CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://zenodo.org/records/12751404

https://www.iajps.com/volumes/volume11-july-2024/12-issue-07-july-24/

Available online at: http://www.iajps.com *Research Article*

TO DESIGN AND PHARMACOLOGICAL STUDY OF POLYHERBAL COMBINATION FOR THE TREATMENT OF GLOMERULONEPHRITIS

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Abstract:
Glomerulonephritis is a kind of kidney disease. It involves damage to the glomeruli, tiny filters inside your kidneys. It's caused by a variety of things, from infections to problems with the immune system. Sometimes glomerulonephritis is mild and soon goes away. Other times it hangs around and leads to kidney failure and other complications. Glomerulonephritis (GN) is a term used to refer to several kidney diseases (usually affecting both kidneys). Many of the diseases are characterized by inflammation either of the glomeruli or of the small blood vessels in the kidneys, hence the name, [1] but not all diseases necessarily have an inflammatory component.As it is not strictly a single disease, its presentation depends on the specific disease entity: it may present with isolated hematuria and/or proteinuria (blood or protein in the urine); or as a nephrotic syndrome, a nephritic syndrome, acute kidney injury, or chronic kidney disease. They are categorized into several different pathological patterns, which are broadly grouped into non-proliferative or proliferative types. Diagnosing the pattern of GN is important because the outcome and treatment differ in different types. Primary causes are intrinsic to the kidney. Secondary causes are associated with certain infections (bacterial, viral or parasitic pathogens), drugs, systemic disorders (SLE, vasculitis), or diabetes.

Keywords: Angelica officinalis Linn, Diuretic, Polyherbal, Glomerulonephritis.

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Please cite this article in press Dipam Nilkanth Mahale et al., **To Design And Pharmacological Study Of Polyherbal Combination For The Treatment Of Glomerulonephritis.,,** *Indo Am. J. P. Sci, 2024; 11 (07).*

1. INTRODUCTION:

Glomerulonephritis (GN) is a term used to refer to several kidney diseases (usually affecting both kidneys). Many of the diseases are characterized by inflammation either of the glomeruli or of the small blood vessels in the kidneys, hence the name, but not all diseases necessarily have an inflammatory component. As it is not strictly a single disease, its presentation depends on the specific disease entity: it may present with isolated hematuria and/or proteinuria (blood or protein in the urine); or as a nephrotic syndrome, a nephritic syndrome, acute kidney injury, or chronic kidney disease. They are categorized into several different pathological patterns, which are broadly grouped into non-proliferative or proliferative types. Diagnosing the pattern of GN is important because the outcome and treatment differ in different types. Primary causes are intrinsic to the kidney. Secondary causes are associated with certain infections (bacterial, viral or parasitic pathogens), drugs, systemic disorders (SLE, vasculitis), or diabetes.

Signs and symptoms of glomerulonephritis may vary depending on whether you have the acute or chronic form and the cause. You may notice no symptoms of chronic disease. Your first indication that something is wrong might come from the results of a routine urine test (urinalysis).

Glomerulonephritis signs and symptoms may include:

- Pink or cola-colored urine from red blood cells in your urine (hematuria).
- Foamy or bubbly urine due to excess protein in the urine (proteinuria).
- High blood pressure (hypertension).
- Fluid retention (edema) with swelling evident in your face, hands, feet and abdomen.
- Urinating less than usual.
- Nausea and vomiting.
- Muscle cramps.
- Fatigue.

Causes

Many conditions can cause glomerulonephritis. Sometimes the disease runs in families and sometimes the cause is unknown. Factors that can lead to inflammation of the glomeruli include the following conditions.

Infections

Infectious diseases can directly or indirectly lead to glomerulonephritis. These infections include:

Post-streptococcal glomerulonephritis. Glomerulonephritis may develop a week or two after recovery from a strep throat infection or, rarely, a skin infection caused by a streptococcal bacteria (impetigo). Inflammation occurs when antibodies to the bacteria build up in the glomeruli. Children are more likely to develop post-streptococcal glomerulonephritis than are adults, and they're also more likely to recover quickly.

Bacterial endocarditis. Bacterial endocarditis is an infection of the inner lining of your heart's chambers and valves. It isn't clear whether the inflammation in the kidneys is the result of immune system activity alone or other factors.

Viral kidney infections. Viral infections of the kidney, such as hepatitis B and hepatitis C, cause inflammation of the glomeruli and other kidney tissues.

HIV. Infection with HIV, the virus that causes AIDS, can lead to glomerulonephritis and progressive kidney damage, even before the onset of AIDS.

2. MATERIALS AND METHODS:

COLLECTION, AUTHENTICATION AND EXTRACTION

Collection and authentication of plant material

Herb authentication is a quality assurance process that ensures the correct plant species and plant parts are used as raw materials for herbal medicines. The proper authentication of herbal raw materials is critically important to the safety and efficacy of herbal medicines.

Angelica officinalis Linn. Carica papaya Linn., Apis Melifica Linn. Were purchased from local market and authenticated in botanical department by botanist.

Extraction of plants material

Extraction Method

Grinding Mill:-

A mill is a device that breaks solid materials into smaller pieces by grinding, crushing, or cutting. Such comminution is an important unit operation in many processes. There are many different types of mills and many types of materials processed in them.

Soxhlet Extraction:-

Soxhlet extraction is a continuous solid / liquid extraction. A solid which contains the material to be

extracted is placed in what is called a thimble. A thimble is made out of a material which will contain the solid but allow liquids to pass through. A lot like filter paper. The thimble containing the material is placed in the Soxhlet extractor. An organic solvent is then heated at reflux. As it boils its vapors rise up and are condensed by a condensor.

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[188].

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- tight containers

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. H2SO4 was added. The formation of a reddish brown ring confirmed the presence of terpenes

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

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

Test for steroids (Liebermann–Burchard reaction): 2ml of acetic anhydride and 2 ml conc. H2SO4 was added into 5ml of the extract in a test tube. Change of colour from violet to blue confirms the presence of steroids

Test for glycosides: 2ml of glacial acetic acid containing one drop of ferric chloride solution and 1 ml of conc. H2SO4 was added into 5ml of the extract in a test tube. The appearance of a brown ring indicates the presence of glycosides

Test for proteins (Biuret test): 4% of NaOH and few drops of 1% CuSO4 solution were added into 3ml of the extract in a test tube. Formation of violet or pink color indicates the presence of proteins

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

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. H2SO4 is slowly added down the sides of the sloping test-tube, without mixing, to form a layer. Violet ring is formed at the interface between the acid and test layers

Test for tannin and phenol (Ferric Chloride Test): 3ml of extract, 3ml of 5% w/w of the FeCl3 solution was added in a test tube. The blue-black colour indicates the presence of tannins and phenols

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.

• **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 potassium iodide) 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 (potassium bismuth iodide) was added in a test tube. The appearance of red brick precipitate indicates the presence of alkaloids.

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.

Kidney weight: The animal kidney weight was measured using digital weighing balance of individual groups (n=6) separately at the end of study.

Determined by auto analyzers using standard test kits.

Table 1: Preparation of working reagent

Volume of working reagent	Add	
	Reagent 1	Reagent 2
10 ml	10 ml	0.2 ml
25 ml	25 ml	0.5 ml
50 ml	50 ml	1.0 ml
100 ml	100 ml	2.0 ml

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.

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.

Table 2: The assay procedure for measuring total cholesterol was;

Pipette into tubes marked	Blank	Standard	Test
Working reagent	1000µl	1000µl	1000µl
Distilled Water	10µl	-	-
Standard	-	10µl	-
Test Sample	-	-	10µl

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

T. cholesterol (mg/dl) = Abs. of test / Abs. of standard λ_{max} Concentration of std. (mg/dl)

Triglycerides: GPO-Trinder method, end point method[207]. The required reagents are; Reagent 1- ATP (2.5 mM/L), Mg2+ (2.5 mM/L), 4-amino antipyrine (0.8 mM/L), 3-5-dichloro-2-hydroxibenzine (1 mM/L).

Table 3: The assay procedure for measuring triglycerides was;

Pipette into tubes marked	Blank	Standard	Test
Working reagent	1000µl	1000µl	1000µl
Distilled Water	10µl	-	-
Standard	-	10µl	-
Test Sample	-	-	10µl

Each reagent were mixed and incubated for 10 min at 37°C. The absorbance for test and standard was recorded against blank at λ_{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).

Table 4: The assay procedure for measuring LDL and HDL was;

Pipette into tubes marked	Blank	Standard	Test
Working reagent 1	450µl	450µl	450µl
Cholesterol calibrator	-	10µl	-
Test Sample	-	10µl	10µl
Working reagent II	150µl	150µl	150µl

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

Estimation of urea and BUN: GLDH-Urease method The required reagents 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 5: The assay procedure for measuring BUN and urea was;

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

Working reagent mixed well and aspirated with standard followed by samples at the reaction temperature 37°C. The absorbance was recorded at wavelength λ_{max} 340 nm for determination of absorbance changes (A) and calculated as;

Estimation of albumin: BCG dye method, end point method[210]. The required reagents are; Reagent 1- Bromocresol green (0.08 mM/L), Sodium azide (1 g/L), Succinate buffer pH 4.2 (50 mM/L), Surfactant (qs) and Reagent 2- Albumin (3.6 g/dl) used as standard.

Estimation of creatinine: Modified Jaffe's method The required reagents are; Reagent 1- Picric acid (40 mM/L), Sodium hydroxide (200 mM/L), Preservative and stabilizer (qs) and Reagent 2- Creatinine (2 mg/dl) used as standard.

Estimation of uric acid: Modified Trinder method[212]. The required reagents were; Reagent 1- 4-amino antipyrine (0.5 mM/L), 2,4,6-tribromo-3-hydroxy benzoic acid (1.75 mM/L), Uricase (>120 U/L), Peroxidase (>500 U/L) Tries buffer pH 8.25 (50 mM/L) and Reagent 2- uric acid (6 mg/dl) used as standard.

Estimation of Calcium (Ca2+) was estimated by o-Cresolphthalein complexone method using Autozyme calcium diagnostic reagent kit. Calcium forms a purple colour complex with cresolphthalein complexone in alkaline medium. This complex absorbs light at 575 nm. The intensity of the colour is directly proportional to calcium concentration in sample

Antioxidant enzyme parameters: The homogenate of kidney tissues was prepared by using homogenizer; sample was stored at 4°C for estimation of following enzymes like SOD, CAT, MDA, GSH, LPO and NO.

Table 6: The assay procedure for measuring SOD was;

Pipette into tubes marked	Test	Control
Tris Buffer	2.85 ml	2.9 ml
Pyragallol	0.1 ml	0.1 ml
Hemolysate sample	0.5 ml	-

Estimation CAT: Aebi H method The decreased absorbance was measured at 240 nm for every 15 seconds interval up to 1 min and the difference in absorbance per unit time was measured. The required reagents are; Phosphate buffer pH 7.4 (60mM), KH2PO4 (6.81 g), Na2HPO4 (8.90 g), H2O2 (19 mM/L).

Table 7: The assay procedure for measuring CAT was;

Pipette into tubes marked	Test	Control
Phosphate buffer	3 ml	3 ml
H2O2	1 ml	1 ml
Hemolysate sample	10 ml	-

Each reagent were mixed well and the initial absorbance was measured immediately at wavelength λ_{max} 420 nm,

FORMULATION AND DEVELOPMENT OF DISPERSIBLE TABLET

Both methanol and aqueous extracts of polyherbal combination were tested for glomerulonephritis activity in animal and as methanol extract has not shown desirable activity so that further studies were made on aqueous extract only.

Characteristics of extract powder

Dried root extract powders were found as heterogeneous because it composed of characteristic particles of different sizes and shapes randomly interspersed with air spaces, so that it seems to be complicated in case of polyherbal extracts powder to determine the rheological properties

Angle of repose

The flow properties of a mixture of polyherbal extracts powder were determined by calculating the angle of repose by the fixed height method. A funnel with 10mm diameter at bottom was fixed at the height of 2cm over the plain and smooth surface.

Bulk density
The bulk densities (BD) of polyherbal powder mixture were determined by pouring gently 25gm of sample mixture with the help of a glass funnel into a 100ml graduated cylinder. The initial volumes occupied by the sample were recorded.

Tapped density
The tapped densities (TD) of polyherbal powder mixture were determined by pouring gently 25gm of sample mixture through a glass funnel into a 100ml graduated cylinder.

Compressibility
The Carr's compressibility of the polyherbal powder mixture was calculated by comparing the bulk density and tapped density which gives a useful empirical direction.

Hausner's ratio
It also indicates the degree of densification of polyherbal powder mixture,

PREPARATION OF POLYHERBAL DISPERSIBLE TABLET

Table 8: Preparation of Polyherbal dispersible tablets

Ingredients (mg/tab)	PHF-1	PHF-II	PHF-1II	PHF-1V	PHF-V	PHF-VI	PHF-VII	PHF-VIII	PHF-IX
Aqueous extracts Powder (3 plants)	25X3 = 75	25X3 = 75	25X3 = 75	25X3 = 75	25X3 = 75	25X3 = 75	25X3 = 75	25X3 = 75	25X3 = 75
β-cyclodextrin	40	40	40	40	40	40	40	40	40
Sod. Starch glycolate	-	-	-	10	15	20	-	-	-
Crospovidone	10	15	20	-	-	-	-	-	-
Croscarmellose sodium	-	-	-	-	-	-	10	15	20
Microcrystalline cellulose	35	30	25	35	30	25	35	30	25
Sodium Saccharin	5	5	5	5	5	5	5	5	5
Mg. Stearate	3	3	3	3	3	3	3	3	3
Talc	2	2	2	2	2	2	2	2	2
Total Weight	170	170	170	170	170	170	170	170	170

EVALUATION OF POLYHERBAL DISPERSIBLE TABLET

Weight variation test

The weight variation of tablets was carried out to ensure that, each of the tablets contains the proper amount of drug.

Hardness test

The hardness of the tablet is defined as the loads required for crushing or fracture a tablet by placing on its edge.

Friability test

The friability test was performed by using tablet friability tester (Veego).

Drug content uniformity test

From each formulation, 20 tablets were taken, weighed and thoroughly triturated. and the absorbance was taken at the wavelength of λmax 200-400nm by using double beam UV spectrophotometer

Disintegration time

From each formulation polyherbal dispersible tablets were randomly selected to determine the disintegration time.

Dispersion time

In-vitro dispersion time of polyherbal dispersible tablet was measured by dropping the tablet in a beaker containing 100ml of normal water.

In-vitro dissolution study

In the regulations for dissolution testing of herbal medicines particularly difficult to oversee authority requirements for dissolution testing of herbal medicines due to widely varying regulations.

Accelerated stability study

Stability study was carried out as per ICH guidelines for polyherbal combination to check the physical, chemical and physiological property of prepared formulation in a short period of time.

3. RESULT AND DISCUSSION:

PHYTOCHEMICAL SCREENING

Table 9: Phytoconstituents present in methanol root extracts of polyherbal plants

Sample	*Boerhavia diffusa*	*Allium Cepa*	*Apis Melifica*
Carbohydrate	+	+	+
Proteins	+	-	+
Lipids	-	+	-
Steroids	+	-	+
Glycosides	+	+	+
Coumarins	-	+	-
Saponins	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+
Tannins	+	+	+
Phenols	+	+	-
Anthraquinones	+	-	-

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

PHARMACOLOGICAL SCREENING

Physical parameters of animal groups

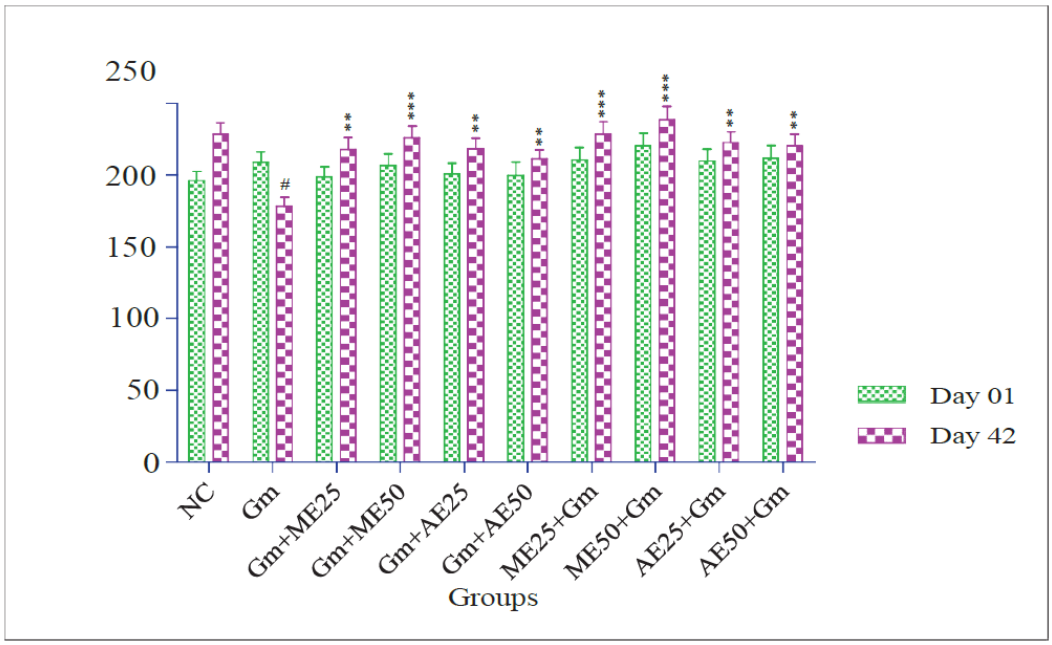
The effect of the polyherbal combination on physical parameters, body weight of all animal groups were observed, while 24 h urinary volumes at first and last day was observed and the kidney weight was recorded at the end of study

Table 10: Effect of polyherbal combination on physical parameters of animal groups

Groups	Body weight (g)		Kidney weight at the end of study (g)		24h Urinary volume (ml)	
	Day 01	Day 42	Left kidney	Right kidney	Day 01	Day 42
NC	196.40±6.23	228.32±8.10	0.663±0.12	0.645±0.05	18.8±1.28	19.6±1.08
DC	208.72±7.35	178.50±6.15$^{\#}$	0.985±0.11$^{\#}$	0.784±0.07	20.6±1.22	14.8±1.04$^{\#}$
Gm+ME25	198.80±7.02	218.06±8.03**	0.886±0.03	0.778±0.04	20.0±2.01	23.0±1.64**
Gm+ME50	206.63±8.12	226.03±8.00***	0.945±0.05	0.662±0.06	19.8±1.48	23.7±1.60**
Gm+AE25	200.81±7.28	218.42±7.28**	0.701±0.07	0.778±0.05	20.5±1.88	22.4±1.90**
Gm+AE50	199.62±9.24	211.46±6.35**	0.693±0.05	0.680±0.07	19.2±2.00	23.6±1.50**
ME25+Gm	210.46±8.75	228.27±8.83***	0.704±0.10	0.742±0.08	21.0±1.20	22.8±1.25**
ME50+Gm	220.28±8.86	238.68±9.18***	0.766±0.08	0.782±0.07	22.0±1.80	23.7±1.76**
AE25+Gm	209.71±8.46	222.62±7.56**	0.605±0.06	0.628±0.08	21.8±1.88	22.4±2.02**
AE50+Gm	212.08±8.35	220.39±7.83**	0.698±0.06	0.782±0.05	22.2±2.00	24.0±1.86**

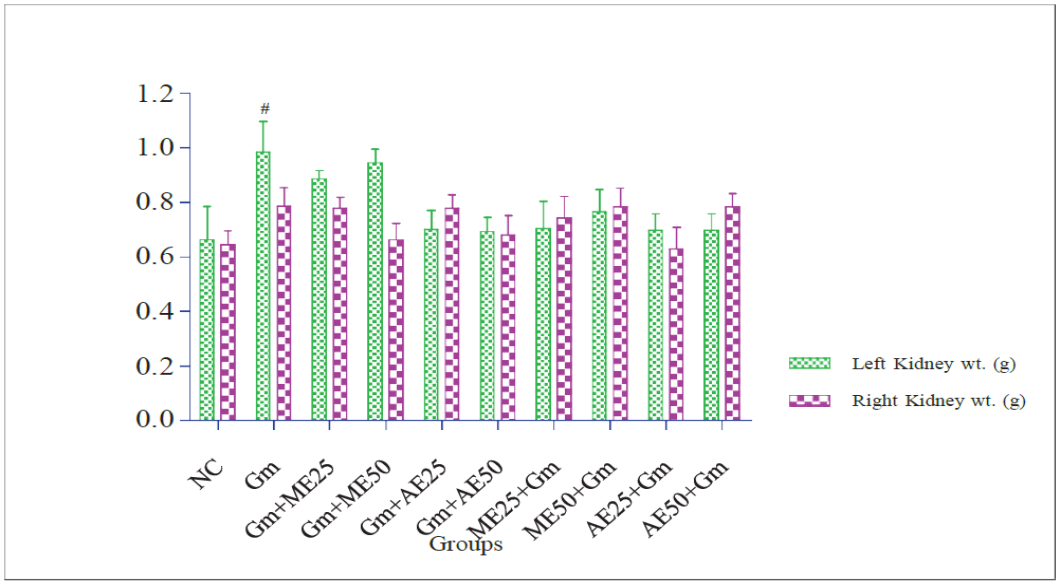
Values are given as Mean±SEM of animal groups (n = 6) and expressed in g & ml. #p±0.05 statistical significance against normal control; **p±0.05 and ***p±0.001 statistical significance against disease control.

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



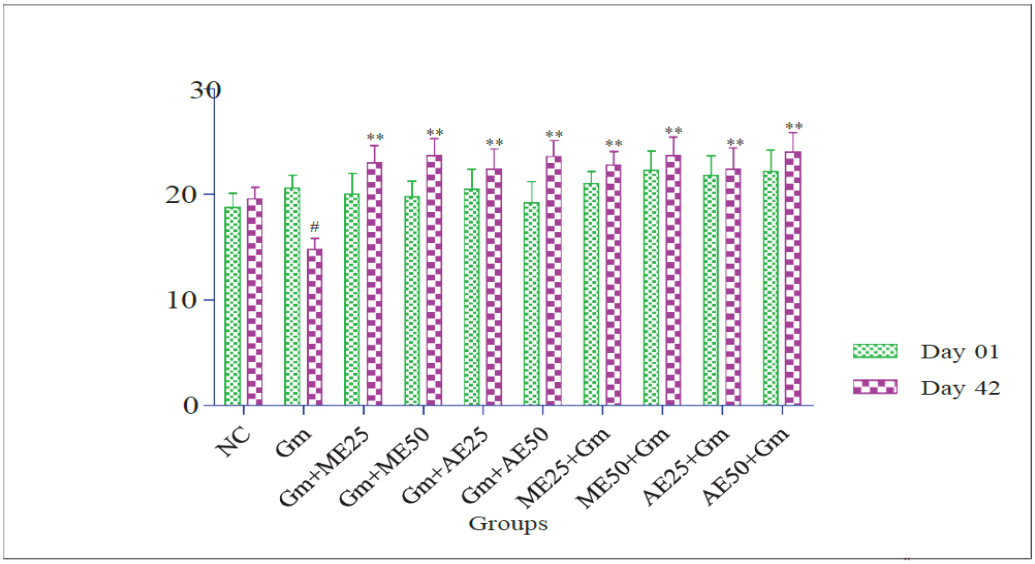
Values are given as Mean±SEM of animal groups (n = 6) and expressed in gm. $^{\#}p0.05$ statistical significance against normal control; $^{**}p\pm0.05$ and $^{***}p\pm0.001$ statistical significance against disease control.

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. $^{\#}p\pm0.05$ statistical significance against normal control and $^{**}p\pm0.05$ statistical significance against disease control.

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



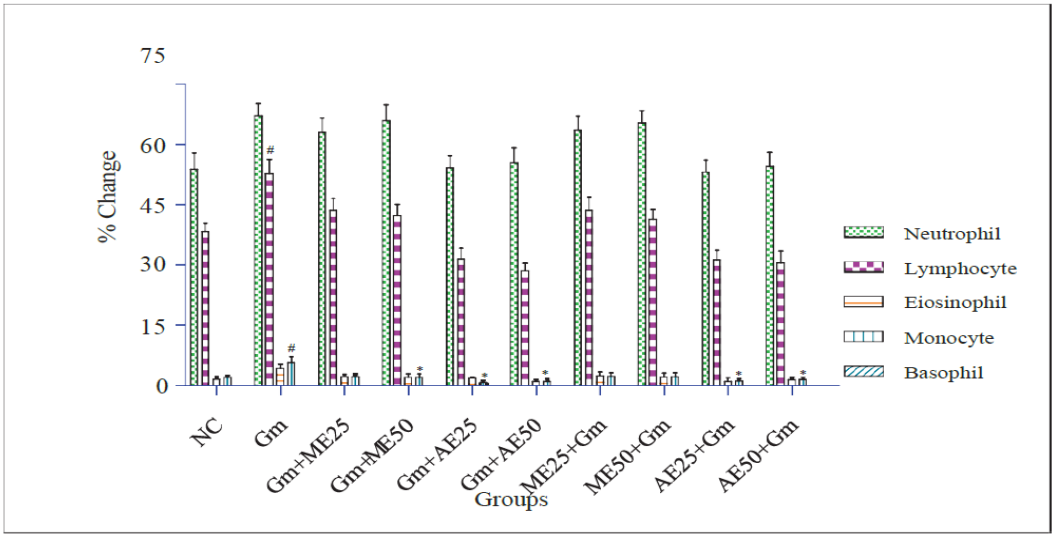
Values are given as Mean±SEM of animal groups (n = 6) and expressed in ml. $^{\#}p\pm0.05$ statistical significance against normal control and $^{**}p\pm0.05$ statistical significance against disease control.

Hematological parameters of animal groups

Table 11: Effect of polyherbal combination on hematological parameters

Groups	Hb (g/dl)	CBC ($x10^3$/µl)	Differential leukocyte count (%)				
			Neutrophil	Lymphocyte	Eiosinophil	Monocyte	Basophil
NC	13.11±1.86	6.80±2.15	53.83±4.12	38.33±2.15	1.50±0.80	2.02±0.60	00
DC	10.80±1.64	5.00±1.36	67.16±2.99	52.64±3.63$^{\#}$	4.28±1.08	5.66±1.58$^{\#}$	00
Gm+ME25	11.18±1.67	5.83±1.58	63.00±3.61	43.50±3.08	2.23±0.63	2.19±0.76	00
Gm+ME50	11.13±1.57	6.00±2.00	65.83±4.02	42.33±2.75	2.00±0.88	2.02±0.86**	00
Gm+AE25	12.92±1.54	6.98±2.16	54.16±2.99	31.56±2.63**	1.88±0.28	0.86±0.45**	00
Gm+AE50	12.90±1.66	6.93±2.01	55.50±3.61	28.50±1.88**	1.00±0.45	1.00±0.73**	00
ME25+Gm	11.15±1.37	5.93±1.51	63.50±3.61	43.50±3.38	2.39±1.03	2.28±0.87	00
ME50+Gm	11.16±1.47	6.11±2.02	65.33±3.02	41.33±2.45	2.08±0.99	2.20±0.96	00
AE25+Gm	12.89±1.94	6.90±2.16	53.16±3.00	31.33±2.38**	1.06±0.90	1.16±0.58**	00
AE50+Gm	12.60±1.77	6.53±2.11	54.50±3.60	30.50±3.00**	1.39±0.73	1.45±0.61**	00

Figure 4: Effect of polyherbal combination on differential leukocyte count in Serum



Values are given as Mean±SEM of animal groups (n = 6) and expressed in % change. $^{\#}p\pm0.05$ statistical significance against normal control and $^{**}p \pm 0.05$ statistical significance against diseasecontrol.

LFT parameters of animal groups

Table 12: The effect of the polyherbal combination on LFT (liver function test)

Groups	Total Bilirubin (mg/dl)	Total Protein (mg/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
NC	0.58±0.04	09.50±0.93	61.82±3.56	33.76±3.25	92.12±4.80
DC	1.38±0.06$^{\#}$	03.58±0.61$^{\#}$	137.30±6.83$^{\#}$	81.39±5.34$^{\#}$	143.67±6.31$^{\#}$
Gm+ME25	0.77±0.05***	05.62±0.72	89.16±5.00***	43.76±3.48***	119.50±5.78**
Gm+ME50	0.85±0.07***	07.50±0.80**	83.98±4.83***	39.69±4.03***	110.89±6.00***
Gm+AE25	0.61±0.04***	09.85±0.68***	63.41±3.89***	34.37±3.22***	96.25±5.31***
Gm+AE50	0.67±0.06***	08.67±0.82***	69.32±3.90***	37.45±3.18***	99.50±5.18***
ME25+Gm	0.75±0.05***	07.26±0.67**	83.72±4.83***	40.88±4.01***	111.07±4.88***
ME50+Gm	0.80±0.07***	08.04±0.70***	79.12±4.76***	36.98±4.00***	104.90±5.04***
AE25+Gm	0.56±0.04***	09.52±0.63***	60.91±3.90***	32.79±3.02***	91.57±4.00***
AE50+Gm	0.63±0.06***	09.01±0.72***	65.20±4.00***	34.65±3.23***	94.59±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

Figure 5: Effect of polyherbal combination on LFT parameters (AST, ALT and ALP)

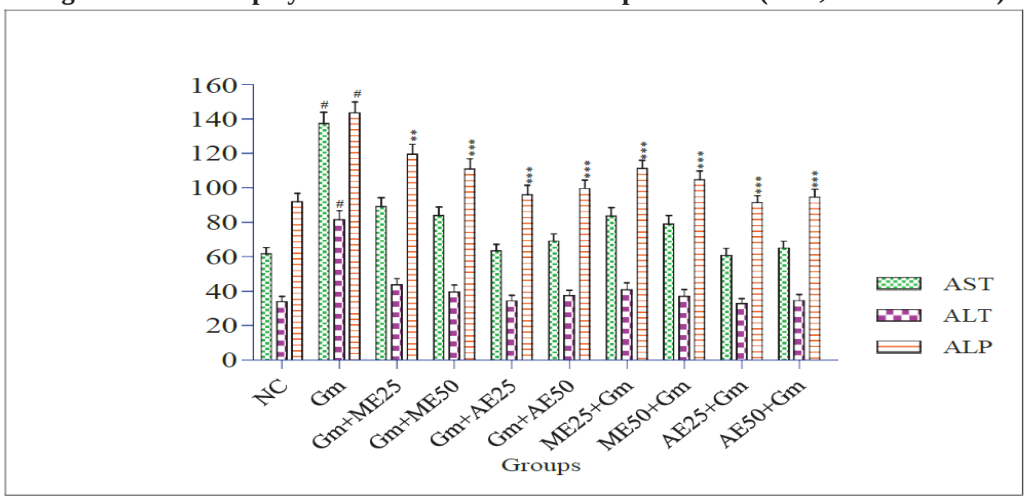
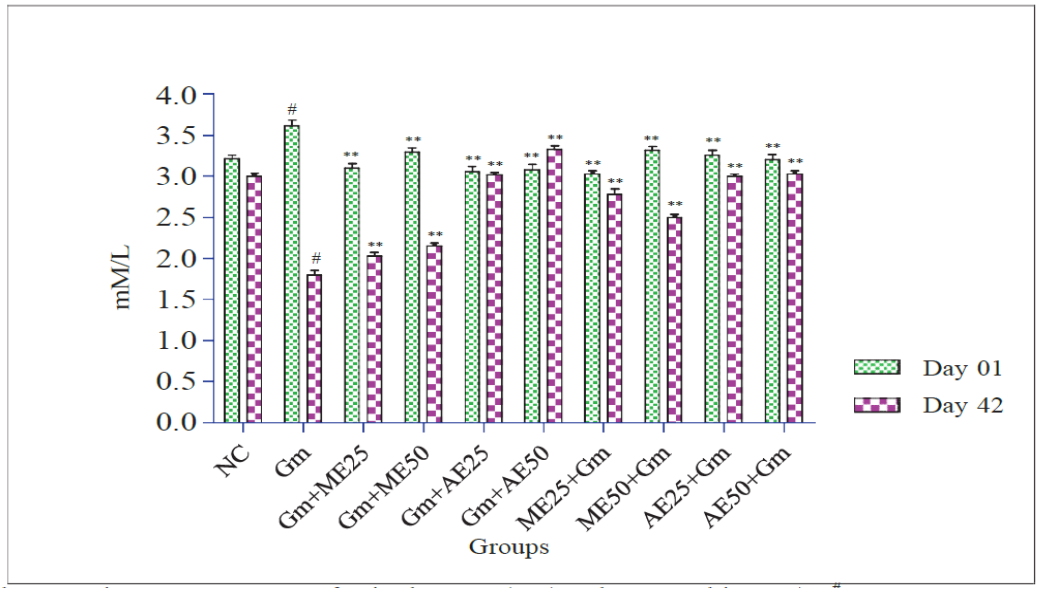
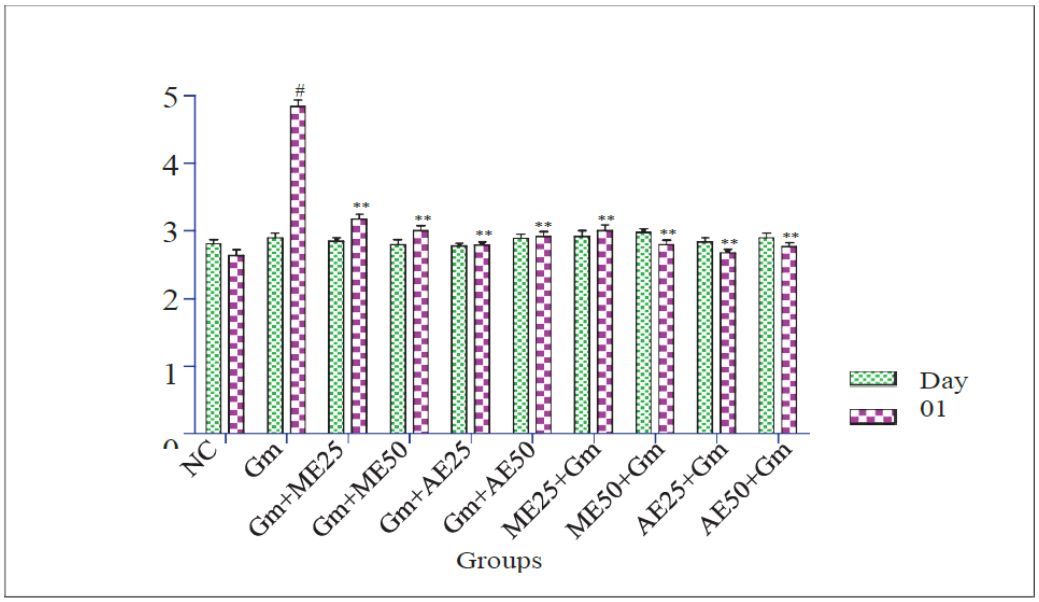


Figure 6: Effect of polyherbal combination on total cholesterol in serum of animalgroups



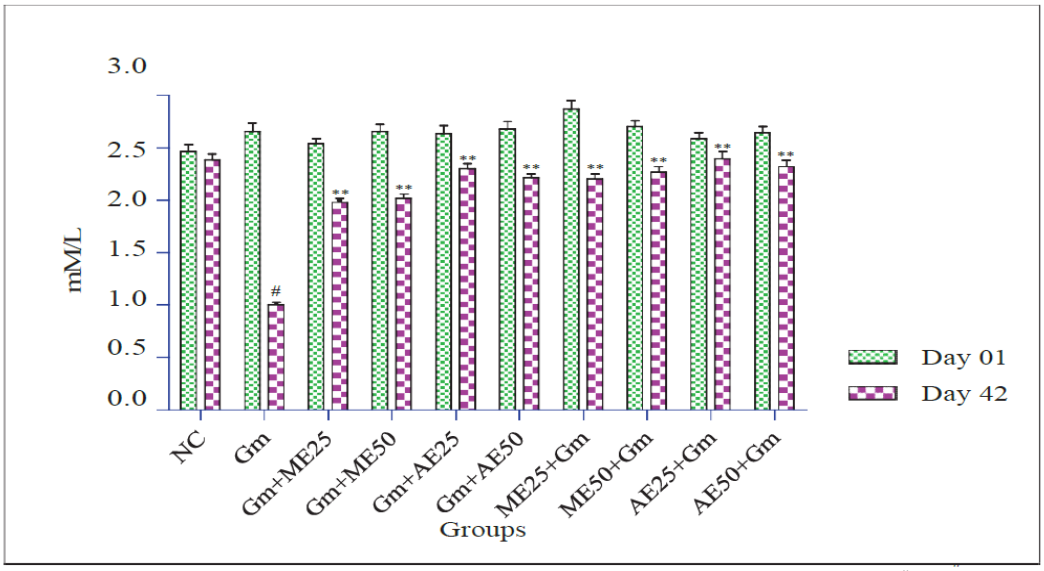
values are given as Mean±SEM of animal groups (n=6) and expressed in mM/L. $^{\#}p\pm0.05$ statistical significance against normal control and $^{**}p\pm0.05$ statistical significance against disease control.

Figure 7: Effect of polyherbal combination on triglycerides in serum of animal groups



Values are given as Mean±SEM of animal groups (n=6) and expressed in mM/L. #p±0.05 statistical significance against normal control and **p±0.05 statistical significance against disease control

Figure 8: Effect of polyherbal combination on low density lipoprotein (LDL) inserum



Values are given as Mean±SEM of animal groups (n=6) and expressed in mM/L. $^{\#}p\pm0.05$ statistical significance against normal control and $^{**}p\pm0.05$ statistical significance against disease control; LDL=Low Density Lipoprotein.

Urinary parameters (protein, albumin, urea, creatinine and uric acid)

Table 13: Effect of polyherbal combination on urinary parameters in urine sample

Groups	U. protein (mg/24h)	Albumin(mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid(mg/dl)
NC	3.30±0.38	06.55±1.07	1.96±0.09	40.02±2.01	125.32±3.20
DC	8.36±1.01$^{\#}$	32.15±3.84$^{\#}$	0.42±0.04$^{\#}$	16.25±1.07$^{\#}$	35.28±2.02$^{\#}$
Gm+ME25	4.35±0.28***	19.83±2.23***	1.27±0.06	27.62±0.36***	108.67±3.33***
Gm+ME50	4.50±0.32***	15.20±1.88***	1.63±0.12**	31.83±0.45***	110.45±3.38***
Gm+AE25	2.96±0.24***	09.23±1.11***	2.80±0.34***	38.28±0.75***	126.08±4.12***
Gm+AE50	3.28±0.45***	12.59±2.02***	2.71±0.41***	41.98±0.94***	118.16±3.50***
ME25+Gm	4.00±0.33***	13.56±2.00***	1.56±0.12**	28.02±0.67***	107.00±3.10***
ME50+Gm	3.90±0.08***	10.00±1.17***	1.78±0.25**	32.19±0.66***	109.34±3.30***
AE25+Gm	2.66±0.07***	05.39±1.00***	2.87±0.42***	39.35±0.54***	124.89±3.62***
AE50+Gm	2.88±0.08***	08.34±1.02***	2.80±0.45***	44.20±0.47***	115.63±3.00***

Significance against normal control; $^{**}p\pm0.05$ and $^{***}p\pm0.001$ statistical significance against disease control

POLYHERBAL FORMULATION AND EVALUATION

Characterization of extract powder

Table 14: The micromeritic properties of polyherbal aqueous root extracts

Formulation	Bulk density (gm/ml)	Tapped density (gm/ml)	% Compressibility	Hausner's ratio	Angle of repose
PHF1	0.38±0.05	0.53±0.08	28.30	1.39±0.14	26.48±1.02
PHF2	0.42±0.04	0.55±0.06	21.82	1.31±0.11	26.24±1.32
PHF3	0.35±0.03	0.49±0.07	28.57	1.40±0.13	24.35±1.00
PHF4	0.40±0.06	0.50±0.06	20.00	1.25±0.09	30.20±2.01
PHF5	0.45±0.04	0.56±0.08	19.64	1.24±0.10	32.12±1.82
PHF6	0.38±0.07	0.50±0.07	24.00	1.32±0.08	26.50±1.22
PHF7	0.43±0.06	0.52±0.05	17.31	1.21±0.09	27.20±1.65
PHF8	0.42±0.05	0.57±0.08	26.32	1.36±0.12	28.32±1.37
PHF9	0.40±0.04	0.54±0.06	25.93	1.35±0.14	28.22±1.56

All values are reported as mean±SD, n=3 measurements, PHF=Polyherbal formulation

Formulation and characterization of tablet

Table 15: characterization of tablet

Sr. No.	Parameter	Result
1.	Color	Yellowish-Brown
2.	Shape	Round, Biconvex
3.	Odor	Characteristic odor
4.	Taste	Pleasant taste
5.	Size in mm	
	i. Thickness	5.12±0.08 mm
	ii. Diameter	12.17±0.01 mm

Table 16: Physical properties of polyherbal dispersible tablets

Formulation Code	Average weight (mg)	Weight variation (%)	Content uniformity (%)	Hardness (kg/cm^2)	Friability (%)
PHF-1	563.15	±2.34	102.38	2.98±0.13	0.90
PHF-2	562.40	±2.20	101.25	2.91±0.09	0.82
PHF-3	563.28	±2.36	105.05	2.99±0.14	0.79
PHF-4	565.31	±2.71	103.10	3.00±0.12	0.86
PHF-5	560.80	±1.93	099.85	2.94±0.13	0.90
PHF-6	563.63	±2.42	101.80	2.95±0.12	0.80
PHF-7	558.34	±1.22	098.96	2.96±0.16	0.85
PHF-8	563.18	±2.34	102.00	3.02±0.18	0.78
PHF-9	564.13	±2.50	099.68	2.97±0.11	0.88

Disintegration time and Dispersion time

Table 17: Disintegration and dispersion time of the polyherbal dispersibletablet

FormulationCode	Disintegration Time (Min)	Dispersion Time (Min)
PHF-1	02.08±0.62	3.18±0.82
PHF-2	01.45±0.28	2.30±0.60
PHF-3	01.10±0.10	2.00±0.45
PHF-4	02.00±0.45	3.00±0.78
PHF-5	02.18±0.51	2.50±0.65
PHF-6	01.55±0.60	2.24±0.58
PHF-7	02.15±0.55	3.25±0.80
PHF-8	02.06±0.70	2.55±0.71
PHF-9	01.50±0.58	3.00±0.82

Stability study

Table 18: Stability data of the polyherbal dispersible tablet (PHF-3)

Time	% Drug content at different storage conditions		
	25°C & 60% RH	30°C & 65% RH	40°C & 75% RH
1 month	98.92	99.10	99.30
2 months	99.20	98.86	98.92
3 months	99.34	98.95	97.67

4. SUMMARY AND CONCLUSION:

The phytoconstituents are playing a significant role in restoring elevated biochemical parameters which affecting the normal biological function. Carbohydrate is the primary source of energy play an important role in protecting blood vessels while protein plays a beneficial role in the maturation of nephron because of maintaining the levels of dyslipidemia and protects the damage of glomerular cells of the kidney as supported by The steroids are strengthening of nephron and protect the damage of glomerular cells in IgA nephropathy and may prevent the loss of kidney function as supported by Nisha,, while glycosides maintain the cellular transport of sodium and potassium in kidney tissues, resulting in the enhancement of renal tubular function. Coumarins and saponins possess a good anti-inflammatory with antioxidant effect, playing a protective role in acute kidney disease, which is also reported by. Flavonoids and alkaloids protect the kidney cells and maintain renal function as well as boosting blood pressure. Terpenoids and tannins are potent therapeutic agents help to prevent nephrotoxicity observed The glomerulonephritis was induced by i.p. administration of gentamicin in animal that causes selective accumulation in the glomerular cells (renal cortex and proximal tubules) which leads to inflammation, lesions of proximal tubules, apoptosis and necrosis of tubular cells. The damaged kidney cells have considered for the decreased in body weight, reduced renal blood flow and decreased GFR that sensitizes tubule cells leads to cell death by reduction of oxygen and ATP availability.

5. ACKNOWLEDGEMENT

The authors are thankful to the Principal, Mansarovar Global University, Sehore M.P, India. Necessary facilities for research work.

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