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Original Research Article

Antinephrotic potential of CATCAB-50 in CCL_4 induced glomerulonephritic animal

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ABSTRACT

Objective: Present study was designed to evaluate antinephrotic potential of CATCAB-50 in CCl_4 induced glomerulonephritis (GN) in *Wistar* rats. Methods: The poly herbal formulation CATCAB-50 has contained fresh dried root extract of six medicinal herbs i. e. *Carica papaya*, *Angelica officinalis*, *Tinospora cordifolia*, *Cissampelos pareira*, *Achyranthes aspera* and *Boerhaavia diffusa*, which was scientifically validated for the management of GN. The antinephrotic potential was evaluated in pre-treating group of rats with dose of 50mg/kg b.w. of CATCAB aqueous extract for 10 days in respect to carbon tetrachloride (CCl_4 1.5ml/kg b.w.) induced GN. Result: The aqueous extract of CATCAB-50 on hematological and biochemical parameters for its pharmacological screening of all groups were measured using standard procedures. Histological studies of rat kidney of all experimental group animals were also completed for its confirmation. Results showed that intraperitoneal injection of CCl_4 induced, significant ($p<0.05$) elevation in the hematological parameter and urine levels of total protein, uric acid, urea and creatinine. Conclusion: However, elevated biochemical parameters were significantly ($p<0.05$) attenuated in rats pre-treated with CATCAB-50, could be mediated by any of the phytocomponents present in it via either antioxidant and/or free radical scavenging mechanism.

1. Introduction

Glomerulonephritis (GN) is a group of diseases that injure the part of the kidney, founded a serious illness which requires immediate treatment. Many herbs represent nature's main store house of raw materials for the management of various renal disorders[1]. As conquer of kidney function leads to kidney failure, several patients almost immediately dependent on hemodialysis, and most of them don't afford the treatment cost while undergoing regular dialysis[2]. The earlier stages of kidney failure patients are exceedingly at risk of drug complications were seen in a community setting, about 10% of patients with renal insufficiency (serum creatinine >1.5 mg/dl) experienced a drug-related adverse event, with greater than one-half of these proceedings considered serious and 4.5% of the events found life-threatening, departed for kidney transplantation[3]. The large number of affected patients even though costs concerned and distressing consequences of medication-related problems like prolong hospitalization, dialysis even organ transplantation etc. presently it is known about the impact of renal impairment on drug disposition, including those drugs eliminated primarily by hepatic pathways play critical role for worsen conditions[4].

The kidney is a comprehensively important organ when it comes to the prognostic evaluation of the safety, efficacy and toxicity of drugs and non-drug chemicals[5]. It is necessary to majored the hematological as well as urological investigations including renal drug clearance during diagnostic confirmation and making goals for the management of these critical condition to early avoiding organ failure which leads to life threatening[6].

The prepared poly herbal formulation CATCAB-50 consist of six major well reported herbs for the management of GN i.e. *C. papaya*, *A. officinalis*, *T. cordifolia*, *C. pareira*, *A. aspera* & *B. Diffusa* (see table 1). The present study was designed to evaluate the protective effect of CATCAB-50 against CCL_4 induced GN in rats[7]. Several studies reported that, the antioxidant and free radical scavenging activity of these herbs shows similar action in renal collapses[8]. Also it was reported that, all these herbs are well known for the same action due to the presence of their chemical constituents such as flavonoids and poly phenolic compounds[9]. The extent of the protective effect was determined by studying serum and urine toxicity markers with histopathology of kidney tissues[10].

2. Materials and methods

2.1. Plant collection and authentication

Fresh dried root parts of these plants were collected from the Himalayan region of Uttarakhand, India[16] and were

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Table No. 1: Description of medicinal plants possessing anti-GN activity

Sr. No.	Scientific Name (Family)	Part	Dose	Activities	Reference
1.	*Carica papaya* (Caricaceae)	Shad dried roots each of 10 gm freshly prepared powdered drugs	Oral administration of the aqueous extract of the roots (50 mg/kg body weight)	Nephro-protective activity	[11]
2.	*Angelica officinalis* (Umbelliferae)			Stimulates kidney action	[12]
3.	*Tinospora cordifolia* (Menispermaceae)			anti-inflammatory, Antioxidant	[13]
4.	*Cissampelos pareira* (Menispermaceae)			Anti-Inflammatory, Antioxidant, Diuretic	[14]
5.	*Achyranthes aspera* (Amaranthaceae)			Pain & Swelling	[15]
6.	*Boerhaavia diffusa* (Nyctaginaceae)			Urinary Infection	[15]

thoroughly made free from foreign matter. The collected plant materials were taxonomically identified and authentication has been done by botanical scientist, the specimen letter no. BSI/NRC/Tech.(Ident.)/2013/755 at Botanical Survey of India (BSI), the northern regional centre, Dehradun, India.

2.2. Experimental animals

Eighteen albino Wistar rats (150-200g) were procured and followed the animal ethical clearance from the animal house of Bhimtal Campus, Kumaun University, Nainital, India (Reg. No. 490/01/a/CPCSEA). All experimental animals were kept in poly propylene cages (6 rats per cage) at 25±2˚C temperature with relative humidity 45-55% under 12 h light and dark cycles. All the animals were acclimatized for laboratory condition as per CPCSEA guideline for a week before uses. They were fed with standard pellet diet and fresh water *ad libitum*. The experimental protocol was in compliance with the ethics committee on research in animals, as well as nationally accepted principles for use and care of the laboratory animals.

2.3. Preparation of extract

CATCAB-50 was prepared by equal proportion of aqueous root extract of selected plants (50gm each) by using cold maceration method. The collected extract was dried by using copper water bath at temperature of 60°C ± 10°C until the entire water get evaporated. The dried extract powder was stored in a well tightly closed container.

2.4. Experimental design

Male Wistar rats (150-220g) were divided into three groups having six animals each. Group I treated as vehicle, was kept as normal control. Group II was treated with a single dose of carbon tetrachloride (CCl_4 1.5 ml/kg in olive oil) was kept as toxic control. Group III was pre-treated with prepared herbal formulation (CATCAB-50) at a dose of 50mg/kg body weight 1 h before CCl_4 administration and continued for 10 days.

2.5. Biochemical estimations

2.5.1. Body weight

Body weight was recorded on the first day and then last day of the study period in each group.

2.5.2. Blood & urine profile

Creatinine, total protein, urea, uric acid levels along with SGOT, SGPT, total bilirubin in blood and same parameter in urine sample along with 24 hours urinary volume were determined by using commercial glucometer kit at the end of experiment.

2.5.3. Histological analysis

Kidney tissues were obtained on the last day after scarification of animals and immediately fixed in 10% buffered neutral formalin solution. The tissue section was stained with hematoxylin and eosin (H & E stain) and examined under light microscope.

2.6. Statistical analysis

All the data were expressed as Mean±S.E.M. followed by one way ANOVA and Tukey's multiple comparison tests. $p \geq 0.05$ represents statistical significance and $p \leq 0.001$ represents statistical significance against control group.

3. Results

3.1. Body weight

CCl_4 treated rat showed a slight decrease in body weight (160g) compared to control rat (200g), while the CATCAB-50 treatment animals showed significantly increased in body weight (185g) as compared to CCl_4 treated rat.

3.2. Estimations of blood profile

Carbon tetra chloride (CCl_4) treated rats showed significant ($^{\#}p \geq 0.05$) increase in levels of total protein, urea, uric acid and creatinine along with SGOT, SGPT & total bilirubin in blood as compared to control group of animals. While pretreatment with CATCAB-50 significantly ($^{**}p \leq 0.001$) maintain the elevated levels of these parameters as compared to CCl_4 treated rats (table 2 & table 3).

Table No. 2: The effect of CATCAB-50 on serum profile in CCl_4 induced GN in rats

Group	Urea	Uric acid	Creatinine	Total protein
Control	18.45+0.18	2.05+0.10	0.72+0.03	8.44+0.20
CCl_4	42.48+0.23$^{\#}$	6.42+0.12$^{\#}$	2.15+0.06$^{\#}$	2.35+0.06$^{\#}$
CATCAB-50+CCl_4	22.37+0.25**	3.00+0.04**	0.88+0.11	7.68+0.08**

Values are given as mean ± SEM and expressed in mg/dl for groups of six animal in each. $^{\#}p\geq0.05$ denotes when disease control was compared with the normal control and $^{**}p\leq0.001$ denotes when treatment group was compared with the normal control.

Table No. 3: The effect of CATCAB-50 of serum marker enzymes in CCl_4 induced GN in rats

Group	Serum marker enzymes at end of study		
	SGPT	SGOT	Total bilirubin
Control	58.64 ± 1.25	142.87 ± 3.05	0.18 ± 0.03
CCl_4	87.32 ± 2.10$^{\#}$	202.58 ± 2.87$^{\#}$	0.22 ± 0.04$^{\#}$
CATCAB-50+CCl_4	62.48 ± 2.45**	148.23 ± 3.02**	0.20 ± 0.05**

Values are given as mean ± SEM and expressed in mg/dl for groups of six animal in each. $^{\#}p\geq0.05$ denotes when disease control was compared with the normal control and $^{**}p\leq0.001$ denotes when treatment group was compared with the normal control.

3.3. Estimations of urine profile

Carbon tetra chloride (CCl_4) treated rats showed significant ($^{\#}p\geq0.05$) increase in levels of total protein, urea, uric acid and creatinine in urine as compared to control group along with estimation of 24 hours urinary volume of all groups. While pretreatment with CATCAB-50 significantly ($^{**}p \leq 0.001$) maintain the elevated levels of these parameters as compared to CCl_4 treated rats (table 4). The estimation of 24 hours urinary volume also shows significant maintenance in urinary volume in CATCAB-50 treatment group (table 5) in comparison to other groups.

Values are given as mean ± SEM and expressed in mg/dl for groups of six animal in each. $^{\#}p\geq0.05$ denotes when disease control was compared with the normal control and $^{**}p \leq 0.001$ denotes when treatment group was compared with the normal control.

Table N0.4: The effect of CATCAB-50 on urine profile in CCl_4 induced GN in rats

Group	Urea	Uric acid	Creatinine	Total protein
Control	28.52+1.32	4.08+0.05	5.35+0.26	41.8+1.05
CCl_4	56.05+3.41$^{\#}$	9.43+0.08$^{\#}$	11.0+0.04$^{\#}$	80.6+3.02$^{\#}$
CATCAB-50+CCl_4	30.30+1.22**	3.85+0.35**	4.50+0.10**	46.2+0.90**

Table No. 5: Estimation of 24 hours urinary volume of all experimental group animals

Group	24 hours urinary volume			
	Initial	3rd Day	6th Day	9th Day
Control	17.50 ± 0.35	16.75 ± 0.40	15.80 ± 0.45	18.00 ± 0.50
CCl_4	37.25 ± 2.00$^{\#}$	40.10 ± 2.40$^{\#}$	33.50 ± 2.35$^{\#}$	28.35 ± 2.25$^{\#}$
CATCAB-50+CCl_4	27.20 ± 2.60**	21.50 ± 2.45**	14.25 ± 3.00**	18.65 ± 2.40**

Values are given as mean ± SEM and expressed in ml for groups of six animal in each. $^{\#}p\geq0.05$ denotes when disease control was compared with the normal control and $^{**}p \leq 0.001$ denotes when treatment group was compared with the normal control.

The urine sample was also taken every alternative day after treatment with CCl_4 and CATCAB-50+CCl_4 respectively. The result showed significant improvement in urine color on each sample collection (figure 1).

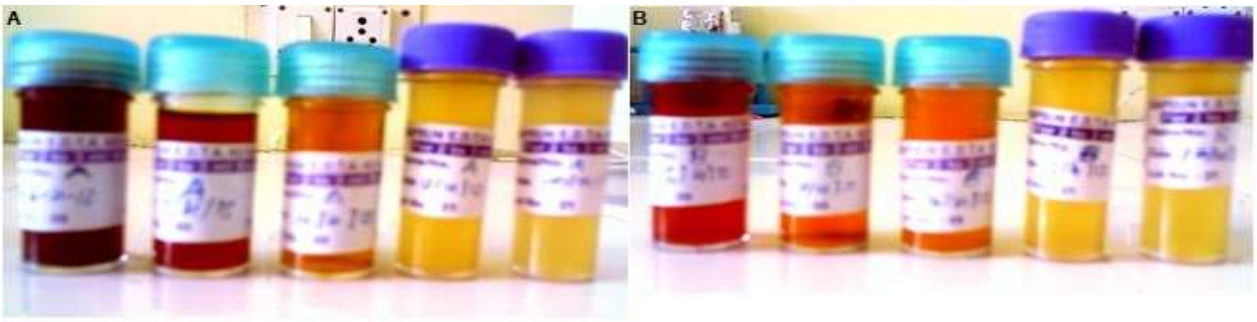

Figure No. 1: Urine sample were collected at every alternate day in the volumetric flask and took only 10 ml for analysis as shown under; (A) CCl_4, (B) CATCAB-50+CCl_4

3.4. Histological studies

Isolated kidney were quickly dipped into 4% para-formaldehyde then embedded in paraffin. Sample sections (1 μm) of the tissue were stained by Mallory and viewed using a light microscope with a computer-assisted analyzer.

Histological changes such as cortical glomerular, peritubular blood vessels congestion, and interstitial inflammation etc. were observed in the CCl_4 and CATCAB-50 + CCl_4 group (figure 2).

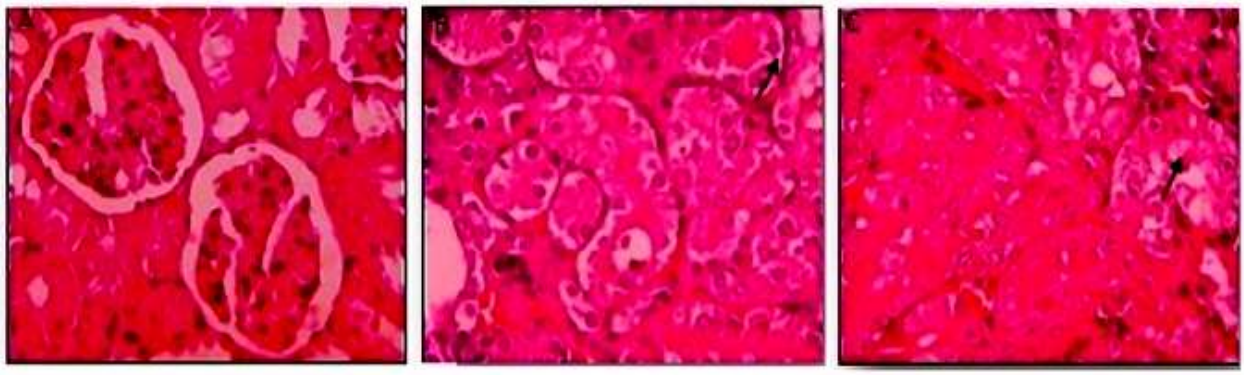

Figure No. 2: Histological appearance of kidney shown as under; (A). Control group, (B). CCl_4 treated, (C). CATCAB-50 + CCl_4

4. Discussion

The herbal plant has been used from ancient time to manage the human ailments without adversely influencing to the body and/or living organs. Kidneys are such vital organ, which is highly influenced by chemical and other synthetics drugs. Current therapies to remove uremic solutes for the renal disorders patient include peritoneal dialysis, hemodialysis, and kidney transplantation, which is costly and time-consuming regimens, and also linked to high morbidity. These types of treatments are predominantly available in developed countries, and therefore patients in developed countries are more likely to have an extended life expectancy. In underdeveloped countries, uremia is generally untreated and patients appear to have a lower life expectancy.

The present study, designed with six medicinal plants possessing antinephrotic activity used to maintain uremic solutes like; uric acid, urea, creatinine protein etc. in CCl_4 induced GN in *Wistar* rats. The poly herbal formulation has been developed by using geometrical dilution method and administered in animal at a dose of 50mg/kg body weight. The pretreatment administration of the drug showed the stun result to maintain the elevated parameter in nephritic rats within very short duration of time.

Our data also indicate that the serum profile in CCl_4 induced GN in rats shows that urea (22.37mg/dl), uric acid (3.00mg/dl), creatinine (0.88mg/dl) and protein (7.68mg/dl) content was observed in treatment group, which was more significant in comparison with disease control group. The constant improvement result was observed in serum biomarkers enzymes like SGPT, SGOT and bilirubin at the end of the study. Simple calculations based on the volume of urine from experimental animals and the difference between uremic and normal rats of 24 h urinary volume estimating that about 15–18 ml of urine per day must be observed with improvements in urine colour.

Our data suggest that the histopathology of the kidney clearly confirms its remodeling and vigorous glomerular capillaries. Our data also proposed that the herbal formulation will remove urea from the uremic animals in an amount that will prove beneficial to rats suffering from any degree of renal insufficiency.

5. Conclusion

The present study suggests that CATCAB-50 provides adequate protection against CCl_4 induced GN as evidenced by body weight, biochemical and histological parameters. The protective effect of CATCAB-50 may be due to its antioxidant potential and/or free radical scavenging mechanism of these herbs which shows a synergistic effect on restoring kidney function. However, further studies are needed to confirm it's a clear mechanism of action on GN and to characterize the phyto constituents responsible for its action on kidney.

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Conflict of interest: None Declared

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