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Phytochemical and Pharmacological characterization of Polyherbal Plant formulation against Glomerulonephritis Kidney Disease

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Abstract

The herbs used in the study have potent anti-nephrotic activity and individually play a crucial role as follows; *Angelica officinalis* responsible for urinary retention, *Boerhavia diffusa* act as excellent diuretic, *Carica papaya* act as immunomodulator and recover kidney tissues, *Cassia Fistula* protects deoxyribose from damage, *Cichorium intybus* has good immunomodulatory action and modulate kidney function, *Ficus hispida* inhibits insulinase activity of liver and kidney, *Fumaria indica* play a crucial role in reduction of granuloma mass, *Crataeva nurvala* support kidney from microbial infection and act as urinary retention, *Solidago virgaurea* stimulates the kidneys with excellent support of kidney health and *Vitex negundo* is an excellent tonic for nephrotic disorder with good diuretic properties.

At present, the polyherbal drugs available in the market are using in very high dose for the treatment of nephritis and observed that it extremely producing workload on the normal function of the kidney. The available Allopathic medicines are used in multiple dosage form for just symptomatic relief that produces side effect and leads to damage filtering units on prolonged exposure. The main purpose of the present study is to prepare a novel polyherbal dispersible tablet using plant's root extract which was scientifically proven and/or folklore medicine and possessing a prominent pharmacological potency for the treatment of glomerulonephritis. This preparation may diminish the economic burden and treatment cost which was not afforded by poor people.

Keywords: Glomerulonephritis, Polyherbal Plant formulation, *Carica papaya, Crataeva nurvala, Vitex negundo.*

Introduction

Kidneys play several delicate tasks, especially when they have to clear unwanted substances (toxins) from the body system[1]. It is constantly collaborating as a precise multi-tasking unit within the body to maintain endocrine function, acid-base balance, blood pressure, erythropoiesis for accomplishing the normal body function. Consequently, it bec me impaired once urinary functions decline due to some ill condition whether have no direct correlation with the pathoph siology of kidney dysfunction[2]. The tiny tuft of blood capillaries within the kidney that responsible for filtration may possibly scarily concern with inflammation and cell proliferation within the glomerulus is termed as glomerulonephritis

Globally the glomerulonephritis (GN) ambiance a variety of immune-mediated disorders that cause inflammation within the glomerulus and other filtering unit of the kidney, known for the most prevalent causes of end-stage renal disease (ESRD)[8].

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Chronic GN is a global warning for ordinary population of under developing countries those can't afford the treatment cost because of the therapies is expensive and lifelong. In India, it is estimated that about 90% patients can't afford such type of treatment cost[9,10].

Epidemiology

Glomerulonephritis is most typical in male than in female patients; however, lupus nephritis is most significantly excepted in females because the frequency is more than two times above than male population [11]. Several cases of GN lead to a delicate, asymptomatic health problems, which was not well recognized in the patient because of the shortage of medical attention and those remains undiagnosed[12]. In many countries like India, Australia, USA, Europe etc. studies have shown that evidence of nephritic syndrome like proteinuria, hematuria, low glomerular filtration rate or the combination of these features have more common for diagnosis of GN[13].

Globally 10% of the population is full of chronic nephritis, in which millions of people per annum rising due to non-affordable of treatment burden. Globally, more than two million people receiving treatment with dialysis or kidney transplant for their usual survival[14]. Individually 10% people of 2 million affected populations those received treatments for nephrosis in United States, Japan, Germany, Brazil and European country represent only 12% of the world populations. Concerning 20% populations were treated among hundred developing countries that frame over 50% of the global community[15].

Material and Methods

Extraction of plants material Methanol and aqueous extracts of each of the above plant were prepared by cold maceration. Roots of above plants were cleaned, shade dried, and powdered by a mechanical grinder **[14]**.

Methanol extracts (MeOH): The grounded root powder of each plant (500g) was separately added to the methanol 99% (2 Liter) and stored at room temperature for 48 hours. Subsequently, extract were separately filtered through Whatman filter paper, concentrated on a rotary evaporator at reduced temperature (40°C), and freeze dried to get the extract powder (yield:18-20% (w/w)) Extract powder(s) were stored in the air- tight containers[15].

Aqueous extracts (H2O): The grounded root powder of each plant (500g) was separately added to the 2 Liter distilled water and stored at room temperature for 48 hours. Thereafter, extracts were separately filtered by using Whatman filter paper, concentrated at reduced temperature (40°C) on a rotary evaporator, and freeze dried to get extract powder [yield 18-20% (w/w)]. Extract powder(s) were stored in the air-tightcontainers [16].

Phytochemical screening methods

The powder extracts were individually evaluated for the presence of different phytoconstituents as per the below mentioned methods:

Test for terpenes: To the 5ml of the extract, 2ml of chloroform and 3ml of conc. H_2SO_4 was added. The formation of a reddish brown ring confirmed the presence of terpenes[17].

Test for flavonoids: A few drops of conc. HCl were added in the small amount of the prepared extracts. The red colour was immediately developed, which confirmed the presence of flavonoids[191].

Test for saponins (*Frothing test):* 0.5ml of the extract was taken into a test tube and dissolved in distilled water. Frothing was persisted on warming, which preliminary shows as evidence of saponins[18].

Test for steroids (*Liebermann–Burchard reaction):* 2ml of acetic anhydride and 2 ml conc. H_2SO_4 was added into 5ml of the extract in a test tube. Change of colour from violet to blue confirms the presence of steroids [19].

Test for glycosides: 2ml of glacial acetic acid containing one drop of ferric chloride solution and 1 ml of conc. H_2SO_4 was added into 5ml of the extract in a test tube. The appearance of a brown ring indicates the presence of glycosides [20].

Test for proteins (*Biuret test):* 4% of NaOH and few drops of 1% $CuSO_4$ solution wereadded into 3ml of the extract in a test tube. Formation of violet or pink color indicates the presence of proteins [21].

Test for reducing sugars (*Fehling test):* 1ml of Fehling's A and Fehling's B solutions was mixed in a test tube, boiled for one minute then added an equal volume of test solution (2ml extract).

The mixed solution was then heated on boiling water bath for 5–10 min. First a yellow then a red brick precipitate was observed [22].

Test for carbohydrates (*Molisch test):* 2–3ml of the aqueous extract, 2 drops of Molisch's reagent (10% alcoholic solution of a-naphthol) was added in a test tube. After mixing, a small amount of conc. H_2SO_4 is slowly added down the sides of the sloping test-tube, without mixing, to form a layer. Violet ring is formed at the interface betweenthe acid and test layers [23].

Test for tannin and phenol *(Ferric Chloride Test):* 3ml of extract, 3ml of 5% w/w of the $FeCl_3$ solution was added in a test tube. The blue-black colour indicates the presenceof tannins and phenols [24].

Test for alkaloids: In 10g of dried extracts 20ml of dilute HCl solution was added with vigorous shaking and then filter. In the filtrate, the following tests were performed[194];

Mayer's Test: 3ml of the filtrates, 1ml of Mayer's reagent (potassium mercuric iodide) was added in a test tube. The appearance of white precipitate confirmed the presence of alkaloids.

Wagner's Test: 3ml of the filtrate, 1ml of Wagner's reagent (iodine in potassiumiodide) was added in a test tube. The emergence of reddish-brown precipitate at the surface indicates the presence of alkaloids.

Dragendroff's Test: 3ml of the filtrate, 1ml of Dragendroff's reagent (potassiumbismuth iodide) was added in a test tube. The appearance of red brick precipitate indicates the presence of alkaloids.

Quantity for preparation of polyherbal combinations: The plants root extract obtained from methanol and aqueous solvent have been composed separately in an appropriate quantity based on lowest dose of each plant as reported for promising activity has been preferred for the preparation of the potent polyherbal combination[195] used in the treatment of glomerulonephritis mention below;

Botanical Name	Quantity
1) *Boerhavia diffusa* Linn.	25mg
2) *Carica papaya* Linn.	25mg
3) *Rheum emodi Linn*	25mg
4) *N. ncifera (flowers)*	25mg
5) *Nelumbo nucifera*	25mg
6) *Crataeva Nurvala* Linn.	25mg

Acute toxicity study of the prepared polyherbal combination

The acute oral toxicity study was conducted as per the OECD guideline-423 designed for the prepared novel polyherbal combination used for the screening of anti-GN activity in experimental animal. The prepared polyherbal formulation at a single oral dose (2000 mg/kg) was administered in rats. A limit test at one dose level of 2000 mg/kg body weight was carried out in six animals due to the available information on the mortality for the individual plant at the highest dose level (2000 mg/kg body weight), then the limit test on polyherbal combination was conducted as per the guideline mentioned bellow (OECD 423-2d). The parameters such as general behaviour, body weight, mortality and necropsy were studied at 14th day of the study[25].

OECD 423-2d: Test procedure with a starting dose of 2000 mg/kg body weight

Observations for general behavioral: The behavioral patterns of animals were observed first 6h and followed by 14h after the administration of polyherbal formulation. There were no significant changes observed in physical parameters viz. Skin, fur, eyes, mucous membrane, salivation and sleep pattern observed for 7 days evaluation[198].

All animals were observed for survival up to 14 days after the administration of polyherbal formulation [199].

Changes in body weights: The mean body weight of rats before and after the oral

administration of polyherbal combination and difference if any was recorded for toxicity evaluation parameter [26].

Necropsy study: The animals were euthanized at the termination of study (day 14) and necropsy analyzed to confirm the toxic effect. Body cavities and vital organs such as heart, liver, kidney and lungs were examined for occurrence of lesions[26].

Pharmacological screening method

Experimental animal: The Wistar rats having weight 180-220g were procured from the Departmental Animal House after the clearance of IAEC, the protocol approval no. is KUDOPS/17. All experimental animals were kept in polypropylene cages at 25±2˚C room temperature under 12h light and dark cycles. All the animals were acclimatized for laboratory condition as per CPCSEA guideline for a week before use. The animals were fed with standard pellet diet and free access of water *ad libitum*.

Procurement of drug and chemicals: Gentamicin (Genticyn, Abbott Healthcare Pvt. Ltd, India) purchased from the local medical shop. All other chemicals and biochemical reagents used in the study were of LR and AR grade obtained from departmental storehouse.

Experimental design: The Wistar rats were divided into ten groups having six animals in each. The study was designed and conducted for 42 days.

Group I: Normal Control (administered saline) and free access to water & diet. (NC) **Group II:** Disease Control (Gentamicin 100mg/kg/day for 8 days) and free access to water & diet. (Gm)

Therapeutic groups:

Group III: Gentamicin 100mg/kg/day for 8 days and then methanol extract of polyherbal combination (25mg/kg/day) for rest 34 days. (Gm+ME25)

Group IV: Gentamicin 100mg/kg/day for 8 days and then methanol extract of polyherbal combination (50mg/kg/day) for rest 34 days. (Gm+ME50)

Group V: Gentamicin 100mg/kg/day for 8 days and then aqueous extract of polyherbal combination (25mg/kg/day) for rest 34 days. (Gm+AE25)

Group VI: Gentamicin 100mg/kg/day for 8 days and then aqueous extract of polyherbal combination (50mg/kg/day) for rest 34 days. (Gm+AE50)

Prophylactic groups:

Group VII: Methanol Extract of polyherbal combination (25mg/kg/day) for 34 days and then Gentamicin 100mg/kg/day for rest 8 days. (ME25+Gm)

Group VIII: Methanol Extract of polyherbal combination (50mg/kg/day) for 34 days and then Gentamicin 100mg/kg/day for rest 8 days. (ME50+Gm)

Group IX: Aqueous Extract of polyherbal combination (25mg/kg/day) for 34 days and then Gentamicin 100mg/kg/day for 8 days. (AE25+Gm)

Group X: Aqueous Extract of polyherbal combination (50mg/kg/day) for 34 days and then Gentamicin 100mg/kg/day for 8 days. (AE50+Gm)

Investigational parameters

Physical parameters: Body weight, kidney weight and 24h urinary volume were considered for the study.

Body weight: The animal body weight was measured using digital weighing balance of individual groups (n=6) separately at day first and end of study. The average weight of animal groups was calculated by Mean±SEM.

Kidney weight: The animal kidney weight was measured using digital weighing balance of individual groups (n=6) separately at the end of study. The kidneys were isolated immediately after scarification of rat and dried by rapping of tissue paper. The average kidney weight of animal groups was calculated by Mean±SEM.

24h urinary volume: The rats were kept in metabolic cages separately for 12 hours, starting at 08:30 PM and ending at 08:30 AM at the next day and also feeded with standard diet and water *ad libitum*. The average urine volume of animal groups was calculated by Mean±SEM.

Fasting condition: To establish the blood collection, animals were fasted for 12 hours (overnight) stay in the metabolic cages, started at 08:30 PM and ending at 08:30 AM at the next day. The hematological parameters, biochemical parameters, LFT and KFT in serum sample were determined by auto analyzers using standard test kits.

Hematological parameters: Hemoglobin, CBC, Neutrophil, Lymphocyte, Eosinophil, Monocyte

and Basophil were considered for the study and estimated by using automated cell counters (Auto analyzer). The blood samples were collected in EDTA tube and mixed by gentle shaking then the sample is loaded into the automated cell counter (CARELAB 200 AUTO ANALYSER). It was forced through a small tube and the automated cell counter through optical or electrical impedance sensors, counted that how many cells go through the tube.

Liver function test (LFT): The blood was withdrawn and serum immediately separated, and then following test were performed for total bilirubin, total protein, AST,ALT and ALP.

Estimation of total bilirubin: Diazo end point (Pearlman and Lee)[202]. The required reagents are; Reagent 1- HCl (100 mM/L), Sulphanilic acid (5 mM/L), surfactant 1.00% and Reagent 2- Sodium nitrite 144 mM/L.

Each ingredient were thoroughly mixed and incubated for 5 min at 37°C. The absorbance for each test and standard sample were recorded at □max 546 nm against blank reagent and calculated as;

T. Bilirubin (mg/dl) = Abs. of test □ Factor(23 for total bilirubin)

Estimation of total protein: Modified Burette, end point assay method[203]. Therequired reagents are; Reagent 1- Copper sulphate (7 mM/L), Sodium hydroxide (200 mM/L), Sodium-potassium tartrate (20 mM/L), Surfactant (qs) Reagent 2- Bovine serum albumin (6.5 g/dl).

Table 1: The assay procedure for estimation of total protein

Pipette into tubes marked	Blank	Standard	Test
Reagent	1000 µl	1000 µl	1000 µl
Serum	-	-	10 µl
Standard	-	10 µl	-

Each ingredient were thoroughly mixed and incubated for 5 min at 37°C. The absorbance for each test and standard sample were recorded at max 578 nm against blank and calculated as;

T. Protein (mg/dl) = Abs. of test / Abs. of std. □ 6.5

Estimation of AST (Aspartate aminotransferase): Modified UV IFCC method[204]. The required reagents are; Reagent 1- Tries buffer pH 7.8 (80 mM/L), L- Aspartate (240 mM/L), Malate dehydrogenate (≥ 600 U/L), Lactate dehydrogenate (≥ 600 U/L) and Reagent 2- a-kitoglutarate (12 mM/L), Nicotinamide adenine dinucleotide (0.18 mM/L).

Preparation of working reagent: Reagent 2 was added into reagent 1 in a ratio of 1:4 after that swirled to dissolve with gentle shaking and allowed to attain at 37°C before performing the test.

Table 2: The assay procedure for estimation of AST was

Pipette into tube marked	Volume
Test/Serum	100 µl
Working reagent	1000 µl

Each reagents were mixed well and aspirated immediately for recording of absorbance after 1 min at wavelength □max 340 nm for the determination of changes in absorbance per min (□A/min) and calculated as;

AST (IU/L) = □ A/min □ K {where, Kinetic factor (K) = 1768}

Estimation of ALT (Alanine aminotransferase): Modified UV IFCC, Kinetic assay method[204]. The required reagents are; Reagent 1- Tries buffer pH 7.5 (100 mM/L), L-Alanine (500 mM/L), Lactate dehydrogenate (≥ 1200 U/L) and Reagent 2- a- kitoglutarate (15 mM/L), Nicotinamide adenine dinucleotide (0.18 mM/L).

Preparation of working reagent: Reagent 2 was added into reagent 1 in a ratio of 1:4 after that swirled to dissolve with gentle shaking and

allowed to attain at 37°C before performing the test.

Table 3: The assay procedure for estimation of ALT

Pipette into tube marked	Volume
Test/Serum	100 µl
Working reagent	1000 µl

Each reagents were mixed well and aspirated immediately for recording of absorbance after 1 min at wavelength max 340 nm for determination of absorbance change per min (□ A/min) and calculated as;

ALT (IU/L) = □ A/min □ K {where, Kinetic factor (K) = 1768}

Estimation of ALP (Alakaline phosphatase): Modified pNPP-AMP (IFCC),Kinetic assay method[205]. The required reagents are; Reagent 1 (AMP; 2-amino-2- methyl-1-propanol)- AMP buffer (300 mM/L), Magnesium acetate (2 mM/L), Zinc suilphate (0.8 mM/L), Chelator (qs) and Reagent 2- p-Nitrophenyl phosphate (pNPP) (10 mM/L), Stabilizer (qs).

Preparation of working reagent: The one vial of reagent 2 reconstituted with 1.5 mlof reagent 1 and swirled to dissolve with gentle shaking and allowed to attain at 8°C.

The assay procedure for estimation of ALP was;

Pipette into tube marked	Volume
Serum	20 µl
Working reagent	1000 µl

Each reagent were mixed well and aspirated immediately for recording of absorbance after 30 sec at wavelength □max 405 nm for determination of absorbance change per min (□ A/min) and calculated as;

ALP (IU/L) = □ A/min □ K {where, Kinetic factor (K) = 2712}

Biochemical parameters: The blood was withdrawn and serum immediately separated, and then following test was performed for total cholesterol, triglycerides, LDL and HDL.

Total cholesterol: CHOD-PAP, end point method[206]. The required reagents are; Reagent 1- Buffer pH 6.4 (100 mM/L), cholesterol oxidase (> 100 U/L), cholesterol esterase (> 200 U/L), Peroxidase (> 3000 U/L), 4-amino antipyrine (0.3 mM/L), Phenol (5 mM/L) and Reagent 2- Cholesterol 200 mg/dl as standard.

Table 4: The assay procedure for measuring total cholesterol

Pipette into tubes marked	Blank	Standard	Test
Working reagent	1000µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Test sample	-	-	10 µl

Each reagent were mixed well and incubated for 10 min at 37°C. The absorbance for test and standard were recorded against blank at wavelength max 505 nm and calculated as;

T. cholesterol (mg/dl) = Abs. of test / Abs. of standard □Concentration of std. (mg/dl)

Triglycerides: GPO-Trinder method, end point method[207]. The required reagents are; Reagent 1-

ATP (2.5 mM/L), Mg^{2+} (2.5 mM/L), 4-amino antipyrine (0.8 mM/L), 3-5-dichloro-2-hydroxibenzine (1 mM/L), Peroxidase (>2000 U/L), Glycerol kinase (>550 U/L), Glycerol phosphate oxidase (>8000 U/L), Lipoprotein lipase (>3500 U/L), Buffer pH 7.0 (53 mM/L) and Reagent 2- Triglycerides (2.3 mM/L)used as standard.

Table 5: The assay procedure for measuring triglycerides

Pipette into tubes marked	Blank	Standard	Test
Working reagent	1000µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Test sample	-	-	10 µl

Each reagent were mixed and incubated for 10 min at 37°C. The absorbance for test and standard was recorded against blank at max 505 nm using auto analyzers and calculated as;
Triglycerides (mg/dl) = Abs. of test / Abs. of standard Concentration of std. (mg/dl)

Estimation of LDL and HDL: PEG-CHOD-PAP, end point method. Low density lipoprotein (LDL) estimated by addition of polyethylene glycol 6000 (PEG). After centrifugation, the high density lipoprotein (HDL) fraction remains in the supernatant and determined by CHOD-PAP method[208]. Required reagents are; Cholesterol oxidase (< 1500 U/L), Peroxidase (< 4 KU/L), N,N-bis (4-sulphobutyl)- m-toludinedisodium (< 1 mM), Ascorbic oxidase (< 3000 U/L), 4-Aminoantipyrine (<1 mM), Preservative (0.1%) and Cholesterol esterase (< 2000 U/L).

Table 6: The assay procedure for measuring LDL and HDL

Pipette in to tube marked	Blank	Standard	Test
Working reagent 1	450 µl	450 µl	450 µl
Cholesterol calibrator	-	10 µl	-
Test sample			10 µl
Mixed and incubated for 5 minutes at 37°C			
Working reagent 2	150 µl	150 µl	150 µl

Each reagent was well mixed with standard and sample respectively and incubated for 5 minutes at 37°C separately. The change in absorbance was measured at 578 nm against reagent blank and calculated as;
HDL (mg/dl) = Abs. of Test /Abs. of standard □ 50 □ 2LDL = TC – (HDL + VLDL); Where, VLDL = TG / 5

Kidney function test (KFT): The blood was withdrawn and serum immediatelyseparated, and then following test was performed for BUN, albumin, urea, creatinine and uric acid.

Estimation of urea and BUN: GLDH-Urease method[209]. The requiredreagents are; Reagent 1- a-ketoglutarate (7.5 mM/L), NADH (0.32 mM/L), Urease (> 8000 IU/L), Glutamate dehydrogenate (> 1000 IU/L), ADP (1.2 mM/L), Tries buffer pH 7.9 (100 mM/L) and Reagent 2- Urea (50 mg/dl), BUN (23.4 mg/dl) used as standard.

Table 7: The assay procedure for measuring BUN and urea

Pipette into tubes marked	Standard	Test
Working reagent	1000 µl	1000 µl
Standard	20 µl	-
Test sample	-	20 µl

Working reagent mixed well and aspirated with standard followed by samples at the reaction temperature 37°C. The absorbance was recorded at wavelength max 340 nm for determination of absorbance changes (A) and calculated as;
Urea (mg/dl) = □A of test / □A of std. □ Concentration of std. (mg/dl)BUN (mg/dl) = □A of test / □A of std. □ Concentration of std. (mg/dl)

Results and Discussion

Table 8: Phytoconstituents present in methanol root extracts of polyherbal plants

Sample	*Boerhaviadiffusa*	*Carica papaya*	*Rheum emodi*	*N. nucifera (flowers)*	*Nelumbo nucifera*	*Crataeva nurvala*
Carbohydrate	+	+	+	+	+	+
Proteins	+	-	+	+	-	-
Lipids	-	-	-	-	-	-
Steroids	+	+	+	+	+	+
Glycosides	+	+	+	-	+	+
Coumarins	-	+	+	+	+	+
Saponins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Anthraquinones	-	+	+	+	-	+

Where: +, indicates presence and −, indicates absence of concentration

The acute oral toxicity study of polyherbal methanol and aqueous extract combination at a single dose of 2000 mg/kg was observed that there were none changes in general appearance and none of mortality recorded in experiment animals. Hence the polyherbal combination was found to be safe at a dose of 2000 mg/kg body weight.

Pharmacological Screening

The pharmacological screening for glomerulonephritis has been conducted on ten groups of Wistar rats (n=6). The glomerulonephritis was induced by i.p. administration of Gentamicin 100mg/kg/day for eight days. The test drugs of polyherbal methanol and aqueous extract were administered by oral route in two different doses i.e., 25 mg/kg and 50 mg/kg respectively for therapeutic and

prophylactic activity. The physical, biochemical, antioxidant and histopathological parameters were evaluated.

Physical parameters of animal groups

The effect of the polyherbal combination on physical parameters, body weight of all animal groups were observed, while 24 h urinary volumes at first and last day was observed and the kidney weight was recorded at the end of study

Table 9: Effect of polyherbal combination on physical parameters of animal groups

Groups	Body weight (g)		Kidney weight at the end ofstudy (g)		24h Urinary volume (ml)	
	Day 01	Day 42	Left kidney	Right kidney	Day 01	Day 42
NC	190.40±6.23	228.32±8.10	0.500 ±0.12	0.645±0.05	16.8±1.28	17.6±1.08
DC	200.72±7.35	178.50±6.15$^{\#}$	0.968±0.11$^{\#}$	0.784±0.07	18.6±1.22	12.8±1.04$^{\#}$
Gm+ME25	190.80±7.02	218.06±8.03**	0.850±0.03	0.778±0.04	20.0±2.01	22.0±1.64**
Gm+ME50	206.63±8.12	226.03±8.00***	0.945±0.05	0.662±0.06	19.8±1.48	21.7±1.60**
Gm+AE25	200.81±7.28	218.42±7.28**	0.701±0.07	0.640±0.05	20.5±1.88	20.4±1.90**
Gm+AE50	199.62±9.24	211.46±6.35**	0.693±0.05	0.690±0.07	19.2±2.00	21.6±1.50**
ME25+Gm	200.46±8.75	228.27±8.83***	0.704±0.10	0.722±0.08	21.0±1.20	20.8±1.25**
ME50+Gm	210.28±8.86	238.68±9.18***	0.766±0.08	0.782±0.07	22.0±1.80	20.7±1.76**
AE25+Gm	190.71±8.46	222.62±7.56**	0.605±0.06	0.628±0.08	21.8±1.88	20.4±2.02**
AE50+Gm	212.08±8.35	220.39±7.83**	0.698±0.06	0.782±0.05	22.2±2.00	22.0±1.86**

Values are given as Mean±SEM of animal groups (n = 6) and expressed in g & ml.
$^{\#}p<0.05$ statistical significance against normal control; $^{**}p<0.05$ and $^{***}p<0.001$ statistical significance against disease control.

The average body weight of rats on 1st day was recorded between 154.40±6.23g (normal control) to 210.28±8.86g (ME50+Gm) of all animal groups and observed thatthe change in b.w. was non-significant in comparison between these groups. At the end of study, the gentamicin treated group showed statistically significant ($^{\#}p<0.05$) decrease in body weight (172.50±6.15g) as compared to normal control. The methanoland aqueous extracts treated groups shows statistical significant ($^{**}p<0.05$) improvement in body weight in comparison to disease control as recorded at the end of study, out of which the rats pre-treated with high dose (ME50+Gm) of polyherbal combination (50 mg/kg) shows statistically significant ($^{***}p<0.001$) improvement in body weight i.e., 238.68±9.18g in comparison to other treatment groups. The rats treated with low lode of aqueous extract (AE25+Gm) also showed significant ($^{**}p<0.05$) improvement in b.w. (222.62±7.56g) in comparison to disease control.

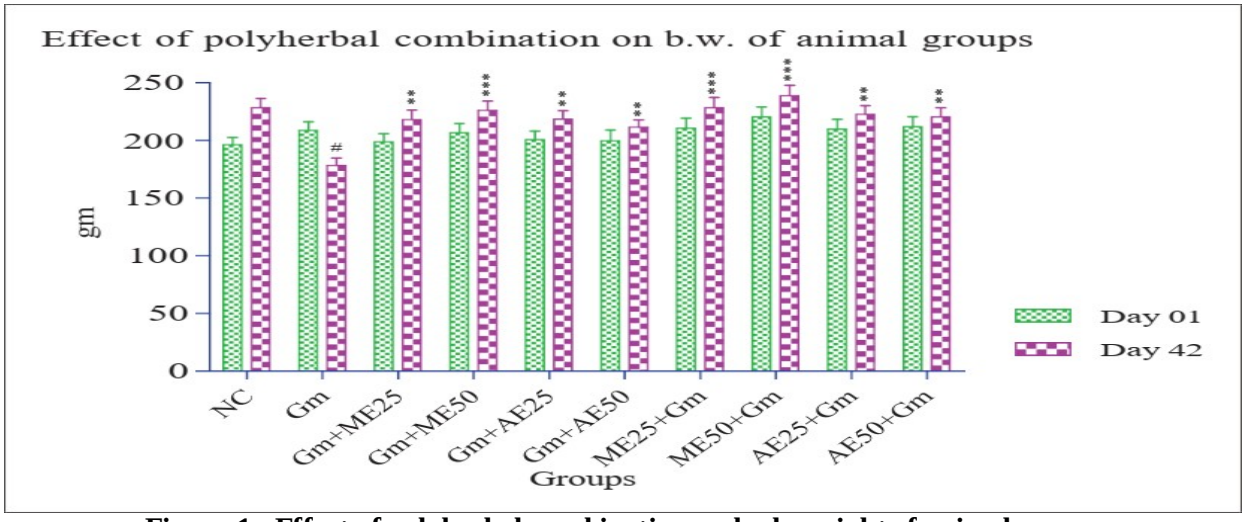

Figure 1: Effect of polyherbal combination on body weight of animal groups

Values are given as Mean±SEM of animal groups (n = 6) and expressed in gm. $^{\#}p<0.05$ statistical significance against normal control; $^{**}p<0.05$ and $^{***}p<0.001$ statistical significance against disease control.

The rat kidney weight was observed at the end of study after scarification of animals, which reviles that the average weight of left kidney varies between 0.625±0.12g (normal control) to 0.945±0.11g (disease control) and showed statistically significant ($^{\#}p<0.05$) difference between these groups. Whereas the average weight of rightkidney varies between 0.625±0.05g (normal control) to 0.754±0.07g (disease control) but did not show statistically significant difference as compared between these groups. The methanol extract treated group showed a little reduction in kidney weight as compare to disease control, while the rats treated with aqueous extracts showed considerable reduction in kidney weight but changes was observed as non-significant. The pre- treated low dose of aqueous extract (AE25+Gm) showed better improvement and the changes was about to comparable with normal control. The reduction inkidney weight is good sign for glomerulonephritis activity.

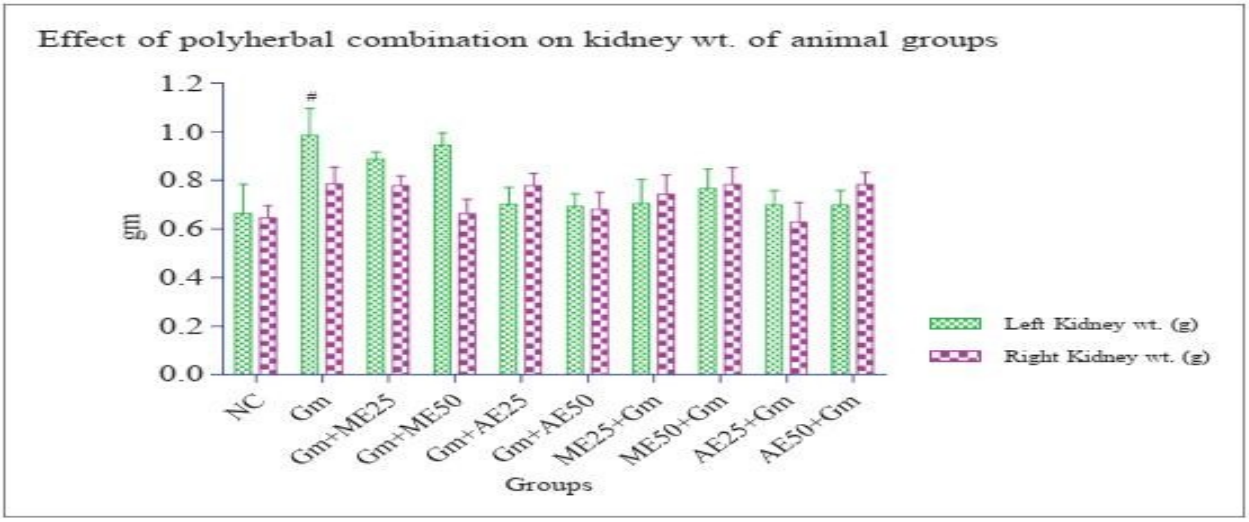

Figure 2: Effect of polyherbal combination on kidney weight of animal groups

Values are given as Mean±SEM of animal groups (n = 6) and expressed in gm. $^{\#}p<0.05$ statistical significance against normal control and $^{**}p<0.05$ statistical significance against disease control.

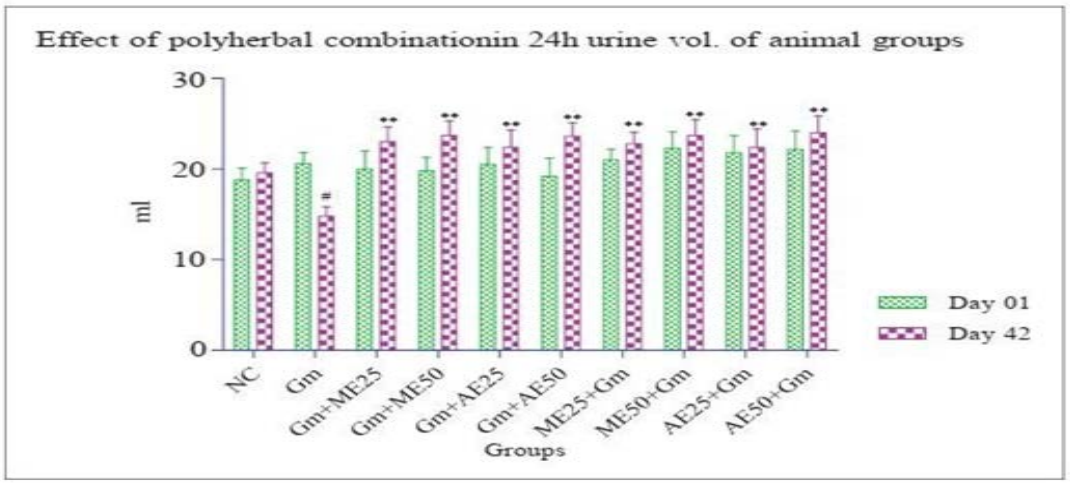

Figure 3: Effect of polyherbal combination on 24h urinary volume of animal groups

Values are given as Mean±SEM of animal groups (n = 6) and expressed in ml. $^{\#}p<0.05$ statistical significance against normal control and $^{**}p<0.05$ statistical significance againstdisease control.

The 24h urinary volume was observed at 1st day and last day of the study, which revealed that the initial amount of the urine volume varies between 15.8±1.06 ml (NC)to 20.2±1.00ml (AE50+Gm) of animal groups and showed statistically non-significant difference between these groups on first day of experiment. At the end of the study 24h urinary volume was observed between 15.8±1.05ml (DC) to 22.0±1.74ml (AE50+Gm) of animal groups and showed statistically significant ($^{**}p<0.05$) difference in comparison to these groups. The other methanol and aqueous extract treated groups also showed statistically significant ($^{**}p<0.05$) increase in urine volume as compared to disease control group (Table 7.6 and Figure 7.6).

Hematological parameters of animal groups

The effect of the polyherbal combination on haematological parameters of animal groups was observed for haemoglobin, CBC parameter and differential leukocyte count (neutrophil, lymphocyte, eosinophil, monocyte and basophil) in serum sampleat the end of study. The observed data of haematological parameters.

Table 10: Effect of polyherbal combination on hematological parameters

Groups	Hb (g/dl)	CBC ($x10^3$/µl)	Differential leukocyte count (%)				
			Neutrophil	Lymphocyte	Eiosinophil	Monocyte	Basophil
NC	11.11±1.86	7.80±2.00	50.83±4.00	37.33±2.25	1.20±0.70	2.35±0.50	00
DC	9.80±1.64	5.00±1.36	67.16±2.99	42.64±3.63$^{\#}$	4.28±1.08	5.66±1.58$^{\#}$	00
Gm+ME25	10.18±1.67	5.75±1.58	63.00±3.61	42.50±3.08	2.23±0.63	2.19±0.76	00
Gm+ME50	13.13±1.57	7.00±2.00	65.83±4.02	41.33±2.75	2.00±0.88	2.02±0.86**	00
Gm+AE25	11.92±1.54	7.98±2.16	54.16±2.99	30.56±2.63**	1.88±0.28	0.86±0.45**	00
Gm+AE50	10.90±1.66	7.93±2.01	55.50±3.61	25.50±1.88**	1.00±0.45	2.00±0.73**	00

ME25+Gm	12.15±1.37	6.93±1.51	63.50±3.61	40.50±3.38	2.39±1.03	2.28±0.87	00
ME50+Gm	11.16±1.47	7.11±2.02	65.33±3.02	40.33±2.45	2.08±0.99	2.20±0.96	00
AE25+Gm	11.89±1.94	7.90±2.16	53.16±3.00	30.33±2.38**	1.06±0.90	1.15±0.58**	00
AE50+Gm	11.60±1.77	7.53±2.11	54.50±3.60	31.50±3.00**	1.39±0.73	1.25±0.61**	00

Values are given as Mean±SEM of animal groups (n = 6) and expressed in % count, $x10^3$/µl & g/dl. #$p<0.05$ statistical significance against normal control and **$p<0.05$ statistical significance against disease control.

The observed haemoglobin values was varying between 11.80±1.64 g/dl (DC) to 12.11±1.86 g/dl (NC) of all experimental groups that showed statistically non- significant difference in comparison between these groups. The animal treated with 25mg/kg (Gm +AE25) of polyherbal combination showed small improvement in hemoglobin concentration, i.e., 11.92±1.54 g/dl in comparison to disease control. Whereas, the methanol extract treated groups didn't showed such type of improvement in Hb parameter as compare to aqueous extract treated groups. In caseof CBC, it was observed that the average concentration varies between 4.00±1.36 $x10^3$/µl (DC) to 6.98±2.16 $x10^3$/µl (Gm+AE25) of animal groups, while the normal control group observed as 7.80±2.15 $x10^3$/µl. The other methanol and high dose of aqueous extract treated groups showed statistically non-significant difference in comparison withdisease control group (Table 7.7 and Figure 7.7).

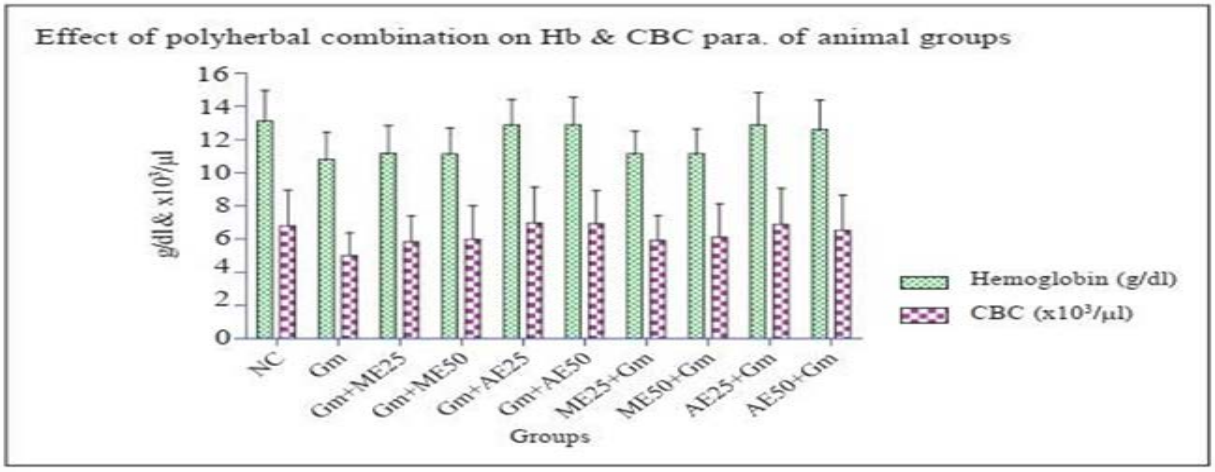

Figure 4: Effect of polyherbal combination on Hb and CBC

Values are given as Mean±SEM of animal groups (n = 6) and expressed g/dl and $x10^3$/µl.

The percentage changes in differential leukocyte counts, i.e., neutrophil, lymphocyte, eosinophil, monocyte and basophil were observed at the end of the experiment. The observed values of neutrophil were varying between 50.16±3.00 (NC) to 67.16±2.99 (DC) that shows statistically non-significant difference in comparison between these groups. In case of lymphocyte the observed values varied between 35.50±3.00. (AE50+Gm) to 50.64±3.63 (DC), the lower dose of aqueous extract treated groups shows statistically significant (**$p<0.05$) difference in comparison to disease control but the methanol extract treated groups did not showed significant improvement as compared to disease group. The observed values of eosinophil were varying between 1.00±0.45 (Gm+AE50) to 3.28±1.08 (DC) shows statistically non-significantdifference in comparison between these groups. The other

treatment groups also did not showed any significant changes in comparison to disease group. In case of monocyte the observed values ranged between 1.00±0.73 (Gm+AE50) to 4.66±1.58 (DC), the lower dose of treatment groups shows statistically significant ($^{**}p<0.05$) improvement in comparison to disease control group. None of the basophil was observed in all animal groups (Table 7.8 and Figure 7.8).

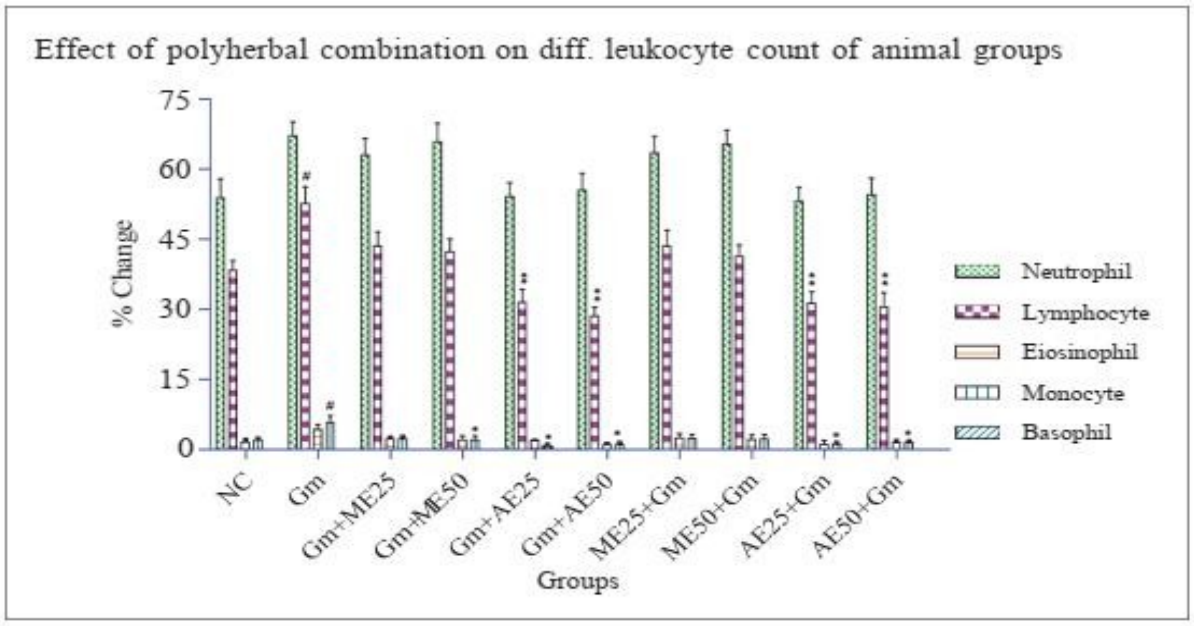

Figure 5: Effect of polyherbal combination on diff. leukocyte count of animal groups

Values are given as Mean±SEM of animal groups (n = 6) and expressed in % change. $^{\#}p<0.05$statistical significance against normal control and $^{**}p<0.05$ statistical significance against diseasecontrol.

LFT parameters of animal groups

The effect of the polyherbal combination on LFT (liver function test) parameters of animal groups were observed for total bilirubin, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP)at the end of study. The observed statistical data

Table 11: Effect of polyherbal combination on LFT (liver function test) parametersin serum of animal groups.

Groups	Total Bilirubin (mg/dl)	Total Protein (mg/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
NC	0.48±0.04	07.50±0.93	61.82±3.56	33.76±3.25	92.12±4.80
DC	1.38±0.06$^{\#}$	01.58±0.61$^{\#}$	127.30±6.83$^{\#}$	71.39±5.34$^{\#}$	153.67±6.31$^{\#}$
Gm+ME25	0.75±0.05***	04.62±0.72	79.16±5.00***	53.76±3.48***	109.50±5.78**
Gm+ME50	0.75±0.07***	06.50±0.80**	73.98±4.83***	49.69±4.03***	100.89±6.00***
Gm+AE25	0.51±0.04***	08.85±0.68***	53.41±3.89***	24.37±3.22***	86.25±5.31***

Gm+AE50	0.57±0.06***	07.67±0.82***	59.32±3.90***	27.45±3.18***	89.50±5.18***
ME25+Gm	0.65±0.05***	06.26±0.67**	73.72±4.83***	50.88±4.01***	100.07±4.88***
ME50+Gm	0.70±0.07***	07.04±0.70***	69.12±4.76***	46.98±4.00***	100.90±5.04***
AE25+Gm	0.46±0.04***	08.52±0.63***	50.91±3.90***	42.79±3.02***	81.57±4.00***
AE50+Gm	0.53±0.06***	08.01±0.72***	55.20±4.00***	44.65±3.23***	84.59±4.61***

Values are given as Mean±SEM of animal groups (n = 6) and expressed in mg/dl & IU/L. #$p<0.05$ statistical significance against normal control; **$p<0.05$ and ***$p<0.001$ statistical significance against disease control. LFT=Liver Function Test, AST=Aspartate Aminotransferase, ALT=Alanine Aminotransferase, ALP=Alkaline Phosphatase.

The average value of total bilirubin was observed between 0.52±0.04 mg/dl (AE25+Gm) to 1.38±0.06mg/dl (DC) at the end of study in animal groups, which showed statistically significant (***$p<0.001$) difference in comparison to disease control and also all treatment groups showed significant improvement in total bilirubin concentration that was almost comparable to normal control group. In caseof total protein, the observed values varied between 2.58±0.61 mg/dl (DC) to 9.85±0.68 mg/dl (Gm+AE25) of the experimental groups and showed statistically significant (***$p<0.001$) improvement in comparison between these groups. The other methanol extract treated groups also showed a significant (**$p<0.05$) improvement in total protein that was almost comparable to normal control, i.e., 7.50±0.93 mg/dl

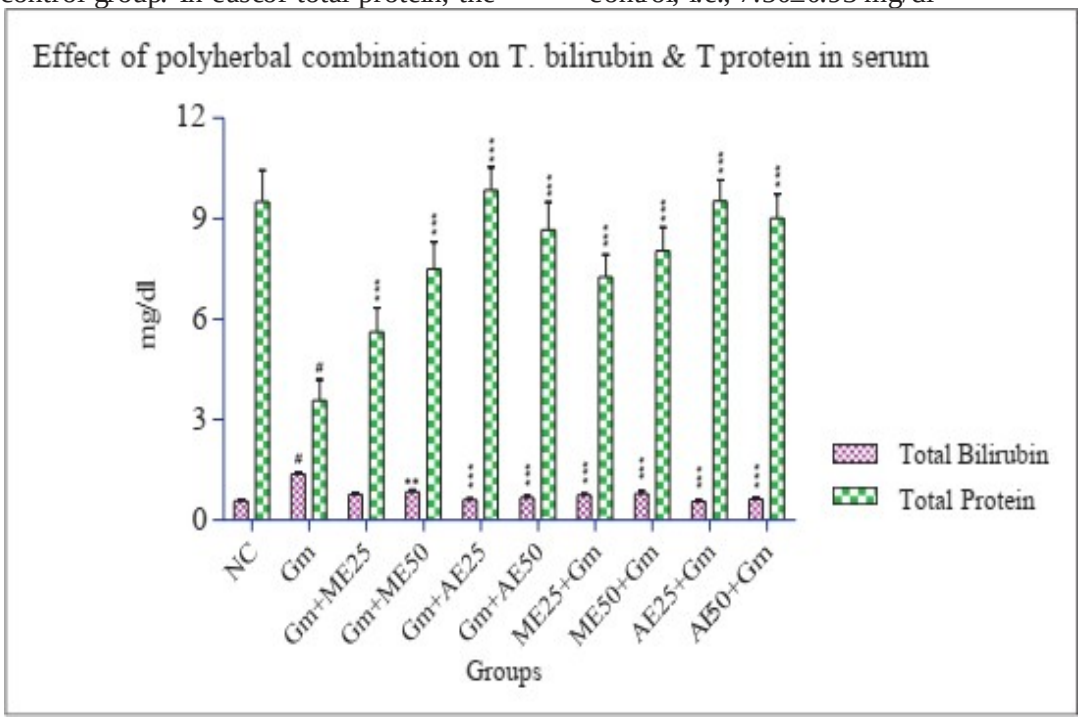

Figure 6: Effect of polyherbal combination on differential leukocyte count in serum

Values are given as Mean±SEM of animal groups (n = 6) and expressed in mg/dl & IU/L. #$p<0.05$ statistical significance against normal control; **$p<0.05$ and ***$p<0.001$ statistical significance against disease control.

The elevated parameter of AST (127.30±6.83 IU/L), ALT (71.39±5.34 IU/L) and ALP (153.67±6.31 IU/L) was observed as increased in the levels with disease control group, which showed statistically significant ($^{\#}p<0.05$) difference in comparison to normal control i.e. AST (51.82±3.56 IU/L), ALT (43.76±3.25 IU/L) and ALP (92.12±4.80 IU/L). The rats treated with low dose of aqueous extract i.e., AST53.41□3.89IU/L), ALT (44.37□3.22IU/L) and ALP (86.25□5.31 IU/L) showed a statistically significant ($^{***}p<0.001$) improvement in these elevated parameters, which was almost comparable to normal control. The pre-treatment with low dose of aqueous extract also showed considerable and statistically significant improvement i.e., AST (50.91□3.90 IU/L), ALT (42.79□3.02 IU/L) and ALP (81.57□4.00 IU/L) in comparison to disease control. The methanol extract treated animal also showedsignificant improvement as compare to DC group.

Conclusion

Kidney is the main target for several toxic xenobiotics including environmental metals, chemicals and drugs. Thus, kidney disorder is the major health challenge in modern society and our society needs urgent solutions. Indian Traditional system of medicine, several formulations and as well single drugs have been used for kidney disease. The hydroalcoholic extract of roots of *Rheum emodi*, and *Boerhaavia diffusa*, bark of *Crataeva nurvala* and flower of *Nelumbo nucifera* has been pharmacologically known for their nephroprotective activity but no information was available for their metabolite profile and mechanism.The present study developed a new polyherbal formulation and defined its complete metabolic profile for its nephroprotective potential. Response surface methodology (RSM) was used for the optimization of extraction conditions to get maximum yield and optimum therapeutic properties. The extract was phytochemically characterized and through randomized block design, a new polyherbal combination was designed. Further, the developed combination was subjected to metabolomics analysis for the identification of bioavailable metabolites present in the formulation.

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