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Prophylactic effects of bi-herbal extracts of *Coriandrum sativum* and *Murraya koenigii* on adenine induced chronic kidney disease in rats

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Abstract
The present study was planned to evaluate the prophylactic efficacy of *Coriandrum sativum* (CS) and *Murraya koenigii* (MK) on adenine-induced chronic kidney disease in rats. The present experiment was conducted on 36 male Sprague-Dawley rats. In order to assess the prophylactic efficacy of bi-herbal aqueous and alcoholic extracts *Coriandrum sativum* (CS) and *Murraya koenigii* (MK), were mixed in a 1:1.5 ratio after determined by *in-vitro* nucleation assay. The rats were randomly divided into six different groups, each group contains six rats. Chronic kidney disease (CKD) was induced in the group II, III, IV, V and VI by adenine @ 200 mg/kg b.wt. daily once through the intragastric route for 28 days. Group I served as control and was given standard pelleted diet. Group II served as adenine control and was given adenine (200 mg/kg b.wt., orally) for 28 days. Groups III and IV received bi-herbal aqueous extracts of CS and MK @ 250 and 500 mg/kg b.wt., for 28 days respectively. Groups V and VI received bi-herbal alcoholic extracts @ 250 and 500 mg/kg b.wt., for 28 days respectively. The blood was collected on 28th day of experiment to analyse the haemato-biochemical parameters. Urine samples were collected by using metabolic cages on 28th day and analyzed for different qualitative and biochemical parameters. The experimental rats were studied for ultrasonographic, gross and histopathological changes in kidneys of different groups. Bi-herbal aqueous and alcoholic extracts treated rats (groups III, IV, V and VI) produced significant improvement in mean body weight and feed consumption as compared to adenine control rats (group-II). The administration of bi-herbal aqueous and alcoholic extracts of CS and MK leaves, along with adenine in prophylactic groups revealed significant improvement on haemato-biochemical and urine parameters. Results of ultrasonographic and histopathological examination of kidney tissues in prophylactic groups was well supported and prevent alterations in kidney associated changes in adenine-induced CKD in rats. Result of the present study showed that the bi-herbal aqueous extracts of CS and MK leaves at dosage of 250 mg/kg b.wt., while bi-herbal alcoholic extracts at dosage of 500 mg/kg b.wt. showed greater efficacy among all prophylactic study groups. Results of the present study showed that aqueous as well as alcoholic bi-herbal extracts of *Coriandrum sativum* and *Murraya koenigii* leaves revealed prophylactic efficacy against adenine induced CKD in rats.

Keywords: *Coriandrum sativum*, *Murraya koenigii*, Bi-herbal extracts, prophylactic effects, CKD

Introduction
The kidneys are the body's main organs for maintaining homeostasis, eliminating metabolic waste products like creatinine, urea, ammonia and uric acid, regulating extracellular fluid volume, blood pressure and controlling systemic pH [1]. Chronic kidney disease (CKD) is gradual loss of function of kidney which is slowly progressive disease, where kidney structure or dysfunction present for three month or more. Six progressive stages of CKD are stages 1, 2, 3a and 3b, 4 and 5. The stages are based on the glomerular filtration rate (GFR) test results [2].
For research on the causes and therapies of CKD, a sneaky illness brought on by kidney damage and characterised by persistent functional decline more than three months, with or without signs of structural deficit, numerous animal models have been developed. The rodent adenine diet model of CKD is an exception. The original adenine diet model resulted in a kidney disease with rapid onset, extensive tubulointerstitial fibrosis, tubular atrophy, crystal formation and obvious vessel calcification.

These chronic adenine diet models enable the characterisation of relatively stable kidney and cardiovascular disease, similar to CKD in humans [3].

Coriandrum sativum L. belongs to the Umbelliferae family. It is known as coriander, dhania in Hindi and dhana in Gujarati [4]. It is well-known for having carminative, digestive, hepatoprotective, diuretic and antihelminthic properties. Flavonoids and polyphenols are the main components of *Coriandrum sativum*, which is a source of phytochemicals with nephroprotective potential [5].

Murraya koenigii (curry leaf tree) belongs to genus "Murraya" and citrus sub-family "Rutaceae", is a native of India and South-East Asia. Leaves of the plant are called "Curry leaves" in English or "Mitha neem" in Hindi and "Mitho limado" in Gujarati [6]. The plant is also reported to have antitumor [7], anti-inflammatory [8], hypoglycemic [9], antihyperglycemic [10]. The leaf extract was discovered to be effective in maintaining normal levels of urine output, urinary creatinine, urinary urea, urinary urea nitrogen, total urinary protein and urinary Na^+. The extract also protected against unilateral renal ischemia reperfusion injury while maintaining the normal pattern in *in vivo* antioxidants, renal myeloperoxidase activity and kidney histology. Many scientific reported studies showed that the extract of this plant is effective in treating rats with kidney problems [11]. The present study was planned to evaluate the nephroprotective effects of bi-herbal aqueous and alcoholic extracts of *Coriandrum sativum* and *Murraya koenigii* leaves on adenine induced CKD in male Sprague Dawley rats through monitoring the haemato-biochemical parameters, urine parameters, ultrasonographic examinations and histopathological alterations.

Materials and Methods

Experimental Animals

The research was conducted using thirty-six healthy male Sprague Dawley rats, aged 8–10 weeks, which were procured from the Zydus Research Centre, Ahmedabad, Gujarat. The rats were kept in the Small Animal House facility at Veterinary College, Kamdhenu University, Anand. The research protocol was accepted by the Institutional Animal Ethics Committee of the College of Veterinary Science and Animal Husbandry (IAEC/373/VPT/2022) and all the animal procedures were carried out according to regulations of Committee for Control and Supervision of Experiments on Animals (CCSEA).

Experimental Design

As a control, Group I received a regular pelleted diet. Group II served as adenine control and was given adenine (200 mg/kg b.wt., orally) for 28 days. Groups III, IV, V and VI were prophylactic group, received bi-herbal aqueous and alcoholic extracts of CS and MK, along with adenine orally, for 28 days. Groups III and IV received bi-herbal aqueous extracts of CS and MK @ 250 and 500 mg/kg b.wt., respectively. Groups V and VI received bi-herbal alcoholic extracts @ 250 and 500 mg/kg b.wt., respectively.

Preparation of Plant Extracts and Ratio Determination

Leaves of *Coriandrum sativum* and *Murraya koenigii* were cut into small pieces and dried under shade, a mechanical grinder was used to make the powder, which was then stored in airtight containers. For preparation of aqueous extracts, 100 g of the *Coriandrum sativum* and *Murraya koenigii* dried leaves powders were soaked in 1 litre of distilled water and shaking three times every day. The obtained aqueous extracts were concentrated at 50-60 °C under reduced pressure in a rotary evaporator, leaving a residue. *Coriandrum sativum* and *Murraya koenigii* aqueous extracts were obtained, and they were put in a petri dish and placed over a hot water bath (50 °C) until the solvent entirely evaporated. For preparation of alcoholic extracts, exactly 100 g of coarse powdered material of *Coriandrum sativum* and *Murraya koenigii* leaves were extracted in soxhlet extractor with solvent alcohol. The extracted materials were concentrated under reduced pressure in a rotary evaporator at 50-60°C, leaving a dark brown residue. Alcoholic extracts of obtained were transferred to a petri dish and kept over hot water bath (50 °C) until the solvent gets completely evaporated. Both the aqueous and the alcoholic extracts were stored for experimental use at 4 °C in a refrigerator. Aqueous and alcoholic extracts of *Coriandrum sativum* (CS) and *Murraya koenigii* (MK) were mixed in a 1:1.5 ratio after determined by *in-vitro* nucleation assay.

Clinical Observation and Mortality

Throughout the experimental period, all the rats from groups I to VI were observed daily for any mortality as well as any unusual physical or behavioural changes.

Feed Consumption

The amount of feed provided to the group of animals placed in each cage varies according to their needs was recorded and the leftover feed was measured every week.

Body Weight

The body weight of all animals in Groups I to VI was measured on the day before starting of the experiment (Day 0) and then further weight was taken on 1st, 2nd, 3rd and 4th week of the experiment.

Collection of Blood and Haemato-Biochemical Evaluation

On the 28th day of the experiment, blood samples were collected from all of the rats via retro-orbital plexuses punctured with a capillary tube under light isoflurane anesthesia. Blood samples (1 mL) collected in K3EDTA test tubes and subjected to estimate different hematological parameters, *viz*. Hemoglobin (g/dL), Total Leukocyte Count (TLC, $10^3/\mu L$), Total Erythrocyte Count (TEC, $10^6/\mu L$), Lymphocytes (%), Granulocytes (%), Monocytes (%) using Automatic Whole Blood Analyzer (BC-2800 Vet, Mindray).

Blood samples (1.5 mL) were collected in centrifuge tubes simultaneously without anticoagulant and at room temperature, allowed to clot (26 ± 2º C). Centrifuging at 3000 rpm for 15 minutes at 10 °C was used to obtain the serum (Eppendorf 5804 R, Germany) and stored at -40 ºC for biochemical analysis. Serum uromodulin concentration was estimated using Enzyme-linked Immunosorbent Assay Kit from MyBioSource, California, USA (MBS2024146). The other serum biochemical parameters like Creatinine (mg/dL), Uric Acid (mg/dL), Blood Urea Nitrogen (BUN) (mg/dL), Aspartate Aminotransferase - AST (U/L), Alanine Aminotransferase - ALT (U/L), Albumin (g/dL), Total Protein (g/dL), Calcium (mg/dL), Phosphorus (mg/dL) were analyzed using standard assay kits by means of CKK 300 Clinical Chemistry Analyzer (Bangalore, India).

Collection of Urine Sample and Urine Analysis

On day 28th after inducing CKD in rats and on day 70th, urine samples from all the rats under experiments were collected. The urine samples were then analyzed for different qualitative

(pH, sp. gravity) and various biochemical parameters, such as urine calcium, phosphorus, total protein and creatinine using a Mindray BS-120 chemistry analyzer.

Ultrasonography

All the experimental rats were examined by ultrasound technology on day 28th using Esaote MyLab40 VET (Esaote Europe B.V., Philipsweg 1, 6227 AJ Maastricht, Netherlands). Real-time B-mode imaging of the kidneys was performed using linear transducers with a frequency range of 3.5 to 12 MHz.

Gross and Histopathological Examination

On 28th day prophylactic group of rats were sacrificed and kidney, liver, lung, spleen, heart was collected. Gross lesions were observed and then formalin was used to fix the tissues. The paraffin wax embedding method of tissue sectioning was used to process the formalin-fixed tissues. By using an automatic section cutting machine (Leica, Automatic Microtome Machine, Germany), sections from all the tissues were cut at a thickness of 5-6 microns at the Department of Pathology and stained with Hematoxylin and Eosin (H&E) stains. Under a microscope, the H&E-stained slides were examined, and lesions were recorded.

Statistical Analysis

One-way analysis of variance (ANOVA) and completely randomized design was used to compare the means of various parameters by using IBM SPSS software (version 26.0). Significant differences ($p<0.05$) between different experimental groups were analysed by Duncan's multiple range test. All the data were presented as Mean ± SE.

Results and Discussion

The findings of the present study to evaluate the prophylactic efficacy of bi-herbal aqueous and alcoholic extracts of *Coriandrum sativum* (CS) and *Murraya koenigii* (MK) on adenine induced chronic kidney disease (CKD) in rats were carried out and results of various parameters like feed consumption, body weight, haemato-biochemical estimation, urine assessment, ultrasonographic examination and histopathological findings in rats are presented.

Clinical Observational Assessments

The majority of general and typical behavioral indications offer important information about the onset, peak and duration of the test drug action. It also provides the spectrum of pharmacological effects produced by the drug. Rats in group I (control group) continued to respond normally throughout the experiment. The adenine control rats (Group II) displayed some aberrant behaviors, such as weakness, lethargy, dullness, depression, diarrhea, dehydration, eating of their own faeces, salivation, polyuria and polydipsia. Rats in groups III, IV, V and VI were given bi-herbal extracts along with adenine and displayed few of the same behavioral symptoms as shown in group II, such as polydipsia, polyuria and weakness, but were relatively more active than adenine control group.

Estimation of Feed consumption

In the present experiment, mean value of feed consumption in adenine control (group II) was significantly lower as compared to control group during week 1 to 4 shown in Table 1. Feed consumption significantly restore in rats of prophylactic groups (III, IV, V and VI) following administration of aqueous and alcoholic extracts of *Coriandrum sativum* and *Murraya koenigii* @ 250 and 500 mg/kg b.wt., orally daily once for 28 days along with adenine. The current findings of feed consumption were accordance with the previously reported results that the rats receiving dietary adenine treatment gradually consumed less feed as compared to normal control rats [12]. Similarly, Li *et al*. (2018) and Rahman *et al*. (2018) also reported that rats with adenine-induced chronic kidney failure consumed less food [13, 14]. In another reported study showed that oral administration of ethanolic extract of *Coriandrum sativum* at the dose of 200 mg/kg/day for 28 days, resulted a significant increase in feed consumption against gentamicin induced renal toxicity in Wistar albino rats [15].

Measurement of Body Weight

The present study demonstrated that mean body weight of adenine treated rats were significantly lower to that of control group shown in Table 2. The administration of aqueous and alcoholic bi-herbal extracts of *Coriandrum sativum* and *Murraya koenigii* leaves along with adenine in groups III, IV, V and VI produced significant improvement in mean body weight as compared to adenine control group. Body weight recorded in the present study were in accordance with the findings of Mori-Kawabe *et al.* (2015) [16], Ali *et al*. (2014) [17] and Rahman *et al.* (2018) [14] reported that significant decrease in body weight following the adenine-induced CKD model in rats. Likewise, oral administration of ethanolic extract of *Coriandrum sativum* at the dose of 200 mg/kg/day for 28 days, resulted in a significant increase in body weight against gentamicin induced renal toxicity in Wistar albino rats [15]. In another reported study founded that oral administration of *Murraya koenigii* aqueous leaves extract @ 300 mg/kg body weight in Wistar albino rats, resulted in a significant increase in body weight in the treated diabetic group as compared to the untreated diabetic controls rats [18].

Haematological Analysis

The result of haematological estimations in different experimental groups and control group on day 28 of experiment have been presented in Table 3. On day 28 of the experiment, the adenine control group-II (CKD) demonstrated a significant decrease in hemoglobin (Hb) levels, lymphocyte counts and total erythrocyte count (TEC) whereas significant increase in granulocyte counts and total leukocyte count (TLC) as compared to normal control group-I. The present finding were in accordance with Munoz Abellan *et al.* (2019) [19], Chang *et al.* (2017) [20] and Ali *et al.* (2014) [17] observed low haemoglobin and low erythropoietin levels in adenine-treated rats. In another reported experiment founded that adenine-induced chronic kidney disease caused significant decreases in TEC [17]. Chang *et al.* (2017) [20] and Muoz Abellan *et al.* (2019) [19] founded increase in white blood cell counts after adenine treatment, due to induced inflammation or iron deficiency in rats.

The administration of bi-herbal aqueous and alcoholic extracts of *Coriandrum sativum* and *Murraya koenigii* leaves along with adenine in all prophylactic groups III, IV, V and VI were showed significantly increased Hb, TEC, lymphocytes and significantly decreased TLC and granulocytes as compared to CKD-induced rats. Among all groups, group III and VI significantly restored the haematological values. The previously reported study founded that the administration of *Murraya koenigii* leaf chloroform extract @150 mg/kg bodyweight significantly increased

hemoglobin (Hb) levels as compared to the lead-intoxicated group in Swiss albino mice [21]. In another study founded that administration of *Coriandrum sativum* incorporated with diet resulted in a significant increase in TEC and Hb in *Catla catla* fish [22]. Similarly, also observed that *Murraya koenigii* aqueous extract @ 250 and 500 mg/kg body weight significantly decrease white blood cell count compared to the control group in rats [23]. Likiwise, in another experimental study observed that bi-herbal *Coriandrum sativum* and *Cichorium intybus* extracts supplementation in broiler chick diets resulted in a significant increase in lymphocyte levels at 6 weeks of age [24].

Serum Biochemistry Analysis

The result of serum biochemical analysis in different experimental groups and control groups on day 28 of experiment have been presented in Table 4. The serum biochemical profile in adenine control group-II showed a significant increase in serum creatinine, BUN, ALT, AST, uric acid and phosphorus levels, along with a significant decrease in serum uromodulin, total protein, albumin and calcium as compared to normal control group. These alterations in the profile provide evidence that the daily dosing of adenine at 200 mg/kg b.wt. for 28 days, along with drinking water, resulted in damage kidney function and induced CKD in rats. The present findings align with other previously reported studies of Chang *et al.* (2017) [20], Rivera-Valdes *et al.* (2017) [25], Mori-Kawabe *et al.* (2015) [16], Ghelani *et al.* (2019) [26], Li *et al.* (2018) [13], Zhu *et al.* (2018) [27], Zhang *et al.* (2016) [28] and Diwan *et al.* (2017) [12] for various serum biochemical parameters. Previously reported study showed that the serum uromodulin concentrations were significantly lower in dogs with chronic kidney disease compared to a control group [29].

Administration of aqueous and alcoholic bi-herbal extracts of *Coriandrum sativum* and *Murraya koenigii* leaves along with adenine in groups III, IV, V and VI showed significantly decreased serum creatinine, uric acid, BUN, ALT, AST, phosphorus and significantly increased serum uromodulin, total protein, serum albumin and calcium as compared to CKD-induced adenine control rats (group II). Groups III and VI showed significant restoration of the serum biochemical alterations, protecting rats against detrimental changes induced on by adenine. These findings highlight the potential prophylactic efficacy of the interventions provided by the bi-herbal extracts from *Coriandrum sativum* and *Murraya koenigii* in mitigating the adverse effects of adenine-induced biochemical changes. Previously reported experiment showed that administration of ethanolic extract of *Coriandrum sativum* @ 200 and 400 mg/kg body weight reduced serum creatinine and BUN level against gentamicin-induced renal damage in rats [5]. Similarly, also observed that administration of aqueous extract of *Murraya koenigii* @200 mg/kg significantly reduced serum creatinine and BUN against unilateral renal ischemia reperfusion injury in male Wistar rats [11]. Mahipal and Pawar (2017) [30] and Molly *et al.* (2017) [31] also observed similar result after administration of *Murraya koenigii* extract in rats. Another report founded that plasma uric acid level in broiler chicks were significantly decreased when fed with a diet supplemented with *Coriandrum sativum* extract [24]. Similarly, experiment also founded that chronic administration of methanolic extract of *Murraya koenigii* for 14 days decrease serum ALT and AST level in Swiss albino rats [32]. Likewise, another study observed administration of *Coriandrum sativum* extract decrease serum ALT and AST in adult male rats with carbon tetrachloride induced liver fibrosis [33]. Likewise, reported study showed that the aqueous extracts of *M. koenigii* revealed hepatoprotective effect at dose dependent manner in rats and results showed significant (p<0.05) reduction in serum ALP, ALT, GGT, AST creatinine kinase, bilirubin and serum creatinine and increase in serum globulin, albumin and total protein level and restored histological structure following administration of aqueous extracts of *M. koenigii* as compared to carbon tetrachloride induced hepatotoxic rats [34]. The report of a significant increase in mean phosphorus values and total protein values after administering adenine and a significantly restored phosphorus in prophylactic groups treated with *Boerhavia diffusa* and *Tribulus terrestris* alcoholic and aqueous extracts in CKD-induced rats [35]. Likewise in another reported study showed antiurolithiatic activity of extract of *Tribulus terrestris* in male wistar rats. The study results revealed the mean value of serum biochemical parameters like urea, BUN, creatinine, uric acid, calcium and phosphorus significantly decreased in *Tribulus terrestris* extracts treated rats as compared to urolithiatic control rats [36]. In the present study, serum biochemistry estimation revealed that bi-herbal extracts of CS and MK administration protected the renal and hepatic damage caused by adenine, but did not entirely prevent CKD in rats. However, these extracts showed potential as nephroprotective prophylactic agents in the context of chronic kidney disease in rats.

Urine Analysis

The result of urine analysis in different experimental groups and control groups on day 28 of experiment have been presented in Table 5. When urine was analysed in the adenine control group-II, it showed that urine pH, urine creatinine and urine phosphorus had significantly decreased, but urine total protein and urine calcium had significantly increased. The alterations in the urine profile indicate that the 28-day daily administration of 200 mg/kg of adenine to rats along with water consumption caused damage to their renal functions and developed CKD. Reported study observed a mild elevation in urine calcium, phosphorus and urine protein levels in adenine-treated rats at a dose of 200/mg/kg body weight [19]. In another study observed a decrease in urinary creatinine levels in a rat model of adenine-induced chronic kidney disease [26].

The present findings align with other previously reported study founded that administration of *Coriandrum sativum* leaf juice orally for 28 days after renal artery ligation-induced nephropathy showed significant increase in urine creatinine level and reduced in urinary total protein in rats [37]. Similarly, also founded that administration of fresh leaves juice of *Murraya koenigii* causes significant reduction of total protein and increase creatinine in urine in rats [11]. Likewise, another study showed that adenine induced CKD in rats causes significant increase in urine total protein levels, while administration of bi-herbal extracts of *Boerhavia diffusa* and *Tribulus terrestris* which causes significant decrease in urine total protein as compared to adenine control group [35].

Renal Ultrasound Examination

Renal ultrasound examination was carried out on day 28 to evaluate the effect on kidneys of all experimental rats. In CKD induced rats, ultrasound examination revealed multiple cortico-medullary junction hyperechoic foci and an unidentified cortico-medullary junction. The kidney showed parenchymal swelling, a spherical form with hypoechoic renal

tissue and an unclear cortico-medullary junction. In contrast, the normal control group displayed a clear cortico-medullary junction. Groups III, IV, V and VI showed a distinct cortico-medullary junction, less hyperechoic and reduced damage severity as compared to the adenine control group, indicating the prophylactic effect of bi-herbal aqueous and alcoholic extracts of CS and MK in rats were depicted in Figure 1.

Histopathological Examination of Kidney

Histopathological examination of kidney revealed many pathological alterations. Adenine-induced groups displayed pathological changes such as the presence of marked tubular atrophy, cystic dilatation, severe fibrosis, congestion, inflammatory exudates and tubular cast in H & E stain. Administration of aqueous and alcoholic bi-herbal extracts of CS and MK leaves along with adenine in groups III, IV, V and VI displayed relatively fewer pathological changes on histopathological examination in comparison to the adenine control group and are depicted in Fig. 2 to 7. The present findings were in accordance with Ali *et al*. (2016) investigated the histopathology of adenine induced CKD in rat kidneys, which showed fibrosis, tubular lumen dilatation and inflammation [17]. The present findings align with reported study that treatment with ethanolic extract of *Coriandrum sativum* @ 200 and 400 mg/kg reduced renal histological lesions against gentamicin-induced kidney injury in rats [5]. Similarly, also reported that treatment with aqueous extract of *Murraya koenigii* @ 200 and 400 mg/kg reduced renal histological lesions, glomerular basement membrane thickening and less matrix expansion when compared to the streptozotocin-induced diabetic male rats [6].

Table 1: Effect of bi-herbal aqueous and alcoholic extracts of *Coriandrum sativum* and *Murraya koenigii* on feed consumption (g/day) in adenine induced CKD rats (Mean ± SE).

Group	Week1 (Day7)	Week2 (Day14)	Week3 (Day21)	Week4 (Day28)
I	24.59±0.40^{d}	28.97±0.98^{c}	27.86±0.24^{e}	31.12±1.17^{e}
II	10.12±0.09^{a}	9.21±0.07^{a}	8.15±0.25^{a}	7.88±0.21^{a}
III	15.42±1.71^{bc}	9.57±0.14^{a}	12.59±0.14^{b}	16.31±0.28^{c}
IV	15.14±1.00^{bc}	11.07±0.36^{a}	14.28±1.71^{bc}	12.35±0.21^{b}
V	18.57±1.43^{c}	18.99±1.87^{b}	17.28±0.29^{d}	18.35±0.21^{d}
VI	11.43±0.66^{ab}	10.64±0.37^{a}	16.71±0.25^{cd}	18.66±0.36^{d}

[Mean values within the column with different superscript (a,b,c,d,e) differ significantly (p<0.05). Group I: Normal control; Group II: Adenine control; Group III: Adenine + Bi-herbal aq. ex. of CS+MK @250 mg/kg; Group IV: Adenine + Bi-herbal aq. ex. of CS+MK @500 mg/kg; Group V: Adenine + Bi-herbal al. ex. of CS+MK @250 mg/kg and Group VI: Adenine + Bi-herbal al. ex. of CS+MK @500 mg/kg].

Table 2: Effect of bi-herbal aqueous and alcoholic extracts of *Coriandrum sativum* and *Murraya koenigii* on body weight (g) in adenine induced CKD rats (Mean ± SE).

Group	BW0	BW1	BW2	BW3	BW4
I	419.66±19.61	451.50±13.68^{b}	488.00±14.22^{d}	523.16±13.55^{c}	522.83±15.98^{c}
II	384.66±12.97	325.50±16.66^{a}	299.00±17.94^{a}	295.66±18.92^{a}	264.33±17.82^{a}
III	381.83±19.66	361.16±19.35^{a}	355.50±16.49^{b}	326.50±16.28^{ab}	408.00±14.81^{b}
IV	368.66±16.70	323.00±16.26^{a}	360.33±5.65^{b}	303.33±18.92^{a}	392.33±20.30^{b}
V	409.16±23.19	349.50±29.74^{a}	331.15±13.47^{ab}	367.33±20.70^{b}	422.33±21.41^{b}
VI	381.33±5.70	337.33±12.14^{a}	354.33±9.64^{c}	329.33±8.97^{ab}	411.00±16.76^{b}

[Mean values within the column with different superscript (a,b,c,d) differ significantly (p<0.05). BW0: Body weight at 0 day; BW1: Body weight at 1st week; BW2: Body weight at 2nd week; BW3: Body weight at 3rd week and BW4: Body weight at 4th week]

Table 3: Effect of bi-herbal aqueous and alcoholic extracts of *Coriandrum sativum* and *Murraya koenigii* on hematological parameter in adenine induced CKD rats (Mean ± SE).

Group	Hb (g/dL)	TEC (×10^{6}/µL)	TLC (×10^{3}/µL)	Lymphocyte (%)	Granulocyte (%)	Monocyte (%)
I	14.02±0.34^{c}	7.28±0.42^{d}	5.50±0.21^{a}	73.83±1.65^{d}	24.05±1.70^{a}	2.11±0.25
II	10.51±0.25^{a}	4.18±0.44^{a}	12.64±0.55^{c}	53.60±1.43^{a}	43.84±1.06^{d}	2.55±0.47
III	13.06±0.42^{c}	6.01±0.05^{bc}	6.21±0.26^{a}	69.44±0.55^{c}	31.40±0.70^{bc}	1.98±0.20
IV	11.96±0.38^{b}	6.02±0.07^{bc}	8.15±0.25^{b}	64.71±1.48^{b}	33.63±1.47^{c}	2.06±0.20
V	11.88±0.31^{b}	5.43±0.42^{b}	8.91±0.34^{b}	65.09±1.68^{b}	33.06±1.86^{c}	2.08±0.23
VI	14.06±0.44^{c}	6.50±0.24^{cd}	6.22±0.36^{a}	70.41±0.66^{cd}	28.12±0.72^{b}	2.03±0.12

[Mean values within the column with different superscript (a,b,c,d) differ significantly (p<0.05). Hb: Hemoglobin; TEC: Total erythrocyte count and TLC: Total leukocyte count]

Table 4: Effect of bi-herbal aqueous and alcoholic extracts of *Coriandrum sativum* and *Murraya koenigii* on serum biochemical parameter in adenine induced CKD rats (Mean ± SE).

Group	Uromodulin (ng/mL)	Creatinine (mg/dL)	Uric acid (mg/dL)	BUN (mg/dL)	AST (U/L)	ALT (U/L)	Albumin (g/dL)	Total protein (g/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)
I	19.60±0.58^{c}	0.56±0.05^{a}	2.38±0.16^{a}	23.23±0.54^{a}	110.29±2.08^{a}	26.23±0.22^{a}	3.68±0.07^{b}	6.62±0.16^{d}	10.36±0.27^{c}	6.83±0.15^{a}
II	10.61±0.34^{a}	3.40±0.10^{c}	5.01±0.14^{c}	154.42±2.53^{d}	117.84±2.96^{b}	51.88±0.44^{d}	2.73±0.12^{a}	5.58±0.14^{a}	7.42±0.19^{a}	9.92±0.26^{d}
III	18.19±0.23^{b}	1.92±0.04^{b}	2.42±0.30^{a}	65.91±2.90^{b}	108.55±2.03^{a}	32.37±0.32^{b}	3.43±0.12^{b}	6.47±0.12^{bcd}	10.10±0.31^{c}	8.42±0.12^{bc}
IV	17.69±0.25^{b}	2.02±0.05^{b}	3.09±0.05^{b}	73.85±2.11^{c}	106.72±1.61^{a}	35.05±0.15^{c}	3.44±0.06^{b}	6.20±0.05^{b}	9.07±0.20^{b}	8.46±0.15^{bc}

V	17.53±0.24^{b}	2.02±0.03^{b}	3.30±0.07^{b}	74.98±1.72^{c}	110.19±2.34^{a}	33.25±0.38^{b}	3.42±0.16^{b}	6.25±0.05^{bc}	9.05±0.19^{b}	8.76±0.09^{c}
VI	18.41±0.23^{b}	2.01±0.05^{b}	2.58±0.09^{a}	66.73±1.40^{b}	112.19±1.56^{ab}	35.34±0.32^{c}	3.63±0.06^{b}	6.58±0.13^{cd}	9.72±0.18^{c}	8.15±0.10^{b}

[Mean values within the column with different superscript (a,b,c,d) differ significantly (p<0.05). BUN: blood urea nitrogen and AST: aspartate aminotransferase, ALT: alanine aminotransferase]

Table 5: Effect of bi-herbal aqueous and alcoholic extracts of *Coriandrum sativum* and *Murraya koenigii* on urine parameter in adenine induced CKD rats (Mean ± SE).

Group	pH	Specific gravity	Total protein (g/dL)	Creatinine (mg/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)
I	8.25±0.11^{d}	1.0143±0.003^{ab}	7.31±0.29^{a}	11.13±0.23^{c}	2.28±0.09^{a}	2.47±0.12^{d}
II	6.16±0.11^{a}	1.0082±0.002^{a}	25.52±0.67^{c}	4.80±0.21^{a}	4.74±0.29^{b}	1.74±0.06^{a}
III	7.58±0.15^{c}	1.0140±0.001^{ab}	8.33±0.22^{a}	10.38±0.18^{c}	2.75±0.37^{a}	2.17±0.11^{bcd}
IV	7.00±0.26^{b}	1.0128±0.004^{ab}	15.70±1.09^{b}	7.80±0.47^{b}	2.89±0.27^{a}	1.83±0.17^{ab}
V	7.25±0.25^{bc}	1.0127±0.003^{ab}	16.88±0.84^{b}	8.61±0.44^{b}	3.07±0.28^{a}	1.90±0.14^{abc}
VI	6.91±0.15^{b}	1.0132±0.003^{ab}	8.42±0.10^{a}	10.52±0.16^{c}	2.51±0.14^{a}	2.23±0.06^{cd}

[Mean values within the column with different superscript (a,b,c,d) differ significantly (p<0.05)]

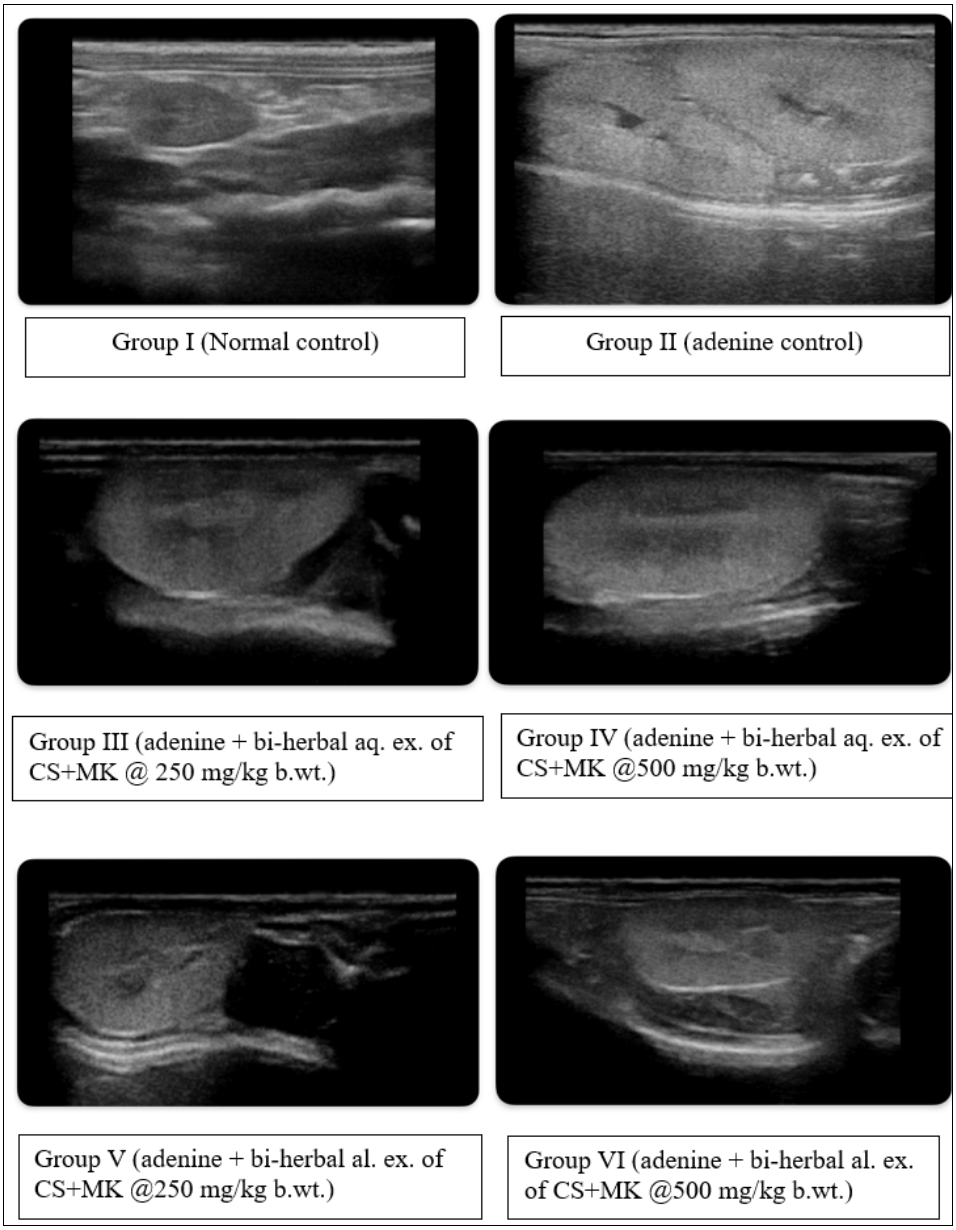

Fig 1: Renal ultrasound examination of normal and prophylactic groups in adenine induced CKD rats.

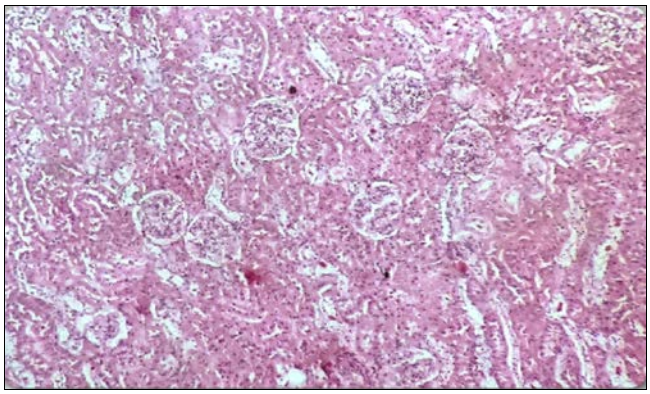

Fig 2: Kidney from group I (Normal control). Microphotographs of kidney showed normal histoarchitecture details of non-treated rats (H&E, 120X).

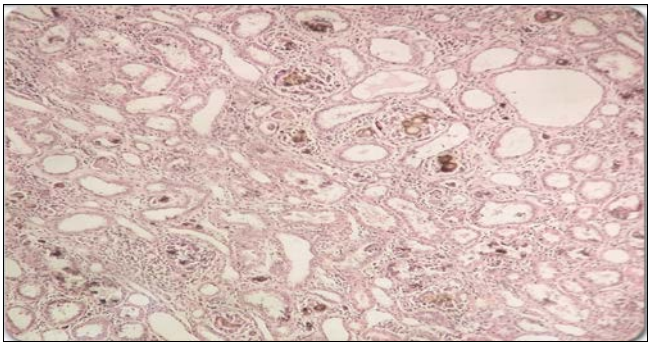

Fig 3: Kidney from group II (adenine control). Microphotographs of kidney showed marked tubular atrophy, cystic dilatation, mild fibrosis, infiltration of mononuclear cell, congestion and tubular cast in adenine treated rats (H&E, 120X).

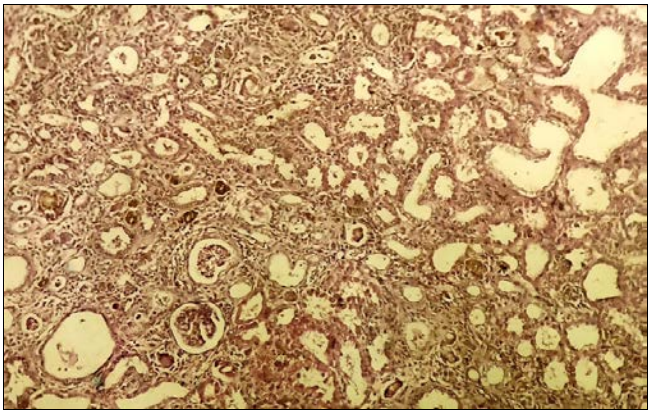

Fig 4: Kidney from group III (adenine + bi-herbal aq. ex. of CS+MK @250 mg/kg b.wt.). Microphotographs of kidney showed mild tubular dilatation and moderate thickening of interstitial by fibrosis and mononuclear cellular infiltrates (H&E, 120X).

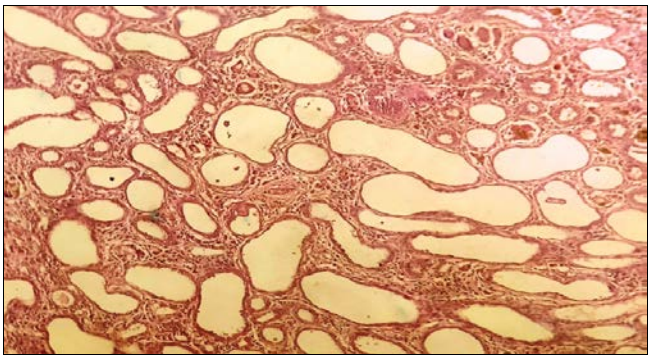

Fig 5: Kidney from group IV (adenine + bi-herbal aq. ex. of CS+MK @500 mg/kg b.wt.). Microphotographs of kidney showed severe tubular dilatation, atrophy and mild interstitial fibrosis (H&E, 120X).

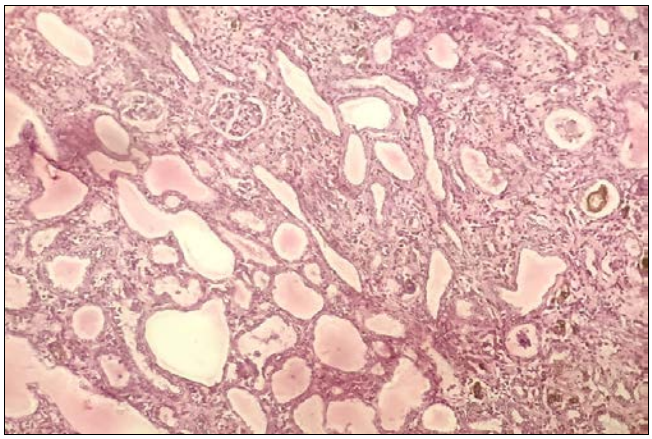

Fig 6: Kidney from group V (adenine + bi-herbal al. ex. of CS+MK @250 mg/kg b.wt.). Microphotographs of kidney showed severe thickening of interstitial and presence of tubular cast (H&E, 120X).

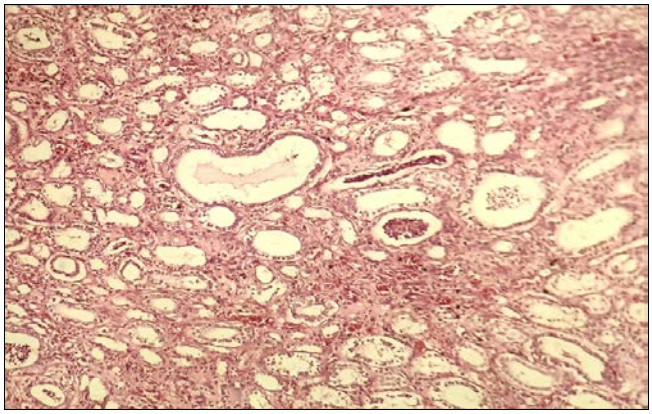

Fig 7: Kidney from group VI (adenine + bi-herbal al. ex. of CS+MK @500 mg/kg b.wt.). Microphotographs of kidney showed mild congestion along with tubular degeneration and dilatation (H&E, 120X).

Conclusions

The bi-herbal aqueous and alcoholic extracts of *Coriandrum sativum* and *Murraya koenigii* leaves at dosage of 250 and 500 mg/kg b.wt., respectively orally for 28 days showed greater prophylactic efficacy in adenine-induced chronic kidney disease in rats based on evaluation of various parameters like body weight and feed consumption, heamato-biochemical analysis, urine assessment, ultrasonographic examination and histopathological evaluation. Furthermore, alcoholic bi-herbal extracts were more effective than aqueous extracts. The prophylactic study results of aqueous and alcoholic bi-herbal extracts in adenine-induced CKD rats suggests its probability for therapeutic potential. Further research will be essential for determining the exact mechanisms of action, discovering bioactive substances and evaluating the possibility for using these extracts in CKD patients.

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Conflict of Interest

All authors state that they have no conflicts of interest.

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