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Therapeutic efficacy of *Glycyrrhiza glabra* and *Curcuma longa* on adenine induced chronic kidney disease in rats

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ABSTRACT

The current investigation was designed to assess the therapeutic efficacy of aqueous and alcoholic bi-herbal extracts of *Glycyrrhiza glabra* (GG) and *Curcuma longa* (CL) on adenine-induced chronic kidney disease (CKD) using 36 male Sprague-Dawley rats. The rats were randomly allocated into six different groups, each group comprising six rats. CKD was induced in groups II to VI by administering adenine at a dose of 200 mg/kg orally once daily for 28 days. Group I served as the control. Group II was adenine control, received adenine (200 mg/kg orally) for 28 days. Groups III, IV, V and VI were therapeutic groups, received adenine @ 200 mg/kg orally once daily for 28 days to induce CKD, after that rats were given bi-herbal aqueous and alcoholic extracts of GG and CL (1.5:1) orally for another 42 days. Groups III and IV, received bi-herbal aqueous extract of GG and CL @ 250 and 500 mg/kg, respectively. Groups V and VI, received bi-herbal alcoholic extracts of GG and CL @ 250 and 500 mg/kg, respectively. Blood samples were collected twice during the experiment, on day 28 and day 70. Various assessments including haematology, serum biochemistry, urine analysis, renal ultrasonography and histopathology were conducted. Adenine administration for 28 days resulted in significant decrease in haemoglobin, total erythrocyte count and lymphocyte, while significant increase in TLC and granulocyte, however treatment with bi-herbal aqueous and alcoholic extracts significantly ameliorated haematological alterations. Adenine induced CKD resulted in elevated serum creatinine, uric acid, BUN, ALT and Phosphorus while significantly reduced levels of serum uromodulin, albumin, total protein, and calcium. Conversely, treatment with aqueous and alcoholic bi-herbal extract significantly improved biochemical changes as compared to adenine control rats. Notably, the therapeutic efficacy was most pronounced in rats treated with bi-herbal alcoholic extracts at the dose rate of 500 mg/kg. In addition, significant increased levels of urine calcium and total protein, with decreased levels of urine creatinine, phosphorus and urine pH were observed in adenine control group as compared to normal control group. These changes were significantly reverted with treatment of aqueous and alcoholic bi-herbal extracts for 42 days. Following CKD induction, treatment with aqueous and alcoholic extracts of GG and CL attenuated ultrasonographic changes and improved histopathological damage in the kidney. Results of the present study showed that the bi-herbal alcoholic extracts of *Glycyrrhiza glabra* and *Curcuma longa* in the ratio of 1.5:1 given at the dose rate of 500 mg/kg once orally daily for 42 days after induction of CKD is more efficacious in the treatment of CKD in rats.

Keywords: CKD, *Glycyrrhiza glabra*, *Curcuma longa*, Therapeutic efficacy, Rats.

INTRODUCTION

Many essential physiological processes, including filtration and elimination of metabolites and hazardous waste products from the body, regulation of the internal fluid environment for sustaining appropriate fluid volume and tonicity, pH balance, electrolyte composition, and critical endocrine functions, are performed by the kidneys. Chronic kidney disease (CKD) is defined as abnormalities of renal structure or function and characterized by progressive knock down of functional unit of kidney. Furthermore, chronic renal failure is caused by a variety of underlying pathophysiologic causes [1]. More than 800 million individuals globally suffer from chronic kidney disease (CKD), a serious public health concern that is predicted to rise in frequency due to growing rates of hypertension and diabetes mellitus [2]. It was predicted that by 2040, CKD will be the fifth most prevalent chronic condition [3]. The prevalence of CKD was annually increase by 8%-16% is alarming and higher than population growth [1]. Although a precise treatment plan for chronic kidney disease (CKD) does not yet exist, it mostly focuses on managing blood pressure, blood sugar, blood lipids, protein and salt intake, and other risk factors. Typically, the first-line treatment for CKD progression is delaying its course with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers. But some patients may not respond well to renin-angiotensin system inhibitors, or they have serious adverse effects, such as hyperkalemia and decreasing renal function, which highlights the current state of therapeutic treatments inadequacies. Because of these pressing issues, scientists are working to develop new medications and approaches to treat CKD [4].

In the modern system of medicine, natural drug compounds have been reported to play important role. Their therapeutic role was supported by the existence of bioactive molecules. Because of their disease-inhibiting properties, they are incredibly valuable as natural medications, providing fundamental bioactive chemicals that are less toxic and more effective, and introducing biological and chemical methods of modifying and extracting natural products into potent drugs [5]. *Glycyrrhiza glabra* Linn. (GG) is a member of the Fabaceae family and has been used as a medicinal and flavouring herb since long times. The chief active component of roots is glycyrrhizin, a combination of 2 to 25% potassium, calcium and magnesium salt of glycyrrhizic acid, a triterpenoid saponin that is almost 50 times sweeter than sucrose and responsible for its medicinal properties. Moreover, it was traditionally used as an insecticide, laxative, anti-inflammatory, anti-ulcer, anti-microbial, antiviral, memory stimulant, anticholinergic, antitussive, antioxidant, anticancer and anti-diuretic agent, due to its action as a monoamine oxidase (MAO) inhibitor [6]. *Curcuma longa* is a member of the Zingiberaceae family and is widely cultivated in Asia's tropical regions, known by many different names including Turmeric in English, Haridra in Sanskrit and Haldi in Hindi, and play vital role in Ayurveda, Siddha, Unani and Chinese medicine. The principal chemical group present in *Curcuma* is curcuminoids, combination of curcumin, monodexmethoxycurcumin, and bisdemethoxycurcumin. The biological properties of turmeric also include anti-oxidant, anti-inflammatory, analgesic, anti-cancer, anti-mutagenic, anti-microbial, anti-obesity, cardioprotective, hepatoprotective and neuroprotective action [7].

However, at presently there has been limited available report focused on nephroprotective effect of *Glycyrrhiza glabra* and *Curcuma longa*. To develop a nephroprotective phytomedicine, the present preliminary study was to undertaken to evaluate the therapeutic effects of aqueous and alcoholic bi-herbal extracts of *Glycyrrhiza glabra* and *Curcuma longa* roots on physiological, haemato-biochemical, urine parameter, ultrasonographic and histopathological changes in CKD model of rats.

MATERIALS AND METHODS

Experimental Animals

The study was conducted on healthy male Sprague-Dawley rats. Thirty-six (36) male rats were procured from Zydus Research Center, Ahmedabad, Gujarat. Prior to study, the experimental protocol was approved by Institutional Animal Ethics Committee (Approval No: IAEC/371/VPT/2022) at College of Veterinary Science and Animal Husbandry, Anand, Gujarat and protocols were followed according to the guidelines of committee for control and supervision of experiments on animals (CCSEA). The animals were housed in standard polypropylene cages and maintained at a humidity of 30% to 70%, and a temperature of 22 ± 3 ºC with 12 h light and 12 h dark cycle. All the rats were fed normal pellet diet and water was provided *ad libitum* throughout the course of the experiment. All the rats were kept under acclimatization for 7 days prior to grouping and initiation of experiment. Rats were under constant observation during entire period of study.

Experimental Design

The rats were randomly divided into 6 different groups. Chronic kidney disease was induced in the group Ⅱ, Ⅲ, Ⅳ, Ⅴ, and Ⅵ by adenine at dose rate of 200 mg/kg body weight daily 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 body weight, orally) for 28 days. Next four group (Group Ⅲ, Ⅵ, Ⅴ, and Ⅵ) was therapeutic group, received bi-herbal aqueous and alcoholic extracts of *Glycyrrhiza glabra* and *Curcuma longa*, after CKD induction, for another 42 days. In group Ⅲ and Ⅵ, bi-herbal aqueous extract given at 250 and 500 mg/kg body weight for 42 days, respectively. Group Ⅴ and Ⅵ received bi-herbal alcoholic extract at the dose rate of 250 and 500 mg/kg body weight for 42 days, respectively.

Preparation of Plant Extracts and Ratio Determination

Roots of *Glycyrrhiza glabra* and *Curcuma longa* were collected locally, cut into small pieces and dried under shade, after then powder was prepared. For aqueous extracts, 100 g of the *Glycyrrhiza glabra* and *Curcuma longa* dried roots powders were soaked separately in 1 liters of distilled water and shaking thrice daily then mixture was filtered through sterilized Whatman no.1 filter paper. Following filtration, the extract was kept in a water bath at 50 °C until the solvent had evaporated entirely. For preparation of alcoholic extracts, 100 g of powdered material of both plants' roots were extracted in Soxhlet extractor with solvent ethanol. The solvent was later separated from the extract with the aid of rotary evaporator at 40°C. The aqueous and alcoholic extracts were stored in a labeled air tight glass bottle at 4°C for further experimental uses. To evaluate efficacy of combined extracts of *Glycyrrhiza glabra* (GG) and *Curcuma longa* (CL) in adenine induced chronic kidney disease, we selected 3/5 part of GG and 2/5 part of CL for both aqueous and alcoholic extracts, determined by *in-vitro* nucleation assay.

Clinical Observations

During the course of the experimental duration, a daily monitoring regimen was implemented to observe any abnormalities in the physical or behavioural changes of the rats, alongside mortality was observed. The quantity of feed allocated to each cage-housed group of animals was measured, with left-over feed quantities assessed on a weekly basis. Additionally, the body weight of all animals within groups Ⅰ to Ⅵ was assessed prior to the commencement of the experiment and subsequently at weekly intervals throughout the study.

Haemato-Biochemical Analysis

Blood samples were collected from all the rats by retro-orbital plexuses puncture under light isoflurane anaesthesia with the help of capillary tube twice, i.e., first on day 28 after induction of CKD and then on 70th day (after 42 days of treatment) of the experimental period. The blood was collected into clean, sterilized micro-centrifuge tubes, with plain tubes used for serum biochemical analysis and tubes containing K_3EDTA added for haematological analysis.

Hematological estimation was carried out using Automatic Whole Blood Analyzer (Mindray BC-2800 Vet, Shenzhen, China). Serum was harvested by centrifuging at 3000 rpm for 15 minutes at 10° C (Eppendorf 5804 R, Germany). Serum biochemical parameters were analyzed using standard assay kits by means of CKK 300 Clinical Chemistry Analyzer (Bangalore, India). Serum uromodulin concentration was estimated using Enzyme-linked Immunosorbent Assay Kit from MyBioSource, California, USA (MBS2024146).

Urine Collection and Analysis

On the day 28th and 70th, animals were placed individually in metabolic cages for collection of urine. Urine pH and specific gravity were determined using Urine Analyzer (Uriscan Optima II) through URISCAN 10 SGL Strip (YD Diagnostics CORP, Korea). Urine biochemical parameters were estimated using a Mindray BS-120 chemistry analyzer.

Ultrasonography of Kidney

All the experimental rats were examined by ultrasound technology on 70th day using Esaote MyLab40 VET (Esaote Europe B.V., Philipsweg 1, 6227 AJ Maastricht, Netherlands). Real-time B-mode imaging of the kidney with a frequency range of 3.5 to 12 MHz was performed using linear transducers.

Histopathological Examination

On 70th day all rats were sacrificed and kidney was collected. The formalin fixed kidney tissues were processed and sections from all the tissues were cut at 5-6 microns' thickness by automatic section cutting machine (Leica, Automatic Microtome Machine, Germany), stained with Hematoxylin and Eosin (H &E) stains (Luna, 1968). The H & E-stained slides were observed under microscope and lesions were recorded.

Statistical Analysis

The study employed a completely randomized design along with one-way analysis of variance (ANOVA) to assess the means of different parameters. IBM SPSS software version 26.0 was utilized for this purpose. Significant variations ($p<0.05$) among the various experimental groups were determined using Duncan's multiple range test. All data were reported as Mean ± Standard Error (SE).

RESULTS AND DISCUSSION

The current study aimed to assess the therapeutic effectiveness of bi-herbal aqueous and alcoholic extracts derived from *Glycyrrhiza glabra* (GG) and *Curcuma longa* (CL) in treating adenine-induced chronic kidney disease (CKD) in rats. The investigation encompassed an evaluation of multiple parameters including feed consumption, body weight changes, hematobiochemical analyses, urine assessments, ultrasonographic examinations, and histopathological findings in the rat subjects. The outcomes of these assessments are comprehensively presented herein.

Clinical Observations

All rats underwent daily observation throughout the entire study duration. Rats in Group I, serving as the control group, exhibited typical active behaviour. Conversely, rats in Groups II to VI displayed various abnormal behaviours such as lethargy, dullness, depression, diarrhoea, dehydration, salivation, polyuria, polydipsia, and weakness. Notably, rats in the therapeutics groups (Groups III, IV, V, and VI) that were treated with bi-herbal extracts demonstrated comparatively higher levels of activity when compared to the adenine control rats (Group II) towards the end of the experiment. Importantly, no mortality incidents were recorded during the study period.

Feed Consumption and Body Weight

The mean value of feed consumption in adenine control was significantly lower compared to control group at the end of experiment as shown in Table 1. However, treatment with bi-herbal aqueous and alcoholic extracts of GG and CL in the therapeutic group shows significant restoration of feed consumption compared to adenine control. Similarly, Diwan *et al.* (2017) reported that administration of adenine decreases feed intake as compared to normal control rats [8]. Gehani *et al.* (2019) also reported significant decrease feed intake in the rats treated with adenine as compared to normal control, while rats treated with aqueous and alcoholic extracts of *Bryophyllum calycinum* and *Acaryanthus aspera* showed significant increased feed consumption in comparison to adenine control group [9].

The body weight of adenine treated rats were significantly lower to that of control group. Nevertheless, in therapeutic study bi-herbal aqueous and alcoholic extract produced dose-dependent significant improvement in body weight value at the end of experiments when compared with the adenine control (Table 2). Similarly, Zhang *et al.* (2016), Arunachalam *et al.* (2021), El-Batsh *et al.* (2021) and Gori *et al.* (2021) also found that adenine administration causes significant reduction in mean body weight compared to normal control [10-13]. Likewise, treatment of aqueous and methanolic extracts of *Curcuma longa* (500 mg/kg body weight) significantly increase body weight in cisplatin-treated rats [14].

Haemato-biochemical Analysis

The administration of adenine for 28 days to induce CKD resulted in significant decrease in haemoglobin, total erythrocyte count and lymphocyte, while significant increase in total leucocyte and granulocyte as shown in Table 3. Conversely, Pathak *et al.* (2014) found that treatment with methanolic extracts of *Curcuma longa* significantly improved total leucocyte count as compared to cisplatin control group but found no significant improvement in total erythrocyte level [14]. In accordance with the present findings Patel *et al.* (2023) also found that adenine administration causes significant decrease in haemoglobin and total erythrocyte count, while increase in total leucocyte and granulocyte [15]. Low total erythrocyte count level in adenine treated rats might be due to the insufficient production of erythropoietin hormone from damaged kidney. However, treatment with bi-herbal aqueous and alcoholic extracts of *Glycyrrhiza glabra* and *Curcuma longa* significantly ameliorated haematological alterations. In another experiment, it was recorded that treatment with several dose of *Glycyrrhiza glabra* aqueous extract significantly improved RBC level and reduced WBC level as compared to untreated group in CCl_4 induced nephrotoxicity [16].

Adenine induced CKD resulted in elevated serum creatinine, uric acid, blood urea nitrogen, alanine transaminase and phosphorus while significantly reduced levels of serum uromodulin, albumin, total protein, and calcium (Table 4). In accordance with our findings, the reported study revealed that serum uromodulin concentrations decreased in dogs with CKD [17]. Serum uromodulin, also known as Tamm-Horsfall protein (THP), is a glycoprotein primarily produced by the cells lining the thick ascending limb of the loop of Henle in the kidneys. They also evidenced that that serum uromodulin may have an advantage over the conventional renal markers in detecting early-stage CKD [17]. Similar to our findings, significant changes in serum biochemical parameters were also reported by Yokozawa *et al.* (1986), Mori-Kawabe *et al.* (2015), Ali *et al.* (2015), Zhang *et al.* (2016), Gori *et al.* (2021) and Singh *et al.* (2022) [10, 13, 18-21]. A possible pathophysiological mechanism behind this is that excretion of nitrogenous compound was supressed by renal tubular occlusion. There was report that in adenine-induced chronic kidney disease, there was significant reduction in serum calcium and increase in serum phosphorus level as compared to healthy control [13, 18]. Conversely, treatment with aqueous and alcoholic bi-herbal extract significantly improved biochemical changes as compared to adenine control rats. Notably, the therapeutic efficacy was most pronounced in rats treated with bi-herbal alcoholic extracts at the dose rate of 500 mg/kg body weight, as they exhibited a restoration of all hemato-biochemical alterations. The results were in accordance with reported study that demonstrated nephroprotective effect of *Curcuma longa* (500 mg/kg, p.o.), recorded aqueous and methanolic extract significantly improved serum creatinine, uric acid level and total protein in cisplatin-induced kidney failure [14]. There was also a report of curcumin improves serum albumin level in Adriamycin-induced nephrotoxicity [22]. Another study also denoted significant improvement in serum albumin level by *Glycyrrhiza glabra* extract in cisplatin-induced nephrotoxicity [23].

Urine Analysis

There was significant increase in levels of urine calcium and total protein, with decreased levels of urine creatinine, phosphorus and urine pH were observed in adenine treated rats as compared to normal control rats. However, the therapeutic groups that was treated with aqueous and alcoholic bi-herbal extracts for 42 days significantly reverted the changes in urine parameter (Table 5). Moreover, aqueous and alcoholic bi-herbal extracts at dosage of 250 and 500 mg/kg showed dose-dependent therapeutic efficacy with regard to restoration of urine parameters. Likewise, Rivera-Valdes *et al.* (2018) developed adenine-induced CKD and observed significant elevation in urine total protein as compared to healthy control [24]. Rahman *et al.* (2018) found that rats with adenine-induced CKD produced significantly more urine because their kidney function was impaired and their

glomerular filtration rate was elevated [25]. According to Levey *et al.* (2003), CKD patients were more likely to experience an increase in urine volume and a fall in urine pH, suggesting that kidney disease is the cause of urine acidification [26]. Similarly, Gehani *et al.* (2020) also found that significant increase in levels of urine output, urine specific gravity, urine calcium, phosphorus, and total protein, with decrease levels of urine creatinine and urine pH observed in adenine treated rats as compared to normal control group. These changes were significantly reverted to near normal levels within next 42 days of daily administration of either single aqueous/alcoholic extract or a combination as biherbal extract of *Bryophyllum calycinum* and *Achyranthes Aspera* [27].

Renal Ultrasound

Renal ultrasound of normal control rats showed normal architecture with distinct corticomedullary junction. Undifferentiated cortex and medulla with hyperechoic structure was typical findings in adenine control rats with increase in size of kidney. However, ultrasonography of therapeutic groups of rats as shown in Figure 1 revealed hyperechoic foci were reduced and the cortico-medullary junction became very much distinguishable when compared with adenine control group. In agreement with the results, Patel *et al.* (2023) also found similar observations in the kidney of rats treated with adenine [15]. Similarly, Patel *et al.* (2023) also found hyperechoic structure and indistinguishable cortex and medulla on kidney of adenine treated rats, while prophylactic treatment with *Coriandrum sativum* and *Murraya koenigii* leaves showed distinct cortico-medullary junction and reduced damage severity on ultrasound examination [28].

Histopathological Examination

The histopathological analysis of the control group revealed intact histo-architectural features (Figure 2). Whereas the microphotograph of the kidney from the adenine control group exhibited pronounced tubular atrophy, cystic dilatation, severe fibrosis, congestion, inflammatory exudates, and tubular casts as on Figure 3. Conversely, the therapeutic groups (Group III to VI) demonstrated relatively fewer pathological changes upon histopathological examination of the kidney (Figure 4-7). Likewise, Patel *et al.* (2023) found that prophylactic treatment with *Coriandrum sativum* and *Murraya koenigii* markedly reduced renal morphological damage and histopathological changes when compared to the adenine-treated group [28]. Consistent findings also reported by Yokozawa *et al.* (1986), Ali *et al.* (2015) and Patel *et al.* (2023) with administration of adenine [15,18, 20].

Table 1: Comparison of Feed consumption (g/day/rat) under different groups

Groups	FC1	FC2	FC3	FC4	FC5	FC6	FC7	FC8	FC9	FC10
I	24.60 ± 0.40^{b}	28.98 ± 0.98^{b}	27.86 ± 0.24^{b}	31.12 ± 1.17^{b}	30.76 ± 0.81^{c}	31.60 ± 1.27^{c}	29.88 ± 1.26^{d}	33.15 ± 1.72^{c}	29.41 ± 0.60^{d}	33.75 ± 1.04^{d}
II	10.13 ± 0.09^{a}	9.21 ± 0.07^{a}	8.16 ± 0.25^{a}	7.89 ± 0.21^{a}	8.43 ± 0.14^{a}	9.54 ± 0.27^{a}	11.03 ± 0.23^{a}	10.77 ± 0.32^{a}	11.54 ± 0.21^{a}	11.85 ± 0.23^{a}
Ⅲ	11.05 ± 1.00^{a}	8.90 ± 0.57^{a}	9.18 ± 0.25^{a}	8.04 ± 0.54^{a}	12.54 ± 0.61^{b}	17.68 ± 0.32^{b}	17.71 ± 0.21^{b}	21.11 ± 2.25^{b}	19.00 ± 0.79^{b}	20.96 ± 0.96^{b}
Ⅳ	11.19 ± 1.00^{a}	10.71 ± 0.24^{a}	9.14 ± 0.33^{a}	9.48 ± 0.57^{a}	14.05 ± 0.24^{b}	19.45 ± 2.88^{b}	21.93 ± 1.88^{c}	24.33 ± 1.00^{b}	25.67 ± 2.86^{cd}	25.81 ± 2.48^{c}
Ⅴ	10.71 ± 0.76^{a}	10.90 ± 0.76^{a}	7.88 ± 0.98^{a}	7.95 ± 1.38^{a}	13.21 ± 0.93^{b}	19.90 ± 0.57^{b}	21.60 ± 1.36^{c}	23.50 ± 1.26^{b}	23.72 ± 1.72^{bc}	24.60 ± 0.60^{bc}
Ⅵ	10.36 ± 0.36^{a}	11.36 ± 1.36^{a}	9.38 ± 0.38^{a}	8.15 ± 1.08^{a}	14.06 ± 0.30^{b}	17.46 ± 0.89^{b}	17.49 ± 0.01^{b}	20.58 ± 0.37^{b}	23.76 ± 0.05^{bc}	26.55 ± 1.12^{c}

Values (Mean ± SE) bearing different superscripts (a, b, c, d) in a column differ significantly (P<0.05). FC=Feed Consumption; I – Vehicle control; II – Adenine control; Ⅲ - Adenine contain solution up to 28 days. After 28 days, aqueous bi-herbal extract @ 250 mg/ kg for another 42 days; Ⅳ - Adenine contain solution up to 28 days. After 28 days, aqueous bi-herbal extract @ 500 mg/ kg for another 42 days; Ⅴ - Adenine contain solution P.O. up to 28 days. After 28 days, alcoholic bi-herbal extract @ 250 mg/ kg for another 42 days; Ⅵ - Adenine contain solution up to 28 days. After 28 days, alcoholic bi-herbal extract @ 500 mg/ kg for another 42 days.

Table 2: Comparison of body weight (g) in rats under different groups

Groups	BW0 (0th day)	BW1 (7th day)	BW2 (14th day)	BW3 (21st day)	BW4 (28th day)	BW5 (35th day)	BW6 (42nd day)	BW7 (49th day)	BW8 (56th day)	BW9 (63rd day)	BW10 (70th day)
I	419.67 ± 19.61	451.50 ± 13.68^{b}	488.00 ± 14.22^{b}	523.17 ± 13.55^{b}	522.83 ± 15.98^{b}	564.33 ± 15.39^{c}	582.67 ± 20.79^{d}	585.33 ± 19.42^{d}	604.67 ± 18.47^{d}	595.17 ± 17.04^{d}	616.67 ± 15.93^{d}
II	384.67 ± 12.97	325.50 ± 16.66^{a}	299.00 ± 17.94^{a}	295.67 ± 18.92^{a}	264.33 ± 17.82^{a}	274.33 ± 17.15^{a}	280.50 ± 11.10^{a}	288.17 ± 13.38^{a}	305.17 ± 15.85^{a}	325.00 ± 13.50^{a}	342.67 ± 10.57^{a}
Ⅲ	397.50 ± 20.97	343.00 ± 18.01^{a}	320.67 ± 17.83^{a}	295.33 ± 16.81^{a}	278.00 ± 16.30^{a}	293.50 ± 18.98^{a}	314.17 ± 22.44^{ab}	358.67 ± 21.76^{b}	385.50 ± 18.92^{b}	403.17 ± 21.02^{b}	423.83 ± 21.34^{b}
Ⅳ	390.00 ± 13.17	367.00 ± 11.65^{a}	351.00 ± 12.52^{a}	343.17 ± 11.48^{a}	311.00 ± 16.78^{a}	364.67 ± 16.45^{b}	400.83 ± 22.88^{c}	432.17 ± 24.62^{c}	468.67 ± 24.93^{c}	486.83 ± 22.30^{c}	493.67 ± 23.74^{c}
Ⅴ	371.00 ± 18.56	345.50 ± 17.10^{a}	342.67 ± 17.04^{a}	336.00 ± 19.94^{a}	312.17 ± 23.34^{a}	366.83 ± 25.52^{b}	405.00 ± 24.82^{c}	437.83 ± 25.55^{c}	454.67 ± 25.23^{c}	469.00 ± 24.97^{c}	490.50 ± 24.60^{c}
Ⅵ	393.50 ± 12.50	327.50 ± 15.24^{a}	310.67 ± 21.59^{a}	301.83 ± 25.73^{a}	291.17 ± 23.72^{a}	314.67 ± 23.53^{ab}	350.50 ± 24.90^{bc}	392.83 ± 17.93^{bc}	424.17 ± 19.31^{bc}	447.83 ± 19.64^{bc}	481.00 ± 14.29^{c}

Values (Mean ± SE) bearing different superscripts (a, b, c, d) in a column differ significantly (P<0.05).

Table 3: Comparison of different haematological parameter on 28th and 70th day in rats under different groups

Groups	Hb (g/dL)		TEC (10^6/µL)		TLC (10^3/µL)		Differential Leukocyte Count (%)			
							Lymphocyte (%)		Granulocyte (%)	
	28th Day	70th Day	28th Day	70th Day	28th Day	70th Day	28th Day	70th Day	28th Day	70th Day
I	14.02 ± 0.34^{b}	15.12 ± 0.60^{c}	7.28 ± 0.42^{b}	7.32 ± 0.33^{bc}	5.51 ± 0.21^{a}	5.71 ± 0.65^{a}	73.83 ± 1.65^{b}	74.66 ± 1.50^{b}	24.05 ± 1.70^{a}	22.16 ± 1.35^{a}
II	10.51 ± 0.25^{a}	7.82 ± 0.55^{a}	4.18 ± 0.44^{a}	3.91 ± 0.31^{a}	12.65 ± 0.55^{b}	14.16 ± 0.69^{d}	53.60 ± 1.43^{a}	54.71 ± 1.64^{a}	43.85 ± 1.06^{b}	42.43 ± 1.42^{b}
Ⅲ	11.56 ±	14.14 ±	4.56 ±	7.34 ±	11.46 ±	8.47 ±	53.38 ±	74.05 ±	44.34 ±	22.91 ±

	0.17[a]	0.58[bc]	0.36[a]	0.28[bc]	0.49[b]	0.53[bc]	1.73[a]	1.51[b]	1.83[b]	1.26[a]
Ⅳ	10.66 ± 0.44[a]	13.12 ± 0.64[b]	4.26 ± 0.29[a]	6.84 ± 0.23[b]	12.70 ± 0.94[b]	9.93 ± 0.77[c]	52.92 ± 1.18[a]	74.14 ± 0.55[b]	44.56 ± 1.34[b]	23.01 ± 0.66[a]
Ⅴ	10.81 ± 0.67[a]	15.32 ± 0.64[c]	4.16 ± 0.05[a]	7.62 ± 0.21[bc]	11.97 ± 0.45[b]	8.44 ± 0.47[bc]	53.97 ± 1.70[a]	74.98 ± 0.86[b]	43.46 ± 1.84[b]	21.88 ± 0.83[a]
Ⅵ	10.45 ± 0.34[a]	15.22 ± 0.81[c]	4.25 ± 0.12[a]	7.92 ± 0.44[c]	12.34 ± 0.25[b]	7.66 ± 0.25[b]	51.52 ± 1.68[a]	72.62 ± 0.81[b]	46.32 ± 1.62[b]	24.19 ± 0.92[a]

Values (Mean ± SE) bearing different superscripts (a, b, c, d) in a column differ significantly (P<0.05).

Table 4: Comparison of different serum biochemical parameters on 28th and 70th day in rats under different groups

Groups	UMOD (ng/mL)		Creatinine (mg/dL)		Uric acid (mg/dL)		BUN (mg/dL)		AST (U/L)		ALT (U/L)	
	28th Day	70th Day	28th day	70th day	28th day	70th day	28th day	70th day	28th day	70th day	28th day	70th day
I	19.60 ± 0.58[b]	19.03 ± 0.63[d]	0.56 ± 0.05[a]	0.61 ± 0.05[a]	2.38 ± 0.16[a]	2.31 ± 0.15[a]	23.23 ± 0.54[a]	25.60 ± 0.74[a]	110.29 ± 2.08	116.07 ± 2.12	26.23 ± 0.22[a]	27.30 ± 0.25[a]
II	10.61 ± 0.34[a]	10.24 ± 0.56[a]	3.40 ± 0.10[b]	3.05 ± 0.09[e]	5.01 ± 0.14[b]	4.90 ± 0.10[d]	154.42 ± 2.53[b]	121.70 ± 2.63[e]	117.84 ± 2.96	116.35 ± 2.51	51.88 ± 0.44[bc]	50.78 ± 0.34[d]
Ⅲ	10.55 ± 0.35[a]	13.67 ± 0.69[b]	3.52 ± 0.09[b]	2.16 ± 0.12[d]	5.04 ± 0.10[b]	3.85 ± 0.18[c]	155.45 ± 2.92[b]	72.89 ± 3.02[d]	119.00 ± 2.54	119.99 ± 3.11	52.25 ± 0.47[bc]	41.15 ± 0.47[c]
Ⅳ	10.60 ± 0.32[a]	15.23 ± 0.86[bc]	3.38 ± 0.10[b]	1.65 ± 0.22[c]	5.11 ± 0.04[b]	3.47 ± 0.12[c]	154.22 ± 2.11[b]	59.06 ± 1.45[c]	116.33 ± 2.29	112.90 ± 3.92	53.30 ± 0.94[c]	38.68 ± 0.72[c]
Ⅴ	10.84 ± 0.44[a]	14.37 ± 0.77[b]	3.33 ± 0.09[b]	1.89 ± 0.18[cd]	5.08 ± 0.11[b]	3.65 ± 0.15[c]	157.68 ± 2.14[b]	65.76 ± 2.76[c]	117.74 ± 5.33	111.34 ± 3.54	51.04 ± 0.65[b]	39.16 ± 2.31[c]
Ⅵ	10.52 ± 0.47[a]	16.98 ± 0.77[cd]	3.43 ± 0.09[b]	1.19 ± 0.08[b]	5.12 ± 0.15[b]	2.94 ± 0.11[b]	157.22 ± 1.62[b]	44.77 ± 2.53[b]	119.50 ± 3.95	120.00 ± 2.70	53.03 ± 0.72[c]	30.91 ± 0.74[b]

Values (Mean ± SE) bearing different superscripts (a, b, c, d, e) in a column differ significantly (P<0.05). UMOD= uromodulin, BUN= blood urea nitrogen, AST=aspartate aminotransferase, ALT=alanine aminotransferase.

Table 4 cont.: Comparison of different serum biochemical parameters on 28th and 70th day in rats under different groups

Groups	Albumin (g/dL)		Total protein (g/dL)		Calcium (mg/dL)		Phosphorus (mg/dL)	
	28th day	70th day	28th day	70th day	28th day	70th day	28th day	70th day
I	3.68 ± 0.07[b]	3.76 ± 0.06[cd]	6.62 ± 0.16[b]	6.49 ± 0.19[c]	10.36 ± 0.27[b]	10.21 ± 0.23[b]	6.83 ± 0.15[a]	7.04 ± 0.17[a]
II	2.73 ± 0.12[a]	2.74 ± 0.11[a]	5.58 ± 0.14[a]	5.75 ± 0.08[ab]	7.42 ± 0.19[a]	8.27 ± 0.09[a]	9.92 ± 0.26[b]	9.41 ± 0.15[c]
Ⅲ	2.63 ± 0.13[a]	2.97 ± 0.28[a]	5.50 ± 0.15[a]	5.69 ± 0.28[a]	7.63 ± 0.23[a]	8.81 ± 0.48[a]	9.90 ± 0.13[b]	8.00 ± 0.24[b]
Ⅳ	2.72 ± 0.22[a]	3.06 ± 0.11[ab]	5.52 ± 0.18[a]	6.16 ± 0.20[ab]	7.66 ± 0.51[a]	9.98 ± 0.42[b]	10.03 ± 0.20[b]	7.65 ± 0.22[b]
Ⅴ	2.92 ± 0.13[a]	3.46 ± 0.13[bc]	5.65 ± 0.09[a]	6.14 ± 0.07[ab]	7.46 ± 0.33[a]	9.90 ± 0.26[b]	9.79 ± 0.25[b]	7.78 ± 0.12[b]
Ⅵ	2.79 ± 0.11[a]	3.96 ± 0.14[d]	5.58 ± 0.17[a]	6.38 ± 0.31[bc]	7.38 ± 0.18[a]	10.31 ± 0.42[b]	9.97 ± 0.25[b]	6.91 ± 0.25[a]

Values (Mean ± SE) bearing different superscripts (a, b, c, d, e) in a column differ significantly (P<0.05).

Table 5: Comparison of different urine parameters on 28th and 70th day in rats under different groups

Groups	pH		Total Protein (g/dL)		Creatinine (mg/dL)		Calcium (mg/dL)		Phosphorus (mg/dL)	
	28th Day	70th Day	28th Day	70th Day	28th Day	70th Day	28th Day	70th Day	28th Day	70th Day
I	8.25 ± 0.11[b]	8.50 ± 0.18[c]	7.32 ± 0.29[a]	7.42 ± 0.24[a]	11.14 ± 0.23[b]	11.69 ± 0.18[e]	2.28 ± 0.09[a]	2.54 ± 0.12[a]	2.47 ± 0.12[b]	2.59 ± 0.14[c]
II	6.17 ± 0.11[a]	5.98 ± 0.18[a]	25.52 ± 0.67[b]	26.54 ± 0.62[c]	4.80 ± 0.21[a]	5.06 ± 0.09[a]	4.75 ± 0.29[b]	5.34 ± 0.32[d]	1.75 ± 0.06[a]	1.37 ± 0.16[a]
Ⅲ	6.50 ± 0.29[a]	7.50 ± 0.26[b]	24.70 ± 0.51[b]	12.25 ± 0.36[b]	4.99 ± 0.29[a]	7.75 ± 0.42[b]	4.71 ± 0.08[b]	3.73 ± 0.19[bc]	1.72 ± 0.06[a]	2.18 ± 0.10[b]
Ⅳ	6.58 ± 0.24[a]	7.75 ± 0.21[b]	25.99 ± 1.01[b]	11.90 ± 0.53[b]	4.84 ± 0.39[a]	9.17 ± 0.46[c]	4.81 ± 0.16[b]	4.22 ± 0.31[c]	1.79 ± 0.04[a]	1.99 ± 0.04[b]
Ⅴ	6.58 ± 0.41[a]	7.92 ± 0.47[bc]	24.61 ± 1.01[b]	12.27 ± 0.35[b]	4.69 ± 0.37[a]	9.90 ± 0.61[cd]	4.94 ± 0.28[b]	3.41 ± 0.43[b]	1.74 ± 0.06[a]	2.30 ± 0.08[bc]
Ⅵ	6.58 ± 0.24[a]	7.75 ± 0.21[b]	25.60 ± 0.38[b]	11.48 ± 0.36[b]	4.86 ± 0.20[a]	10.32 ± 0.31[d]	4.74 ± 0.29[b]	3.36 ± 0.26[b]	1.73 ± 0.06[a]	2.13 ± 0.06[b]

Values (Mean ± SE) bearing different superscripts (a, b, c, d, e) in a column differ significantly (P<0.05).

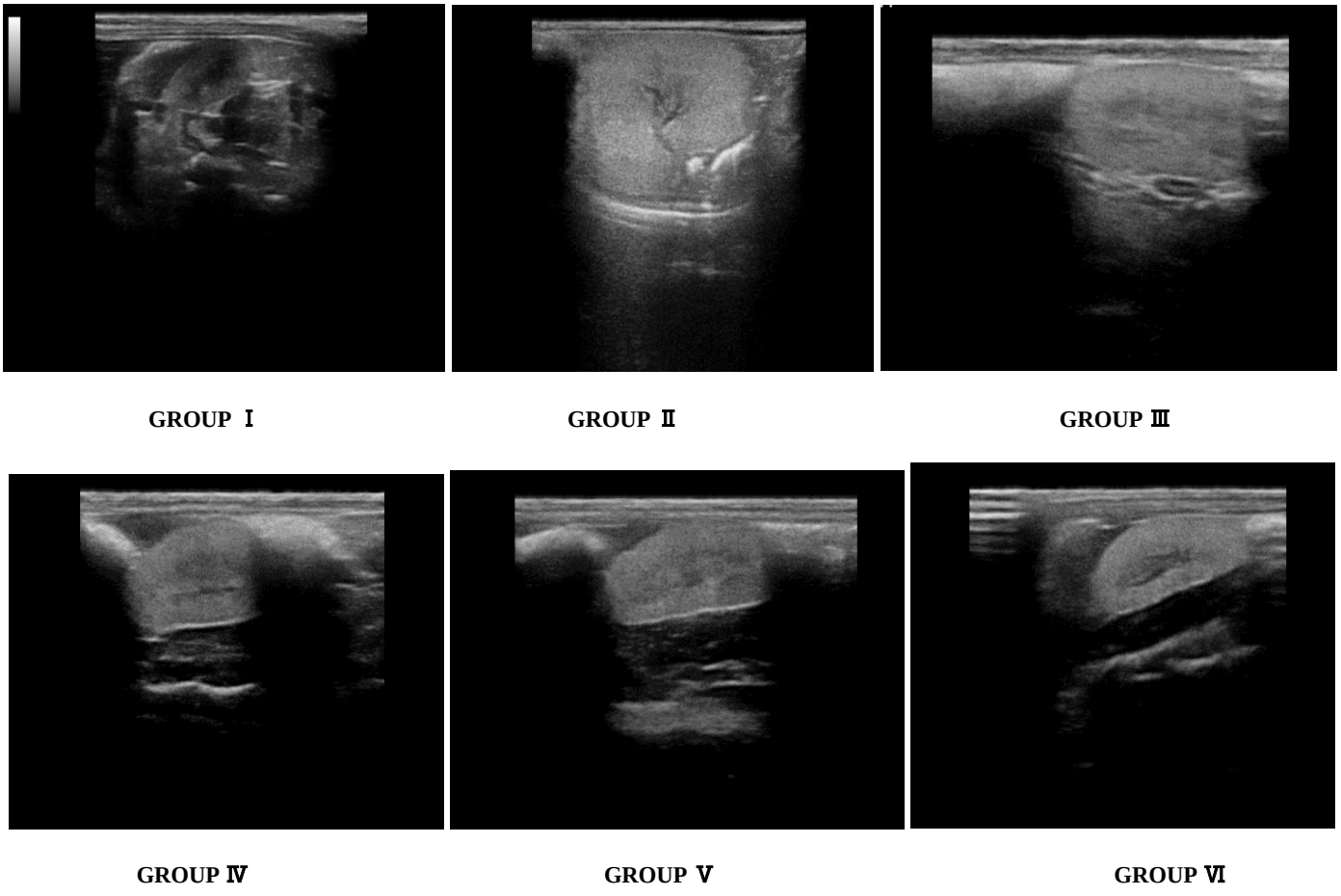

Figure 1: Ultrasonographic images of kidney from different groups on day 70 in rats

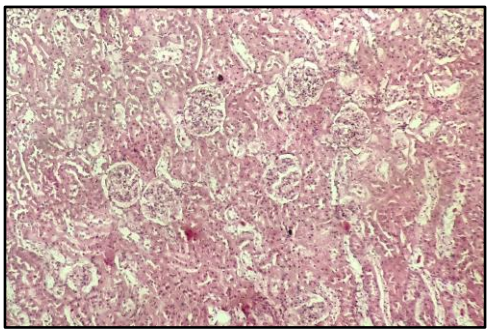

Figure 2: Kidney showed normal histo-architexture details in rats of control group I (H&E, 120X)

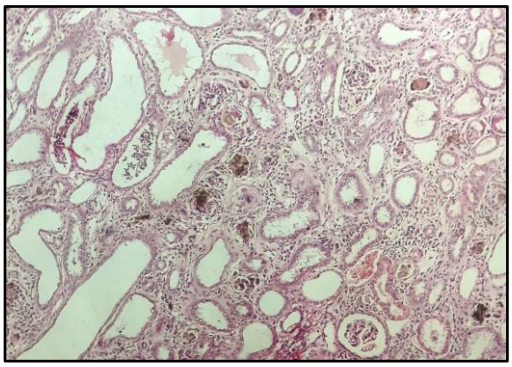

Figure 3: Kidney (Group II) showed marked tubular atrophy, cystic dilatation, mild fibrosis, congestion, inflammatory exudates and tubular cast in adenine treated rats (H&E, 120X)

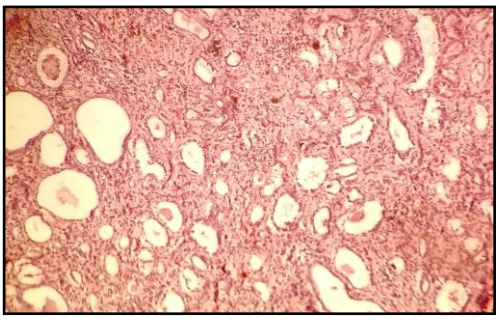

Figure 4: Kidney (Group III) showed severe fibrosis of parenchyma with tubular dilation in rats treated with bi-herbal aqueous extract @ 250 mg/kg after CKD induction (H&E, 120X)

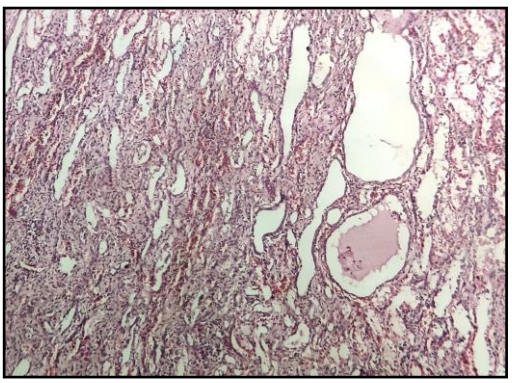

Figure 5: Kidney (Group IV) showed moderate fibrosis of parenchyma with tubular dilation, congestion and presence of cast in rats treated with bi-herbal aqueous extract @ 500 mg/kg after CKD induction (H&E, 120X)

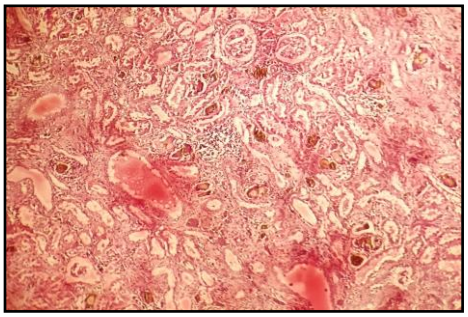

Figure 6: Kidney (Group V) showed moderate degenerative changes in tubular epithelial cells and presence of eosinophilic cast with mild infiltration of inflammatory cells in rats treated with bi-herbal alcoholic extract @ 250 mg/kg after CKD induction (H&E, 120X)

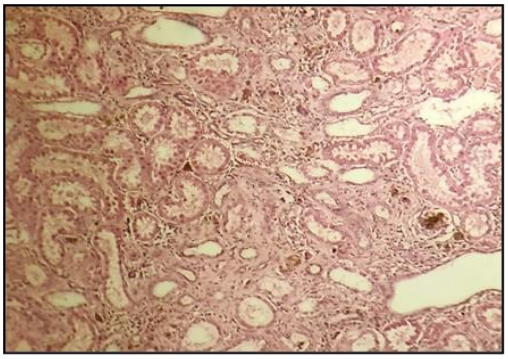

Figure 7: Kidney (Group VI) showed only mild degenerative changes in tubular epithelial cells and mild tubular dilatation in rats treated with bi-herbal alcoholic extract @ 500 mg/kg after CKD induction (H&E, 120X)

CONCLUSION

The aqueous and alcoholic bi-herbal extracts of *Glycyrrhiza glabra* and *Curcuma longa*, administered at a dosage of 500 mg/kg body weight, exhibited promising efficacy as compared to the dosage of 250 mg/kg. This efficacy was substantiated through various parameters including body weight and feed consumption, hematobiochemical analysis, urine assessment, ultrasonographic examination and histopathological evaluation. Particularly noteworthy was the greater effectiveness of the alcoholic bi-herbal extracts when compared to the aqueous extracts. The bi-herbal alcoholic extract of *Glycyrrhiza glabra* and *Curcuma longa*, in a ratio of 1.5:1, administered orally at the dose rate of 500 mg/kg body weight daily once for 42 days after CKD induction, demonstrated greater efficacy in CKD treatment and warrants further large-scale research investigation for better exploration.

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Conflict of interest

The authors declared no conflict of interest.

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