Effect of Extracts of *Bryophyllum calycinum* and *Achyranthes Aspera* on Urine Profile in Male Wistar Rats having Adenine Induced Chronic Kidney Disease

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**Abstract**

The study was undertaken to evaluate the therapeutic efficacy of aqueous and alcoholic uniherbal and biherbal extracts of *Bryophyllum calycinum* and *Achyranthes aspera* on adenine induced chronic kidney disease (CKD) in male Wistar rats. Forty-eight rats were randomly divided into eight equal groups, each of six animals. The rats of 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.) daily along with drinking water for 28 days. After the 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 either as single extract or a combination as biherbal extracts (3:1) in 0.5 % sodium bicarbonate using syringe and rat gavage needle. CKD was confirmed by evaluating urine parameters. Significantly (p <0.01) 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 near normal levels within next 42 days of daily administration of either single aqueous/alcoholic extract or a combination as biherbal extract (3:1), without statistical differences among formulations with regard to therapeutic/nephroprotective efficacy against CKD in terms of reducing the altered urine values towards near-normal by 42 days of oral administration.

**Keywords:** *Achyranthes aspera*, *Bryophyllum calycinum*, Herbal extracts, Nephroprotective effect, Urine analyses, Wistar rats.

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**Introduction**

Chronic kidney disease (CKD) is a major growing public health problem globally that has been linked to poor health outcomes, including decreased length and quality of life due to cardiovascular dysfunction, neurohumoral dysfunction, and the development of end-stage renal disease (ESRD). Till date, 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 the normalization of insulin, glucose, and blood pressure. Therefore, the development of either novel therapies or dietary supplements is the need of the day either to abate the effects of the disease or to slow down /reverse the deterioration in kidney function (Ali et al., 2017).

In our earlier paper, we have reported the effect of extracts of medicinal plants, *Bryophyllum calycinum* (Crassulaceae), and *Achyranthes aspera* L. (Amaranthaceae) on serum biochemistry of CKD induced Wistar rats (Gehani et al., 2019). Large numbers of phytochemical constituents from these plants which possess activities like diuretic, purgative, laxative, antasthmatic, hepatoprotective and anti-allergic properties have been identified. (Srivastav et al., 2011; Chandraker and Sharma, 2014). The present study was aimed to report the nephroprotective effect of herbal extracts of *Bryophyllum calycinum* and *Achyranthes aspera* through urine analysis following adenine induced chronic kidney disease in male Wistar rats.

**Materials and Methods**

Experimental Design

The project was approved by the Institutional Animals Ethics Committee (IAEC) of the College (Approval No: 271/2018). The work was carried out on 48 healthy mature (12-15 weeks old) male Wistar rats. Rats divided into 8 equal 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.) daily 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 *Bryophyllum calycinum* and *Achyranthes aspera* @ 300 mg/kg b.wt. orally either as single extract or a combination as biherbal extracts (3:1) in 0.5% sodium bicarbonate using syringe and rat gavage needle for next 42 days, i.e., till day 70th of an experiment (Table 1).

**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 g 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 (residues) were preserved in a refrigerator at 4°C for a subsequent experiment.

**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* @ 300 mg/kg each, respectively for 42 days. Similarly, the rats under groups V and VI were given aqueous and alcoholic extracts of *Achyranthes aspera* @ 300 mg/kg each, respectively for 42 days, while the rats under groups VII and VIII were given aqueous biherbal extracts and alcoholic biherbal extracts of *Bryophyllum calycinum* and *Achyranthes aspera* @ 300 mg/kg (in 3:1 ratio), respectively, for 42 days, i.e., till day 70th of experiment (Table 1).

**Urine Collection and Analyses**

After induction of CKD, i.e., on day 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). The rats had free access to drinking water during the urine collection period. 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 estimations for urine calcium, phosphorous, creatinine, uric acid, and total protein by using kits on standard auto-analyzer (CKK-300 Ark diagnostics, Bangalore). The data were statistically analyzed using a one-way-analysis of variance (ANOVA) to compare the effects of groups within the period using SPSS software (Version 20). Paired ‘t’ test was used to compare urine parameters before and after treatment in each group (Snedecor and Cochran, 1990).

**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 28 and day 70 of an experiment are shown in Tables 1-2 and Figures 1-2.

In the present study, highly significant (p<0.01) 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 normal control group; however, there was no any discernible change in the urinary uric acid levels among eight groups (Table 1-2; Fig. 1-2). 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 *A. aspera* for 42 days significantly reduced these changes, i.e., by 70th day of experiment in most of the groups, 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

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*Figure 1: Urine calcium and phosphorus (mg/dl) levels in rats under different CKD groups*
extract in reducing the adenine effects (Table 1, 2). However, the same biherbal alcoholic extract had shown better efficacy in bringing the altered serum biochemical profiles to near normal levels over the aqueous biherbal extract and even single aqueous or alcoholic extract in adenine induced CKD rats in our earlier study (Gehani et al., 2019).

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. Levey et al. (2003) concluded that the CKD patients had higher chances to increase the volume and decrease the pH of urine, which indicated that the acidification of urine occurs due to kidney damage. Jia et al. (2013) reported a non-significant change in urine calcium in adenine induced mice, while Rathva (2016) found a significant increase in urine calcium level following adenine treatment that decreased after treatment with extracts of Achyranthes aspera and Solanum xanthocarpum in rats. However, a significant increase in urine phosphorus was recorded in adenine induced mice by Jia et al. (2013). Similarly, Patel (2018) observed significant increase in urine phosphorus level in adenine induced CKD rats, which decreased following 42 days of treatment with extracts of Bryophyllum calycinum and Solanum xanthocarpum.

Table 1: Urine volume, pH, specific gravity and uric acid levels on 28th and 70th day in CKD induced and herbal treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urine Volume (ml)</th>
<th>Urine pH</th>
<th>Urine Specific Gravity</th>
<th>Urinary Uric Acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28th day</td>
<td>70th day</td>
<td>28th day</td>
<td>70th day</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>7.80 ± 0.36</td>
<td>7.94 ± 0.02</td>
<td>6.80 ± 0.50</td>
</tr>
<tr>
<td>II</td>
<td>Adenine Control</td>
<td>20.89 ± 1.18</td>
<td>21.40 ± 0.74</td>
<td>11.43 ± 0.10</td>
</tr>
<tr>
<td>III</td>
<td>AQ. EX. BC</td>
<td>20.67 ± 2.02</td>
<td>11.09 ± 0.19</td>
<td>10.67 ± 0.25</td>
</tr>
<tr>
<td>IV</td>
<td>AL. EX. BC</td>
<td>21.56 ± 1.36</td>
<td>11.17 ± 0.33</td>
<td>11.25 ± 0.19</td>
</tr>
<tr>
<td>V</td>
<td>AQ. EX. AA</td>
<td>20.00 ± 1.21</td>
<td>11.17 ± 0.23</td>
<td>11.25 ± 0.19</td>
</tr>
<tr>
<td>VI</td>
<td>AL. EX. AA</td>
<td>21.89 ± 1.35</td>
<td>11.06 ± 0.35</td>
<td>11.25 ± 0.19</td>
</tr>
<tr>
<td>VII</td>
<td>BI AQ. EX (BC+AA)</td>
<td>21.56 ± 1.36</td>
<td>11.17 ± 0.33</td>
<td>11.25 ± 0.19</td>
</tr>
<tr>
<td>VIII</td>
<td>BI AL. EX (BC+AA)</td>
<td>21.11 ± 1.21</td>
<td>11.17 ± 0.33</td>
<td>11.25 ± 0.19</td>
</tr>
</tbody>
</table>

Means with different superscripts (a,b,c,…) within the row differs significantly (p < 0.05).
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In contrast, Abellan et al. (2019) reported significant decrease in mean value of urinary phosphorus in adenine fed rats. Rivera-Valdes et al. (2017) found significantly decreased urinary creatinine in adenine fed rats. In an earlier study, the adenine-exposed mice did not have increased proteinuria compared to controls (Jia et al., 2013). This was likely explained by the C57BL/6 strain's known resistance towards the development of proteinuria in combination with the tubular interstitial nature of the renal damage in this model.

From the study, it was concluded that the administration of aqueous or alcoholic extract of *B. calycinum* and *A. aspera* at the dose rate of 300 mg/kg b.w.t. 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.

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**References**


