RESEARCH ARTICLE

Effect of Herbal Extracts of *Bryophyllum calycinum* and *Achyranthes aspera* on Serum Biochemical Profile of Rats Having Adenine Induced Chronic Kidney Disease

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Abstract

The experiment was undertaken to study the therapeutic efficacy of aqueous and alcoholic extracts of *Bryophyllum calycinum* and *Achyranthes aspera* on adenine induced chronic kidney disease (CKD) in male Wistar rats. The rats (n = 48) were divided randomly into eight equal groups (n = 6 each). Group I and II served as normal and adenine control, respectively. In group II to VIII, CKD was induced by administration of adenine (200 mg/kg) along with drinking water for 28 days. After 28th day, the rats of CKD induced Groups III to VIII were given aqueous and alcoholic plant extracts of *B. calycinum* and *A. aspera* @ 300 mg/kg b.wt. orally as either single or combination of both herbal extracts (3:1) in 0.5 % sodium bicarbonate using syringe and rat gavage needle. CKD was confirmed by evaluating serum biochemical parameters before and after herbal treatment. Increased levels of serum glutamic pyruvic transaminase, urea nitrogen, uric acid, creatinine and phosphorus, while decreased levels of serum total protein were observed in all CKD groups by 28th day as compared to 0 day or normal control group, which was significantly reverted to near normal levels by day 70th following administration of single aqueous/alcoholic extract or a combination as biherbal extracts (3:1) from day 28 to 70 of experiment. The serum albumin and calcium, however, did not show significant alteration in any of the groups. Results of serum biochemistry revealed that aqueous and alcoholic extracts of *B. calycinum* and *A. aspera* possess good therapeutic/nephroprotective efficacy against CKD. The effect of biherbal alcoholic extract of the plants was much better in restoring the biochemical values of CKD induced rats by 42 days of administration.

Key words: *Achyranthe saspera, Bryophyllum calycinum,* Herbal extracts, Nephroprotective effect, Serum biochemistry, Wistar rats.

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Introduction

Chronic kidney disease (CKD) is a major growing public health problem in both developed and developing countries that has been linked to poor health outcomes, including decreased length and quality of life. There is high prevalence of morbidity and mortality associated with it, mainly due to cardiovascular dysfunction, neurohumoral dysfunction, and the development of end-stage renal disease (ESRD), which can annually cost more than one trillion dollars globally in clinical care. Till now, there is no single drug to treat kidney dysfunction in CKD patients, and the current therapeutic approaches to slow down its progression are limited to a normalization of insulin, glucose, and blood pressure (BP). Therefore, the development of either novel therapies or dietary supplements (especially from natural products) is highly needed to either abate the effects of the disease or slow/reverse the deterioration in kidney function (Ali *et al*., 2017).

The plant, *Bryophyllum calycinum* (Crassulaceae), commonly known as air plant, love plant, miracle leaf, life plant, zakham-e-hyat, panfutti, ghayamari, has been accepted as a herbal remedy in almost all parts of the world. It is a crassulescent herb of about 1 metre in height, with opposite, glabrous leaves (with 3–5 deeply crenulated, fleshy leaflets), growing primarily in the rain forest. It grows widely and used as folk medicine in tropical Africa, India, China, Australia, tropical America, Madagascar, Asia and Hawaii (Yadav *et al*., 2016). *Achyranthes aspera* L. (Family: Amaranthaceae)

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is an important medicinal plant which is found as a weed throughout India, predominantly on roadsides, in fellow/ uncultivated lands and along the boundaries of the cultivated fields. It grows up to an altitude of 2100 *m. A. aspera* is found in South Andaman Islands (Chandraker and Sharma, 2014). Large numbers of phytochemical constituents from this plant have been identified which possess activities like a diuretic, purgative, laxative, antiasthmatic, hepatoprotective, and anti-allergic properties (Srivastav *et al*., 2011). The present study was aimed to evaluate the nephroprotective effect of herbal extracts of *Bryophyllum calycinum* and *Achyranthes aspera* on adenine induced chronic kidney disease in male Wistar rats through monitoring the serum biochemical profile.

Materials and methods

Experimental Design

The project was approved by the Institutional Animals Ethics Committee (IAEC) of the College (Approval No: AAU/GVC/ CPCSEA/ IAEC/ 271/2018 dated 17/11/2018). The work was carried out on 48 healthy mature (12-15 weeks old) male Wistar rats. For the induction of CKD, adenine was used. Rats divided into eight groups (n = 6, each) were kept in separate cages. Group I and II served as Normal and Adenine control, respectively. In group II to VIII, CKD was induced by administration of adenine (200 mg/kg b.wt.) along with drinking water for 28 days. After the 28th day, the rats of CKD induced groups (III–VIII) were given aqueous and alcoholic plant extracts of *Bryophyllum calycinum* and *Achyranthes aspera* @ 300 mg/kg b.wt. orally either as a single extract or a combination as biherbal extracts (3:1) in 0.5% sodium bicarbonate using syringe and rat gavage needle for the next 42 days, *i.e*., till day 70th of the experiment as detailed below.

Preparation of Plant Extracts

Fresh leaves of *Bryophyllum calycinum* and roots of *Achyranthes aspera* were air-dried and powdered. A coarse powdered material of both the plants (100 gm of each) was extracted with water and alcohol in Soxhlet apparatus for 24 hours. The extract was evaporated under reduced pressure to give solid residue. The aqueous and alcoholic extracts were preserved in refrigerator at 4°C for subsequent experiments.

Therapeutic Study

After 28 days of induction of CKD, the rats under groups III and IV were given aqueous and alcoholic extracts *of Bryophyllum calycinum* orally @ 300 mg/kg, respectively, for 42 days. Similarly, the rats under groups V and VI were given aqueous and alcoholic extracts of *Achyranthes aspera* orally @ 300 mg /kg, respectively for 42 days, while the rats under group VII were given aqueous biherbal extracts and group VIII were given alcoholic biherbal extracts of *Bryophyllum calycinum* and *Achyranthes aspera* orally @ 300 mg/kg (3:1), respectively, for another 42 days, *i.e*., till day 70 of experiment.

Blood Collection and Serum Biochemical Analysis

Blood samples were collected from all the rats by retro-orbital plexuses puncture under mild diethyl ether anesthesia with the help of capillary tube thrice, *i.e.,* first on day 0, after 28th days of induction of CKD and then on 70th day (after 42 days of treatment) of the experimental period. Serum was harvested by centrifugation at 3000 rpm for 15 minutes at 10°C (Eppendorf 5804 R, Germany) and stored at -40°C until used for biochemical analysis using standard auto-analyzer (CKK-300 Ark diagnostics, Bangalore). The biochemical parameters studied were serum glutamic pyruvic transaminase (SGPT), serum urea nitrogen (SUN), uric acid, creatinine, calcium and phosphorus, total protein (TP) and albumin.

Statistical Analysis

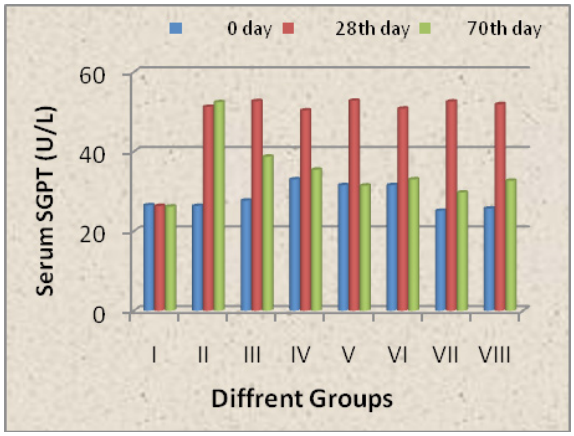
One-way analysis of variance (ANOVA) was used to compare the effects of groups within the period using SPSS software (Version 20). Paired 't' test was used to compare serum parameters before and after treatment (Snedecor and Cochran, 1990).

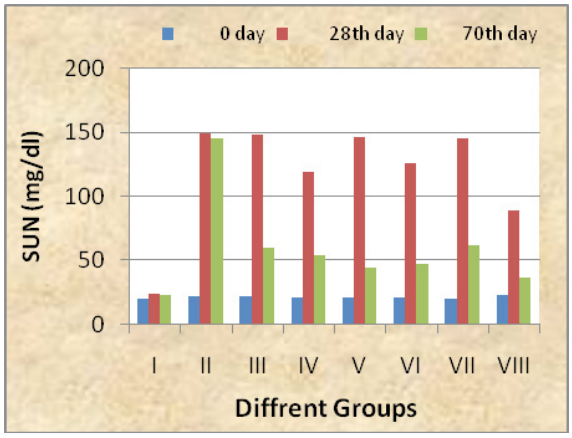
Results and discussion

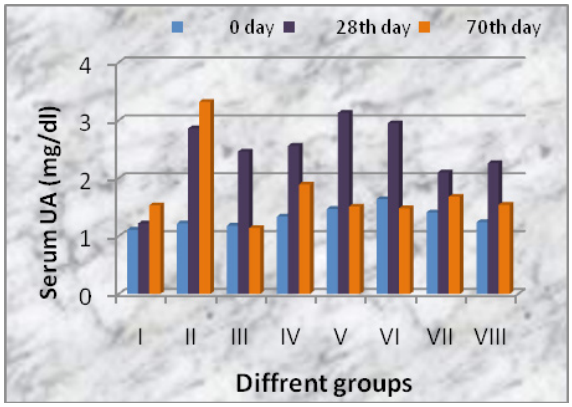
The findings on renal function evaluated by measuring serum total protein, SGPT, SUN, uric acid, creatinine, calcium, phosphorus, and albumin in group I–VIII are presented in Table 1, 2, and Figure 1.

In the present study, the decreased levels of serum total protein and increased levels of serum SGPT, SUN, creatinine, phosphorus, and uric acid were observed in the CKD induced groups by day 28 of adenine treatment as compared to day 0 (when experiment was started) and of normal control group (Table 1). 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. These observations were in accordance with the reports of Jia *et al*. (2013) and Ali *et al*. (2016) for one or the other constituents. The increased serum SGPT might also be due to the leakage of this enzyme into the general circulation from the collateral circulation (Ali *et al.,* 2016). The elevated SUN in blood serum of kidney failure patients may also be due to the degradation of food and tissues such as muscle with its impaired mechanism of elimination from glomeruli (Nisha *et al*., 2017).

Administration of either single extract or the co-treatment subsequently with aqueous and alcoholic extracts of *B. calycinum* and *A. aspera* for 42 days significantly ($p < 0.05$) restored these changes, *i.e.,* by 70th day of experiment in most of the groups. Biherbal alcoholic extract compared to mono-herbal extract of the said plants was better in restoring the values of serum total protein, SGPT, SUN, creatinine, phosphorus, and uric acid. The serum SGPT and total protein values came down nearer to the vehicle control group by the 70th day. Rahman *et al*. (2018) recorded a significantly elevated







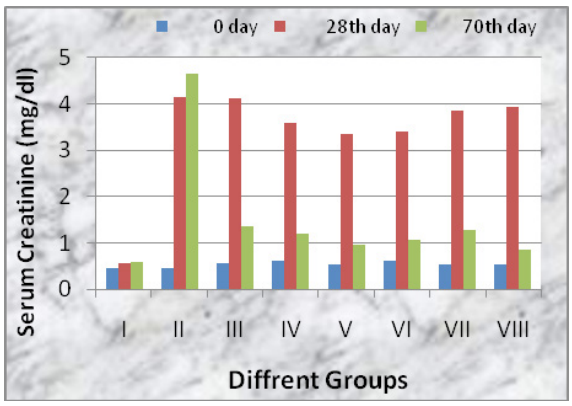


Fig.1: Serum biochemical changes in rats under different CKD induced and herbal treated groups in relation to initial values (Gr. III to VIII) and of negative and positive control groups (Gr. I and II).

Table 1: Serum total protein, albumin, SGPT, and SUN concentrations on day 0, 28 and 70 in CKD induced and herbal treated rats

Groups		Total protein (g/dL)			Serum albumin (g/dL)			SGPT (U/L)			SUN (mg/dL)		
No.	Name	'0' day	28th day	70th day	'0' day	28th day	70th day	'0' day	28th day	70th day	'0' day	28th day	70th day
I	Normal control	6.65^{a} ± 0.63	6.15^{a} ± 0.34	6.43^{ab} ± 0.25	4.83 ± 0.17	4.60 ± 0.30	4.87 ± 0.18	26.50^{b} ± 0.92	26.33^{b} ± 0.84	26.15^{c} ± 4.26	20.33_{x} ± 1.38	24.00^{b} ± 1.15	23.50^{d} ± 0.88
II	Adenine control	$6.34^{a}{}_{y}$ ± 0.68	$5.75^{ab}{}_{x}$ ± 0.13	$5.93^{c}{}_{x}$ ± 0.17	4.20 ± 0.15	4.65 ± 0.13	4.01 ± 0.21	$26.33^{b}{}_{x}$ ± 0.68	$51.22^{a}{}_{y}$ ± 4.45	$52.40^{a}{}_{y}$ ± 7.46	22.33_{x} ± 1.59	$148.56^{a}{}_{y}$ ± 7.78	$145.40^{a}{}_{y}$ ± 5.01
III	AQ. EX. of B.C.	$5.93^{ab}{}_{y}$ ± 0.53	$5.67^{b}{}_{x}$ ± 0.80	$6.17^{bc}{}_{y}$ ± 0.19	4.60 ± 0.20	4.07 ± 0.16	4.09 ± 0.39	$27.67^{ab}{}_{y}$ ± 0.86	$52.67^{a}{}_{x}$ ± 2.83	$38.71^{b}{}_{z}$ ± 1.74	21.78_{x} ± 1.14	$148.22^{a}{}_{z}$ ± 9.70	$60.29^{b}{}_{y}$ ± 9.99
IV	AL.EX. of B.C.	$6.02^{ab}{}_{y}$ ± 0.39	$5.15^{b}{}_{x}$ ± 0.53	$6.89^{a}{}_{y}$ ± 0.18	4.99 ± 0.13	4.83 ± 0.20	4.48 ± 0.27	$30.00^{a}{}_{y}$ ± 1.50	$50.33^{a}{}_{x}$ ± 2.74	$35.43^{b}{}_{y}$ ± 4.82	20.78_{x} ± 0.95	$138.67^{a}{}_{z}$ ± 9.95	$54.14^{b}{}_{y}$ ± 10.27
V	AQ.EX. of A.A.	$5.98^{ab}{}_{y}$ ± 0.38	$5.18^{b}{}_{x}$ ± 0.58	$6.18^{bc}{}_{y}$ ± 0.19	4.13 ± 0.16	4.16 ± 0.16	4.83 ± 0.30	$31.56^{a}{}_{x}$ ± 1.60	$52.78^{a}{}_{y}$ ± 3.13	$31.43^{bc}{}_{x}$ ± 3.57	20.89_{x} ± 0.30	$146.11^{a}{}_{z}$ ± 8.36	$44.00^{bc}{}_{y}$ ± 5.30
VI	AL.EX. of A.A.	$5.63^{ab}{}_{y}$ ± 0.57	$5.12^{b}{}_{x}$ ± 0.72	$6.58^{ab}{}_{y}$ ± 0.15	4.59 ± 0.14	4.96 ± 0.14	4.20 ± 0.15	$31.56^{a}{}_{x}$ ± 1.54	$50.78^{a}{}_{y}$ ± 1.56	$33.00^{bc}{}_{x}$ ± 2.73	21.67_{x} ± 0.57	$125.33^{a}{}_{z}$ ± 10.12	$47.43^{b}{}_{y}$ ± 5.21
VII	BI AQ EX. of B.C.+ A.A.	$6.02^{ab}{}_{y}$ ± 0.50	$5.03^{b}{}_{x}$ ± 0.57	$6.89^{a}{}_{y}$ ± 0.97	4.96 ± 0.19	4.84 ± 0.20	4.58 ± 0.22	$25.11^{b}{}_{y}$ ± 0.77	$52.56^{a}{}_{x}$ ± 1.98	$29.71^{bc}{}_{y}$ ± 4.49	20.33_{x} ± 0.68	$145.00^{a}{}_{z}$ ± 11.06	$62.00^{b}{}_{y}$ ± 12.94
VIII	BI AL EX. of B.C.+A.A.	$5.74^{b}{}_{y}$ ± 0.58	$5.13^{b}{}_{x}$ ± 0.37	$6.33^{bc}{}_{y}$ ± 0.10	4.51 ± 0.14	4.99 ± 0.17	4.52 ± 0.12	$25.67^{b}{}_{x}$ ± 2.06	$51.89^{a}{}_{y}$ ± 1.67	$32.67^{b}{}_{x}$ ± 1.76	22.89_{x} ± 1.04	$138.56^{a}{}_{z}$ ± 3.75	$36.83^{c}{}_{y}$ ± 2.18

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

Table 2: Serum uric acid, creatinine, calcium, and phosphorus concentrations on day 0, 28 and 70 in CKD induced and herbal treated rats

Groups		Uric acid (mg/dL)			Creatinine (mg/dL)			Calcium (mg/dL)			Phosphorus (g/dL)		
No.	Name	'0' day	28^{th} day	70^{th} day	'0' day	28^{th} day	70^{th} day	'0' day	28^{th} day	70^{th} day	'0' day	28^{th} day	70^{th} day
I	Normal control	1.11 ± 0.15	$1.22^{a}{}_{z} \pm 0.24$	$1.53^{ab}{}_{z} \pm 0.09$	0.45 ± 0.10	$0.55^{a}{}_{y} \pm 0.06$	$0.58^{a}{}_{x} \pm 0.10$	6.65 ± 0.63	6.15 ± 0.34	6.43 ± 0.25	6.91 ± 0.36	$6.95^{a}{}_{x} \pm 0.17$	$6.61^{ab}{}_{y} \pm 1.75$
II	Adenine control	$1.25_{y} \pm 0.21$	$2.86^{b}{}_{x} \pm 0.21$	$3.32^{c}{}_{z} \pm 0.17$	$0.46_{x} \pm 0.08$	$4.14^{b}{}_{y} \pm 0.49$	$4.66^{c}{}_{y} \pm 0.23$	6.34 ± 0.68	6.75 ± 1.13	5.93 ± 0.17	$6.48_{y} \pm 0.21$	$7.98^{b}{}_{x} \pm 0.90$	$7.82^{c}{}_{x} \pm 0.46$
III	AQ. EX. B.C.	$1.18_{y} \pm 0.17$	$2.46^{b}{}_{x} \pm 0.20$	$1.14^{a}{}_{y} \pm 0.29$	$0.55_{x} \pm 0.10$	$4.12^{b}{}_{y} \pm 0.30$	$1.36^{b}{}_{x} \pm 0.25$	5.93 ± 0.53	6.67 ± 0.80	6.17 ± 0.19	$6.06_{y} \pm 0.24$	$9.15^{bc}{}_{x} \pm 0.97$	$6.42^{ab}{}_{y} \pm 0.90$
IV	AL.EX. B.C.	$1.34_{y} \pm 0.04$	$2.56^{b}{}_{x} \pm 0.20$	$1.89^{ab}{}_{y} \pm 0.20$	$0.62_{x} \pm 0.05$	$3.59^{b}{}_{y} \pm 0.38$	$1.20^{b}{}_{x} \pm 0.17$	6.02 ± 0.39	5.95 ± 0.53	5.89 ± 0.18	$6.26_{y} \pm 0.24$	$7.38^{b}{}_{x} \pm 0.56$	$6.87^{ab}{}_{y} \pm 0.19$
V	AQ.EX. A.A.	$1.47_{y} \pm 0.10$	$3.13^{bc}{}_{x} \pm 0.17$	$1.51^{ab}{}_{y} \pm 0.07$	$0.53_{x} \pm 0.10$	$3.36^{b}{}_{y} \pm 0.36$	$0.95^{ab}{}_{x} \pm 0.10$	6.98 ± 0.38	5.81 ± 0.58	6.18 ± 0.19	$6.14_{y} \pm 0.31$	$7.43^{b}{}_{x} \pm 0.22$	$6.88^{ab}{}_{y} \pm 0.70$
VI	AL.EX. A.A.	$1.64_{y} \pm 0.23$	$2.95^{b}{}_{x} \pm 0.19$	$1.48^{ab}{}_{y} \pm 0.09$	$0.62_{x} \pm 0.05$	$3.40^{b}{}_{y} \pm 0.31$	$1.06^{ab}{}_{x} \pm 0.13$	6.63 ± 0.57	5.71 ± 0.72	5.58 ± 0.15	$6.77_{y} \pm 0.36$	$7.37^{b}{}_{x} \pm 0.45$	$6.34^{a}{}_{y} \pm 0.38$
VII	BI AQ EX. (B.C.+A.A.)	$1.41_{y} \pm 0.06$	$2.10^{b}{}_{x} \pm 0.17$	$1.68^{ab}{}_{y} \pm 0.14$	$0.52_{x} \pm 0.08$	$3.85^{b}{}_{y} \pm 0.49$	$1.27^{b}{}_{x} \pm 0.46$	6.02 ± 0.50	6.03 ± 0.57	6.89 ± 0.97	$6.14_{y} \pm 0.54$	$7.66^{b}{}_{x} \pm 0.84$	$6.18^{a}{}_{y} \pm 1.05$
VIII	BI AL EX. (B.C.+A.A.)	$1.24_{y} \pm 0.09$	$2.26^{b}{}_{x} \pm 0.22$	$1.54^{ab}{}_{y} \pm 0.06$	$0.54_{x} \pm 0.07$	$3.94^{b}{}_{y} \pm 0.39$	$0.85^{ab}{}_{x} \pm 0.08$	6.74 ± 0.58	5.79 ± 0.37	6.33 ± 0.10	$6.76_{y} \pm 0.52$	$9.28^{bc}{}_{x} \pm 0.70$	$6.90^{ab}{}_{y} \pm 0.41$

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

level of serum creatinine in the adenine model group. Tani *et al*. (2017) however did not observe a significant change in serum calcium level in modified adenine CKD induced rats, while Patel *et al*. (2018) found significant increase in serum calcium level in CKD induced rats, which decreased after treatment with extracts of *Bryophyllum calycinum* and *Solanum xanthocarpum*. Rathva (2016) reported a significant increase in serum phosphorus level in CKD induced rats that decreased after treatment with extracts of and *Solanum xanthocarpum* and *Achyranthes aspera*.

It was concluded that alcoholic extract of *B. calycinum* and *A. aspera* at the dose rat of 300 mg/kg 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.

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References

Ali, B.H., Al Za, M., Adham, S.A., Yasin, J., Nemmar, A. and Schupp, N. (2016). Therapeutic effect of chrysin on adenine-induced chronic kidney disease in rats. *Cell. Physiol.& Biochem*., 38(1), 248-257.

Ali, B.H., Cahliková, L., Opletal, L., Karaca, T., Manoj, P., Ramkumar, A., and Locarek, M. (2017). Effect of aqueous extract and anthocyanins of calyces of *Hibiscus sabdariffa* (Malvaceae) in rats with adenine induced chronic kidney disease. *J. Pharmacy & Pharmacol*., 69(9), 1219-1229.

Chandraker and Sharma (2014). Plant review: Phytochemical constituents and their pharmacological activities of *Achyranthes aspera L*. (Chirchira) *Intl. J. Pharmacol. & Pharmaceutical Sci*., 2(2), 13-19.

Jia, T., Olauson, H., Lindberg, K., Amin, R., Edvardsson, K., Lindholm, B., and Larsson, T. E. (2013). A novel model of adenine-induced tubulointerstitial nephropathy in mice. *BMC Nephrology*, 14(1), 116.

Nisha, R., Srinivasa Kannan, S.R., Thanga Mariappan, K., and Jagatha, P. (2017). Biochemical evaluation of creatinine and urea in patients with renal failure undergoing hemodialysis. *J. Clin. Path.& Lab. Med*., 1(2), 1-5.

Patel, D.B., Raval, S.K., Mandali, G.C., Patel, A.C. and Pande, A.M. (2018). Nephroprotective effect of herbal extracts of *Bryophyllum calycinum* and *Solanum xanthocarpum* on induced urolithiaisis in Wistar rats: Haemato-biochemical evaluation. *Indian J. Vet. Sci. & Biotech*., 13(3), 49-54.

Rahman, A., Yamazaki, D., Sufiun, A., Kitada, K., Hitomi, H., Nakano, D., and Nishiyama, A. (2018). A novel approach to adenine-induced chronic kidney disease associated anaemia in rodents. *PloS one*, 13(2), e0192531.

Rathva, A.N. (2016). *Evaluation of therapeutic efficacy of plants Solanum xanthocarpum and Achyranthes aspera on ethylene glycol induced urolithiasis in Wistar rats*. MVSc Thesis, Anand Agricultural University, Anand, India.

Snedecor, G.W., and Cochran, W.G. (1990*). Statistical Methods*. 6th *ed*., Oxford and JBH Publishing, New York.

Srivastav, S., Singh, P., Jha, K.K., Mishra, G., Srivastava, S., Karchuli, M.S., and Khosa, R.L. (2011). Diuretic activity of whole plant extract of *Achyranthes aspera* Linn. *European J. Exptl. Biol.*, 1(2), 97-102.

Tani, T., Orimo, H., Shimizu, A., and Tsuruoka, S. (2017). Development of a novel chronic kidney disease mouse model to evaluate the progression of hyperphosphatemia and associated mineral bone disease. *Scientific Reports*, 7(1), 2233.

Yadav, M., Gulkari, V. D., and Wanjari, M. M. (2016). *Bryophyllum pinnatum* leaf extracts prevent formation of renal calculi in lithiatic rats. *Ancient Science of Life*, 36(2), 90.