats in various groups, at baseline day 11 and day 33. The food and water intake of all rats in all groups was comparable on day 11. The rats in group 1,2,4,5 showed a significant weight gain over 33 days as compared with day 11. However, the rats in group 3 did not show a significant change in food and water intake weight from the day11.

Table 3: Effect of water and food intake levels

Groups	**Day 11**		**Day 33**	
	Water (ml)	Food (gram)	Water (ml)	Food (gram)
1 (control)	239.4	94.8	238.6	116
2 (BD extract only)	235.7	92.9	240.6	132.8
3 (CKD)	186.8	57	95.5	25.3
4 (CKD+BD)	205.3	58.1	205.3	66.9
5 (CKD+PFD)	197.5	60.3	197.5	56.5

(Values are expressed as mean standard deviation, with paired t-test significance levels of * $p< 0.05$, ** $p<0.01$ as compared with respective day 11)

3. **Effect of bio chemical levels in rat model of adenine induced chronic kidney disease rats**

At day 11, all groups' levels of glucose, urea, serum creatinine, and total proteins were comparable, as shown in **Table 4**. The group 3 animals' serum creatinine, glucose, and urea levels dramatically rose on day 33 compared to day 11 while their levels of total proteins significantly reduced. Comparatively to day 11, post-treatment groups 4 and 5 significantly increased the levels of total protein, and decreased serum creatinine, and glucose urea on day 33.Since day 0, group 1 and 2 have maintained their normal glucose, urea, serum creatinine, and total protein levels.

TABLE 4: Effect of bio chemical levels in rat model of adenine induced chronic kidney disease rats

Group	Glucose (mg/dl)		Urea (mg/dl)		Crea (mg/dl)		Total protein (g/dl)	
	Day11	Day33	Day11	Day33	Day11	Day33	Day11	Day33
1 (control)	_	84.00±5.477	-	12.50±.356**	-	.66±.007**		6.6±.1136**
2 (BD extract only)	_	81.67±4.082	-	12.50±.429**	-	.67±.008**		6.6±.063**
3 (CKD)	213.38 ±55.949	253.50± 115.169	57.08±7.79	67.12±228.27**	1.45±.287	2.0±.82**	5.9±.092	5.2±.192**
4 (CKD +BD)	207.75 ±18.568	117.13± 9.433	57.50±8.66	17.06±3.20**	1.68±.383	.68±009**	6.08±.145	6.8±.074**
5 (CKD +PFD)	193.25 ±14.469	131.38± 10.042	49.37±8.94	21.72±3.68	1.7±.320	1.03±.177**	6.1±.092	6.9±.148**

(crea: serum creatinine)

(Values are expressed as mean standard deviation (SD), **P<0.01, *P<0.05 using paired t-test (as compared with respective day 11)

4. **Effect of hematological parameter in rat model of adenine induced chronic kidney disease rats**

In comparison to Hb, RBC, WBC, and platelets on day 11, group 3 levels in day 33 decreased while those of groups 4 and 5 increased. On days 11 and 33, the control group and the BD group had normal levels. **Table 5.**

Table 5: Effect of hematological parameter in rat model of adenine induced chronic kidney disease rats

Groups	Hb (g/dl)		RBC (million cells/mcl)		WBC (micro litre)		Platelets (micro litre)	
	Day11	Day33	Day11	Day33	Day11	Day33	Day11	Day33
1 (control)	_	13.63±.121**	-	7.5±.282**	-	7250±388.5**	-	6.3±.108**
2 (BD extract only)	_	14.2±.273**	-	7.5±.240**	-	7250±388.5**	-	6.4±.117**
3 (CKD)	9.4±.785	8.4±.738**	4.3±.306	3.8±.261**	6237±417.2	5612±408.6**	5.3±.370	4.5±.365**
4 (CKD+BD)	9.6±.636	13.6±.261**	4.3±.331	7.4±.124**	6600±450.3	7450±512.6**	5.4±.169	6.5±.204**
5 (CKD+PFD)	9.5±.592	12.7±.292**	4.2±.243	6.9±.145**	6350±420.8	6950±389.1**	5.3±.180	6.4±.141**

(Hb: haemoglobin, RBC: Red blood cells, WBC: White blood cells)

(Values are expressed as mean standard deviation (SD), **P<0.01, *P<0.05 using paired t-test (as compared with respective day 11)

5. **Effects of differential counts in rat model of adenine induced chronic kidney disease rats**

In comparison to day 11 and day 33, there are no appreciable differences in monocyte and eosinophil counts in any of the five groups. However, group 3 has lower MCV and lymphocyte levels compared to day 11 and day 33.increased in groups 4 and 5.**Table 6**

Table 6: Effects of differential counts in rat model of adenine induced chronic kidney disease rats

Groups	Lymphocytes (%)		Eosinophils (%)		Monocytes (%)		MCV (cubic microns)	
	Day11	Day33	Day11	Day33	Day11	Day33	Day11	Day33
1 (control)	_	67.2±.809**	-	2.3±.516**	-	1.6±.516*	-	50.0±.083*
2 (BD extract only)	_	67.1±.789**	-	2.0±.632**	-	2.0±.632*	-	50.3±.332*
3 (CKD)	59.5±1.414	56.5±1.60**	1.8±.835	5.5±.535**	1.7±.707	11.5±17.18*	46.0±1.16	36.5±14.8*
4 (CKD+BD)	60.7±1.488	67.3±1.06**	2.2±.886	1.3±.518**	2.1±.641	1.3±.518*	46.3±1.033	50.4±.584*
5 (CKD+PFD)	59.7±1.66	66.5±.354**	2.2±.886	1.2±.463**	2.5±.756	1.5±.535*	45.2±2.389	49.3±.476*

(MCV: Mean corpuscular volume)

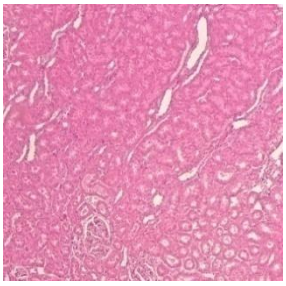
(Values are expressed as mean standard deviation (SD), **P<0.01, *P<0.05 using paired t-test (as compared with respective day 11)

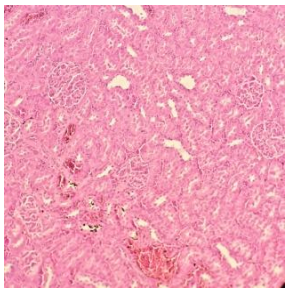
6. **Effect of kidney histopathology**

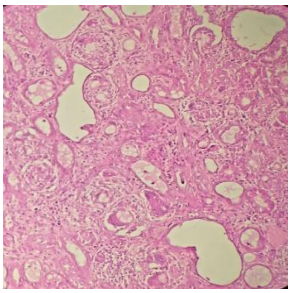
Group 1, 2 and 4showes normal renal architecture in H&E (**Fig 1**) and MTS (**Fig 2**) stain, and group 5 shows mild tubular atrophy and mild fibrosis in H&E stain unremarkable changes in MTS stain. Group 3 (CKD group) shows enlarged glumerulai, increased mesangial cellularity, thickend mesengial matrix and basement membrane, diffuse tubular necrosis in H&E stain increased mesengial matrix with irregular glomeruli seen in MTS stain.

Fig 1: H&E

Group 1 (Control) **Group 2 (BDE)** **Group 3 (CKD)**

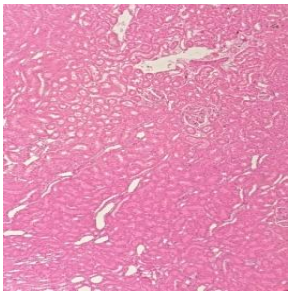


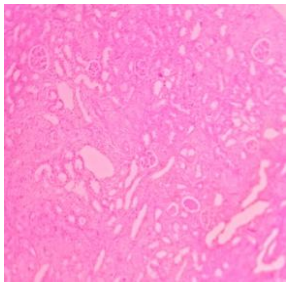




(a) **(b)** **(c)**

Group 4 (CKD+BDE) **Group 5 (CKD+PFD)**



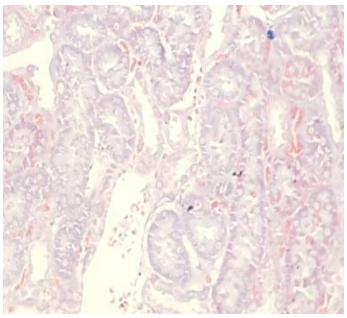


(d) **(e)**

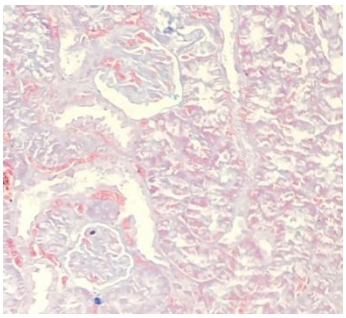
(BDE:*Boerhavia diffusa* extract CKD :chronic kidney disease PFD :pirfenidone)

Fig: 2 MTS (Massiontrichrom)

Group 1 (Control) **Group 2 (BDE)** **Group 3 (CKD)**



(a)



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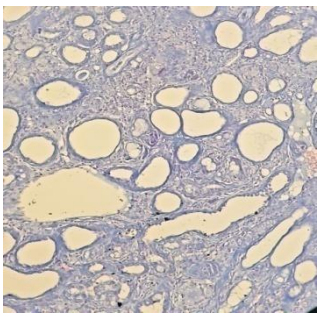
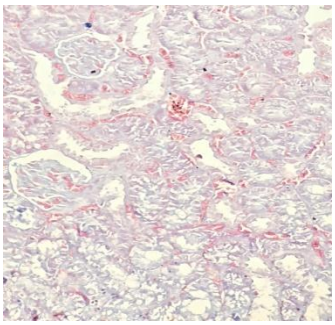
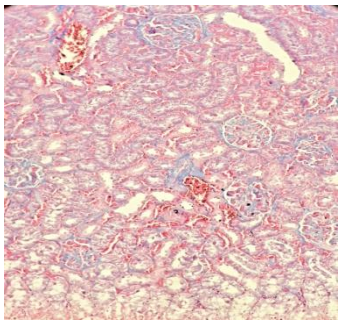


(c)

Group 4 (CKD+BDE) **Group 5 (CKD+PFD)**



(d)



(e)

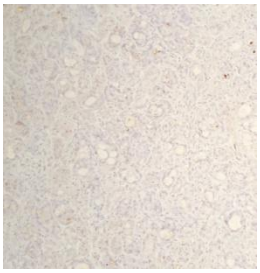
(BDE:*Boerhavia diffusa* extract CKD :chronic kidney disease PFD :pirfenidone)

7. **Immunohistochemistry**

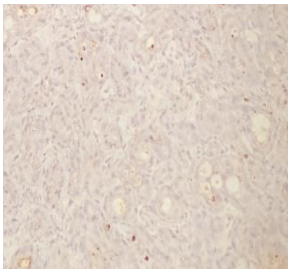
On immunohistochemistry analysis kidney tissues from group 3 rats showed a significant rise in tgf beta. The levels of tgf beta in the renal tissues of group 4 *Boerhavia diffusa* 400mg/kg treated animals were dramatically lowered. Kidney tissues from group 5 animals displayed a low level of glumerulus cytoplasmic positivity. **Fig(3)**

Fig:3Immunohistochemistry

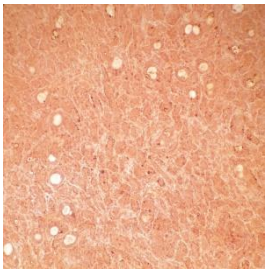
Group 1 (control) **Group 2(BDE)** **Group 3 (CKD)**



(a)

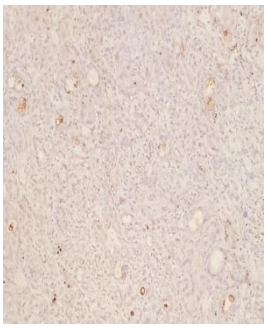


(b)

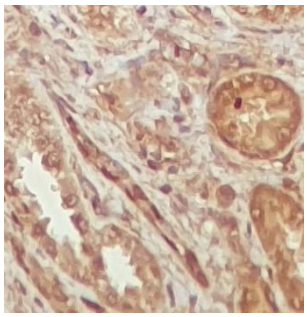


(c)

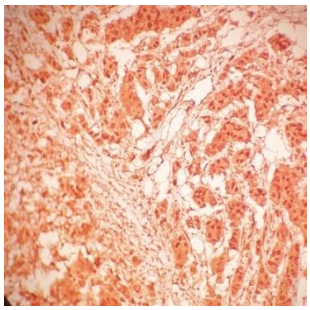
Group 4 (BDE+CKD) **Group 5 (CKD+PFD)** **Positive control**



(d)



(e)



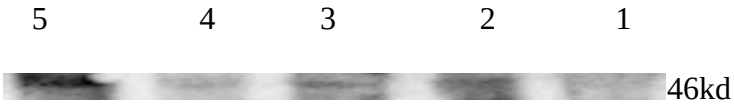
(f)

(BDE:*Boerhavia diffusa* extract CKD :chronic kidney disease PFD :pirfenidone)

8. **Western blot and RT-PCR**

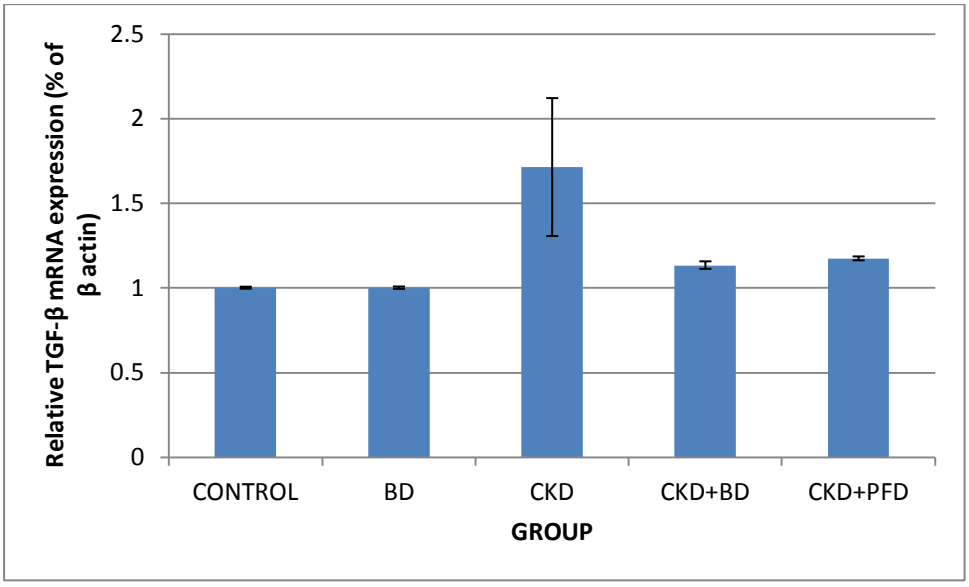
Western blot (**Fig 4**) and Real-time PCR (**Fig 5**) analyses showed that group 3 rats had significantly expressed genes and proteins with normal expression, while group 5 rats had moderately expressed genes and proteins in compared to normal control rats. Groups 2 and 4 displayed normal expression in contrast.

Fig 4: Western blot



(1:control 2:BD 3:CKD 4:CKD+BD 5:CKD+PFD)

Fig 5: RT-PCR



(BDE:*Boerhavia diffusa* extract CKD :chronic kidney disease PFD :pirfenidone)

Discussion

The development of effective medication for the best and most cause-specific management of renal failure in the clinical setting has benefited from the use of animal models of drug-induced renal injury. The causes, processes, and treatment strategies of chronic kidney disease and its consequences are frequently studied using a variety of animal models. The well-known animal models, such as the 5/6 nephrectomy, produce anatomical and functional changes that are comparable to CKD in people. This model has a significant mortality rate and necessitates the execution of sophisticated surgery. In addition to the 5/6 nephrectomy

model, other models utilised for CKD research include streptozotocin-induced diabetes, genetically engineered diabetes, diabetic neuropathy, and modified mice models. Adenine-induced CKD model, which mimics human CKD and has a lower death rate, has been used in numerous research(23,24 and 25).The mechanisms of adenine toxicity have been thoroughly investigated in experimental settings. A purine base is adenine. When adenine is taken orally, it breaks down into the poorly soluble 2,8-dihydroxy adenine (DHA), which is then eliminated in the urine. Adenine overconsumption results in excessive DHA synthesis, which is then retained in tubular epithelial cells, causing inflammation and tubulointerstitial fibrosis as a result (26).In our study, we administered adenine a dose of 600 mg/kg/day for 10 days.

The disease control developed CKD after receiving adenine 600 mg/kg for 10 days, as seen by a considerable rise in blood creatinine,urea and blood glucose. In the model of adenine-induced CKD, researchers have observed comparable findings of an increase in serum creatinine and levels of nitrogenous waste products after 10 days of 600 mg/kg adenine oral treatment (20).

The normal control group demonstrated a physiological rise in body weight over 14 days that was notable relative to its baseline weight, whereas the illness control group failed to gain weight significantly despite drinking more water. This disparity in weight gain may be attributable to the catabolic condition brought on by chronic kidney disease brought on by adenine.

Acute or chronic renal failure are not phrases used by Ayurvedic science to describe kidney problems.However, it uses specific words to refer to renal function issues.comprises numerous indications and symptoms, such as dysuria (Mutrakrucchra), urinary incontinence (Mutraghata), and urine retention (Mutravrodha) (27). BD is a member of the class of plants that are frequently used to treat certain ailments. Edema (shotha) from a variety of causes has

also been treated with BD, either by itself or in conjunction with other plants. BD is a component of various polyherbal remedies (such as Vidarighrita and Bhadravahaghrita) that are used to treat symptoms thought to be brought on by renal impairment (28).

In the current investigation, we found that BD had a nephroprotective effect in preventing rats with adenine-induced chronic kidney disease from harming their kidneys. The results of our investigation show that the kidneys of rats with adenine-induced chronic renal disease are protected by the greater dose of 400/kg/mg of BD ethanolic extract. Additionally, it enhances the physical activity of rats (food and water intake) (29) regulates blood biochemical changes, and enhances renal ultrastructural changes in rats with adenine-induced chronic kidney disease. The outcome is the same as that reported in this dosage deponent for Acetaminophen-Induced Nephrotoxicity in Rats (30).

According to this study, the adenine-treated group's serum levels of creatinine, urea, and glucose were considerably higher than those of the healthy control group. In compared to the treated group with adenine, BD considerably ($p< 0.001$) decreased the serum creatinine, urea, and glucose. This result also reported by the cisplatin induced nephrotoxicity rats various dosage (31).

In our study, administration with 400mg/kg of bd extract for two weeks also improves urinary parameters such urine colour, appearance, RBCs, epithelial cells, and albumin levels. Highly significant differences between the illness control group and the positive control groups. This study revealed the pioneering researcher.

After two weeks of treatment, our study considerably reduces haemtocrit and different blood counts. RBC levels were considerably lowered and diseased controls' anaemia was clearly visible these results were in line with those of Nethaji Lokeswar Oburai (32).

Additionally, in our investigation, the histology of the kidneys of rats given 400 mg/kg/day of BD treatment revealed a substantial improvement compared to the disease control. The proximal tubular cells in the renal cortex were normal in appearance, and there was less eosinophilia and inflammatory infiltration overall. Only isolated desquamation, affecting about half of the tubules, was visible. The glomeruli were unaltered. In kidneys treated with gentamycin, BD had demonstrated a similar improvement in histological damage. BD ability to prevent kidney damage brought on by gentamycin was dose-dependent. However, our results demonstrate that the acute tubular necrosis brought on by adenine can be prevented to the same amount (as demonstrated by functional, biochemical, and histological characteristics) by an ethanolic extract of the root of BD given at a dose of 400 mg/kg/day(21,33,34and35)

The typical and terminal stage of practically all end-stage renal diseases is interstitial fibrosis. TGF-β is a well-known and significant molecular marker of organ fibrosis, but due to its diverse roles, it cannot be used as a targeted therapeutic target during the fibrosis process. TGF-β stimulates local fibroblasts, myofibroblasts, and tubule epithelial cells to create ECM components and inhibits ECM breakdown in the tubulointerstitial region of the glomerulus, which results in tubulointerstitial fibrosis. Whether the disease begins in the glomeruli, tubuli, or renal arteries, TGF-β is a crucial component in CKD progression. In any case, as they are drawn into the degeneration, TGF-β takes centre stage at all injured compartments (36).

Our result unequivocally shows that renal tissue damage observed after a pathological evaluation utilising MTS staining was consistent with the mesenchymal matrix rate. These findings imply that adenine can damage kidneys by increasing the mesangial matrix of renal tissue. It agrees with reports in the literature. Nevertheless, BD therapy, given at a concentration of 400 mg/kg, significantly protects the kidney tissue from the harmful effects of adenine by demonstrating antifibrotic activities.A well-known model of drug-induced

nephrotoxicity, kidney damage caused by acetaminophen (200 mg/kg/day for 14 days), was found to be protected against by the aqueous extract of BD root. In this investigation, when compared to the disease control, BD at a dose of 400 mg/kg/day for 14 days enhanced the histology(30). In kidneys treated with acetaminophen, BD had demonstrated a similar improvement in histological damage. BD has a dose-dependent protective effect against acetaminophen-induced kidney damage (37). However, our results show that the 400 mg/kg/day dose of an ethanolic extract of BD offers the same level of protection against adenine-induced CKD. Thus, future research may be advised to use the BD dose of 400 mg/kg/day.According to the immunohistochemical findings of this study, 400mg/kg of *BD* causes the expression of TGF-β to be slowed down in comparison to rats in the disease control group. In this study, we came to the conclusion that *BD* possesses antifibrotic properties and may lessen fibrosis in renal illness.Results from IHC and western blotting showed that the disease control group had much higher levels of TGF-β expression, which also suggested that kidney fibrosis was a process. However, the treatment group displayed a decrease in TGF-β expression, indicating that *BD* has an antifibrotic action. This report is consistent with the earlier report (38).In the current investigation, treatment with *BD* 400 mg/kg for 14 days reduced the expression of TGF-β in adenine-induced CKD.

According to recent studies, BD has a wide range of compounds, including steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins. Alkaloids, flavonoids, lignins, sugars, glycoprotein and proteins. It has been well established that alkaloids, flavonoids, sterols glycosides, and have antioxidant properties . The beneficial benefits shown in our study could be attributable to any one of these components, alone or in combination.

Ayurveda treatments using BD have been shown to be successful, and numerous investigations have shown this to be the case so far. In addition, to determine the active

ingredients in the ethanolic extract of BD and to assess their potential renoprotective effects, more research is necessary.

The majority of studies that assess a substance's capacity to prevent toxin-induced damage do so while simultaneously administering the substance or as a preventative measure. We gave BD and a large dose of adenine at the same time during this trial. Aging, hypovolemia, comorbidities like diabetes or hypertension, concurrent therapy with nephrotoxic medications, the presence of preexisting renal disease or septic shock, etc. are some risk factors that are more frequently linked to adenine-induced nephrotoxicity.

It's intriguing to think about how these compounds might be used therapeutically to alleviate renal diseases brought on by drugs or quicken the recovery from nephrotoxicity. The established chronic kidney damage brought on by adenine or other drugs with comparable negative effects may be reversible with BD.

Conclusion

The current findings imply that BD may be a useful treatment for kidney damage brought on by adenine. More experimental and clinical study should be carried out in order to demonstrate and employ this plant medicine's protective function in drug-induced kidney injury because it is often utilised by Ayurvedic practitioners to treat the symptoms of renal disorders. TGF-β appears to be downregulated by BDextract, which may have a favourable effect in lowering renal fibrosis. BD, a plant that is frequently consumed as a vegetable, can be utilised as a nutraceutical or therapeutic food for the prevention and treatment of CKD and other related conditions. To determine its clinical relevance and therapeutic potential, additional in-depth research are needed.

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CONFLICT OF INTEREST

There is no potential conflict of interest to declare.

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