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FORMULATION AND PHARMACOLOGICAL STUDY OF POLYHERBAL COMBINATION FOR THE TREATMENT OF GLOMERULONEPHRITIS.

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Abstract:
The study revealed that the methanol and aqueous root extracts of polyherbal combination possess several potent phytoconstituents observed in the methanol extract showed the presence of carbohydrates, steroids, glycosides, coumarins, saponins, flavonoids, alkaloids, tannins and phenols approximately in all plants. Whereas proteins and anthraquinones were reported in few plants and none of the plant showed the presence of lipids. The aqueous extracts also showed the presence of carbohydrates, steroids, glycosides, saponins, flavonoids and tannins approximately in all plants. Whereas, coumarins, phenols and anthraquinones reported in few plants only and none of the plat showed the presence of proteins, lipids and alkaloids. The phytoconstituents are playing a significant role in restoring elevated biochemical parameters which affecting the normal biological function. Carbohydrate is theprimary 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, 2017[239], while glycosides maintain the cellular transport of sodium and potassium in kidney tissues, resulting in the enhancement of renal tubular function.

Keywords: *Glomerulonephritis, phytoconstituents, Rheumatoid arthritis.*

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INTRODUCTION:

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.

- The reason glomerulonephritis appears is often unknown. But causes may include:
- Genetics, meaning it runs in the family (this is rare).
- Anti-GBM disease (formerly Goodpasture syndrome), a group of diseases affecting the lungs and kidneys.
- Secondary to endocarditis, an infection in the heart valves.
- Secondary to other viral infections, such as strep throat, HIV or hepatitis C.
- Problems with the immune system attacking healthy parts of the body, such as with lupus.
- Rare diseases that inflame blood vessels like granulomatosis with polyangiitis (formerly Wegener's disease), microscopic polyangiitis, Henoch-Schönlein Purpura, or eosinophilic granulomatosis with polyangiitis (formerly Churg-Srauss Syndrome).

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 become impaired once urinary functions decline due to some ill condition whether have no direct correlation with the pathophysiology 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.

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.

Boerhavia diffusa Linn., Allium Cepa Linn., Apis Melifica Linn. were purchased from local market and authenticated in botanical department by botanist.

Extraction of plants material:

Extraction Method:

a. 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.

b. 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[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 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.

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.

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

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

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

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 store house.
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)

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.

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 total protein: Modified Burette, end point assay method[203]. The required 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 2: The assay procedure for estimation of total protein was

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

Each ingredient were thoroughly mixed and incubated for 5 min at 37°C. The absorbance for each test and standard sample were recorded at λ_{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 3: The assay procedure for estimation of AST was;

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

Each reagents were mixed well and aspirated immediately for recording of absorbance after 1 min at wavelength λ_{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 4: The assay procedure for estimation of ALT was;

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

Each reagents were mixed well and aspirated immediately for recording of absorbance after 1 min at wavelength λ_{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 ml of reagent 1 and swirled to dissolve with gentle shaking and allowed to attain at 8°C.

Table 5: The assay procedure for estimation of ALP was;

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

Each reagent were mixed well and aspirated immediately for recording of absorbance after 30 sec at wavelength λ_{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 6: 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

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 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), 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 7: 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). 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 8: 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

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 2 LDL = TC – (HDL + VLDL); Where, VLDL = TG / 5
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 9: 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;
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)
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.
The reagents were mixed well. The initial absorbance of the standard was recorded after 30 seconds (AS1) and the final absorbance was recorded after an interval of 120 seconds (AS2) at 505 nm. Then absorbance reading for test was recorded accordingly at 37°C and calculated as;
Creatinine (mg/dl) = AT2-AT1 / AS2-AS1 □ 2
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.
Urinary parameters: Urinary protein, albumin, urea, creatinine and uric acid were measured using urine depiction test kit assay method. The Cystitis test kit (Urinalysis, Reagent Strips, Mission©) was used for measuring these parameters by matching the colour changes at particular time interval mentioned on strip kit
Electrolyte concentrations in serum and urine sample:
The blood was withdrawn and serum immediately separated, while the urine samples were freshly collected and then following test was performed for Sodium (Na+), Potassium (K+) and Calcium (Ca2+) were considered for the study.

Estimation of Sodium (Na+) and Potassium (K+):
Estimated by flameless ion-selective electrode method by using KNA2 Sodium-Potassium analyzer (Copenhagen and Denmark Radiometer). The concentration of Na+ and K+ was measured in serum after centrifugation of blood and in urine directly sipping into electrolyte analyzer (Auto-analyzer) using Na+ and K+ ion-selective electrodes (ISE)[214].

Procedure: The KNA2 Sodium-Potassium Analyzer was set-up with mode button to obtain P mode to analyze sodium and potassium respectively. The heparinized blood sample was aspirated through the inlet stub by using a catheter tube and immediately pressing the aspirate button, till the busy lamp glowed. Then the tube was removed and the inlet flap was closed. The total sample needed for measurement is 125 µl. The activity in the aqueous phase was measured by ISE (F2211 Na SELECTRODE®) within 47 seconds and expressed in mmol/L.

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.

Estimation of SOD: Marklund S and Marklund G (1974) method. The SOD inhibits auto oxidation of pyragallol, which can be determined as an increase in absorbance per two minutes at 420 nm on a spectrophotometer. Required reagents are; Tris Buffer (50mM), EDTA (1mM), HCl (50mM) and Pyragallol (20mM).

Table 10: 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	-

Reagent was mixed well and absorbance was taken after 1 min 30 sec and 3 min 30 sec at λ_{max} 420 nm. The absorbance per two minutes was recorded and calculated as;
SOD Unit/ml = C-T/C×50×100×1/0.05
= C-T/C×50×2000
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 11: 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, then the decrease in absorbance was measure at every 15 sec interval up to 1 min on UV-VIS spectrophotometer and calculated as;
Catalase activity = A/min × total vol. of assay / 0.081 × sample vol. × mg of protein Where, □A= difference in the absorbance reading
The molar extinction coefficient for H2O2 = 0.081

Estimation of MDA: Modified Jean CD method[218]. One molecule of MDA reacts with two molecules of TBA with the elimination of water to yield pink crystalline pigment MDA-TBA adducts with an absorption maximum wavelength at532 nm. Required reagents are; Perchloric acid liquid (7%), Thiobarbituric acid pH 7.4 (0.8%), 3 n- butanol (3ml).

Estimation of GSH: Reduced glutathione in the kidney tissue homogenate was determined according to the method of Moron et al., 1979. The acid soluble sulfhydryl groups (non-protein of which more than 93% is reduced glutathione) formed yellow colored complex with dithionitrobenzene (DTNB).

Procedure: In a test tube 0.1 ml of 25 % TCA was mixed with 0.5 ml of the tissue homogenate and kept on ice for few minutes after that centrifugation at 3000 rpm for few minutes for settled the precipitate. 0.3 ml of the supernatant was mixed with 0.7 ml of 0.2 M sodium phosphate buffer (pH 8) and 2 ml of 0.6 mM DTNB (prepared in
0.2 M buffer pH 8). The obtained yellow color was measured after 10 min at 412 nm against blank which contained 0.1 ml of 5% TCA in place of the supernatant. A standard graph was prepared using different concentrations (10-50 n moles) of GSH in 0.3 ml of 5 % TCA. The GSH content was calculated with the help of the standard graph and expressed as nM/mg protein or U/mg protein[219].

Estimation of LPO: The level of lipid peroxidation in the kidney tissue homogenate was measured same method used in measuring MDA, which was developed by Ohkawa 1979. Initially, the tissue of malondialdehyde was allowed to react with TBA. The MDA-TBA adduct formed during the reaction in acidic medium was extracted toward the organic layer and the absorbance was measured at 532 nm.

Estimation of NO: The assay was performed according to the method described by Sreejayan 1997. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to nitrite ions, which was measured by Griess reagent. The reaction mixture (3 ml) containing 10 mM nitroprusside in phosphate buffered saline and the fractions or the extracts at different concentrations (50–800 µg/ml) were incubated for 150 min at 25°C. An aliquot (0.5 ml) of incubated sample was removed at 30 min intervals and 0.5 ml Griess reagent was added. The absorbance of produced chromophore was measured at 546 nm. Inhibition of the Nitric oxide generated was measured by comparing the absorbance values of control and extracts

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. About 10gm of a thoroughly mixed sample was slowly passed beside the wall of the funnel until the tip of the pile formed and touches the bottom of the funnel. A rough circle drowned around the pile base and the radius of the powder cone was measure

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. The cylinder was tapped from the height of 2 inch until to achieved the constant volume or 100 tapping[226]. The final volume occupied by the sample after tapping were recorded

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:

Polyherbal dispersible tablets were prepared separately through geometrical dilution mixing and direct compression method (Punching machines, Cadmach CMS-15 No. H/513/11-12), using powdered extracts, disintegrating agents, talc and other excipients. All the ingredients were passed through mesh sieve no. 120 and then mixed each other as per geometrical dilution method to maintain uniformity. The powder mixtures possess good flow properties and good packing ability, so the mixtures were directly compressible[229,230]. Nine formulations were prepared by using all ingredients in appropriate quantity but the disintegrants was used in different concentration only and denoted by PHF-1 to PHF-3

Table 12: 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. The test was performed by weighing 20 tablets individually using an analytical balance, then calculated average weight and compared the individual tablet weights. The percentage weight variation was calculated by using the equation 6.

$$\% \text{ wt variation} = \frac{\text{Average wt} - \text{Individual wt} \times 100}{\text{Average wt}}$$

Hardness test:

The hardness of the tablet is defined as the loads required for crushing or fracture a tablet by placing on its edge. The confrontation of tablets for capping, abrasion or breakage under storage conditions, transportation and handling before usage depend on tablet hardness (kg/cm2). The hardness test was performed by using Monsanto hardness tester (Harrison's). The instrument measures the force required to break the tablet when the force (Kilogram-force) generated by anvils to the tablet. The tablet was placed between two anvils; the force applied to the anvils and the crushing strength that causes the tablet to break was recorded and the crushing strength test was performed on 20 tablets of each formulation.

Friability test:

The friability test was performed by using tablet friability tester (Veego). Ten tablets of each formulation were weighed and tested at a speed of 25 rpm for 4 min (100 rotations). After removing of dust, tablets were re-weighed and friability percentage was calculated according to equation 7.

$$\% \text{ Friability} = \frac{\text{Tablet wt before friability} - \text{Tablet wt after friability} \times 100}{\text{Tablet wt after friability}}$$

Drug content uniformity test:

From each formulation, 20 tablets were taken, weighed and thoroughly triturated. The sufficient amount of the powder equivalent to 250mg of the drug was accurately weighed and diluted in 300ml of 0.1N HCl for 10min with vigorous shaking. Further, this prepared mixture was diluted with 0.1N HCl up to 400ml and then filtered. 10ml of this filtrate diluted in 100ml distilled water and the absorbance was taken at the wavelength of λmax 200-400nm by using double beam UV spectrophotometer

Disintegration time:

From each formulation 6 polyherbal dispersible

tablets were randomly selected to determine the disintegration time. The acidic buffer (pH 1.2) was used as a disintegration medium and temperature was maintained at 37±0.5°C. The average disintegration time of six tablets was taken

Dispersion time:
In-vitro dispersion time of polyherbal dispersible tablet was measured by dropping the tablet in a beaker containing 100ml of normal water. Two tablets from each formulation were randomly selected and in-vitro dispersion time was determined. A smooth dispersion is produced, which passes through a sieve screen with a nominal mesh aperture of 710µm[234].

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. Since the phytoconstituents of medicinal plant and other ingredients of the polyherbal combination covers the multiple constituents, so that the development of dissolution method becomes more complex than for defined single constituent. Hence the in-vitro dissolution test was not performed for polyherbal dispersible tablet

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. The optimized polyherbal formulation was subjected to accelerated stability studies and the formulations were packed in bottles at specified conditions of temperature and relative humidity i.e., 25°C/60% RH, 30°C/65% RH and 40°C/75% RH for 3 months

Statistical Analysis:
All data observed from experimental animal were expressed as mean±S.E.M. The statistically significant difference between animal groups were assessed by one-way ANOVA followed by Bonferroni's multiple comparison test plus factorial analysis, student's t-test for a number of animals (n=6) and Bartlett's test for equal variances also applied during analysis. Significance of data was expressed as #$p<0.05$ against normal control; $p<0.05$ and $p<0.001$ against disease control. The Chi-Square test for the qualitative data and Wilcoxon-signed-rank test for non-parametric data were performed for the calculation and comparison of results between the groups.

RESULT AND DISCUSSION:

Phytochemical screening:

Table 13: 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:
The pharmacological screening for glomerulonephritis has been conducted on ten groups of Wistar rats (n=6).

Physical parameters of animal groups

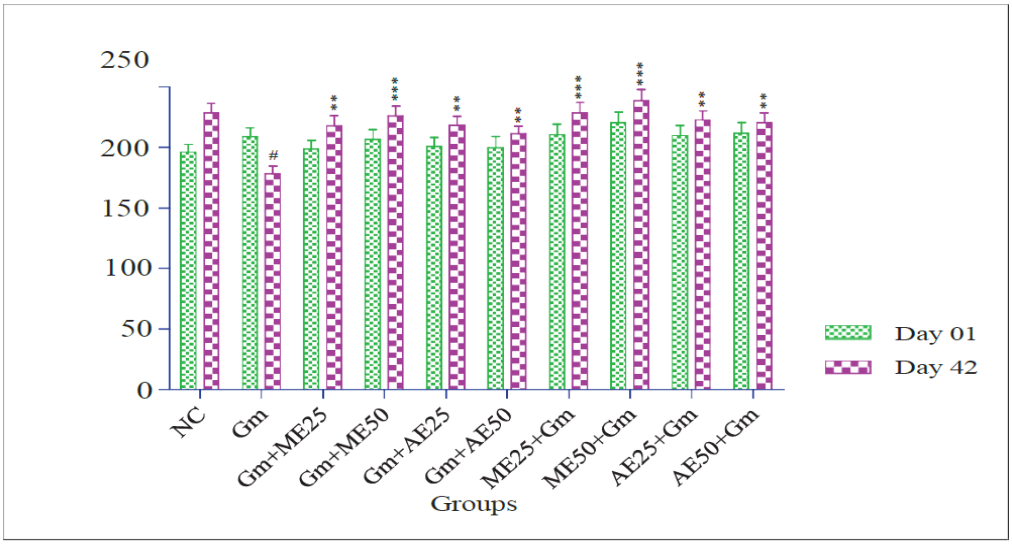
The effect of the polyherbal combination on physical parameters, body weight of all animal groups were observed, while 24 h urinary volumes at first and last day was observed and the kidney weight was recorded at the end of study

Table 14: 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**

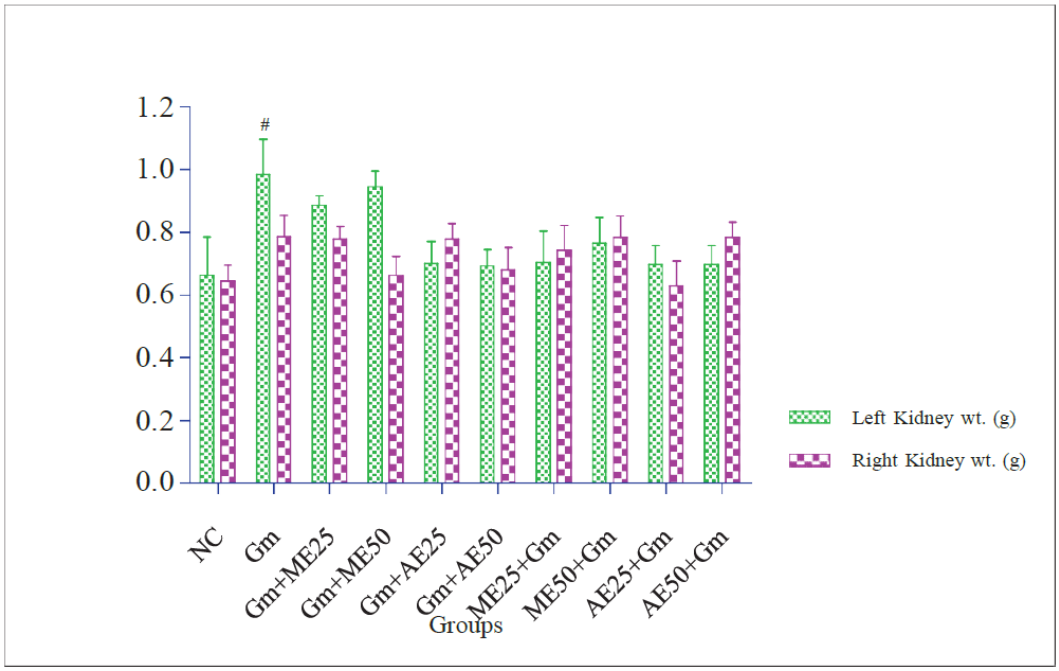
Values are given as Mean±SEM of animal groups (n = 6) and expressed in g & ml. #p±0.05 statistical significance against normal control; **p±0.05 and ***p±0.001 statistical significance against disease control.

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



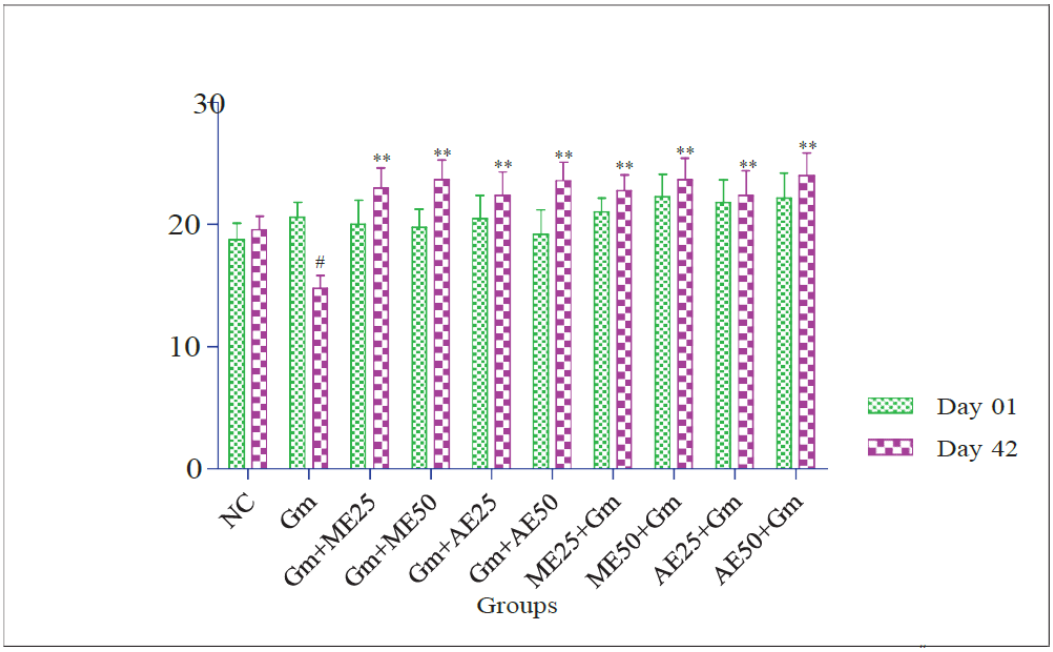
Values are given as Mean±SEM of animal groups (n = 6) and expressed in gm. $^{\#}p0.05$ statistical significance against normal control; $^{**}p\pm0.05$ and $^{***}p\pm0.001$ statistical significance against disease control.

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



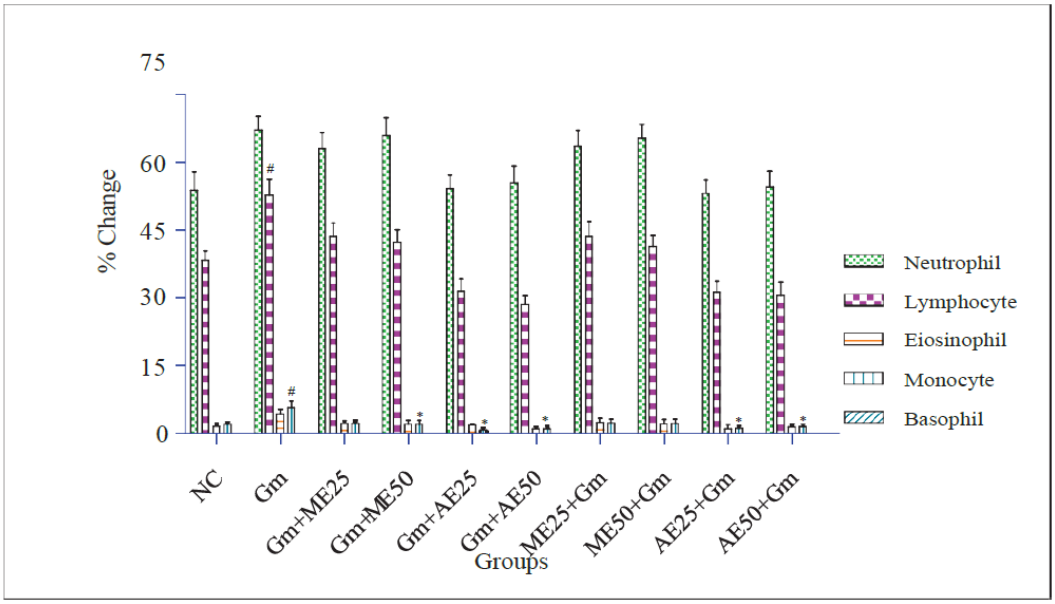
Values are given as Mean±SEM of animal groups (n = 6) and expressed in ml. $^{\#}p\pm0.05$ statistical significance against normal control and $^{**}p\pm0.05$ statistical significance against disease control.

Hematological parameters of animal groups

Table 15: Effect of polyherbal combination on hematological parameters

Groups	Hb (g/dl)	CBC ($x10^3/\mu l$)	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

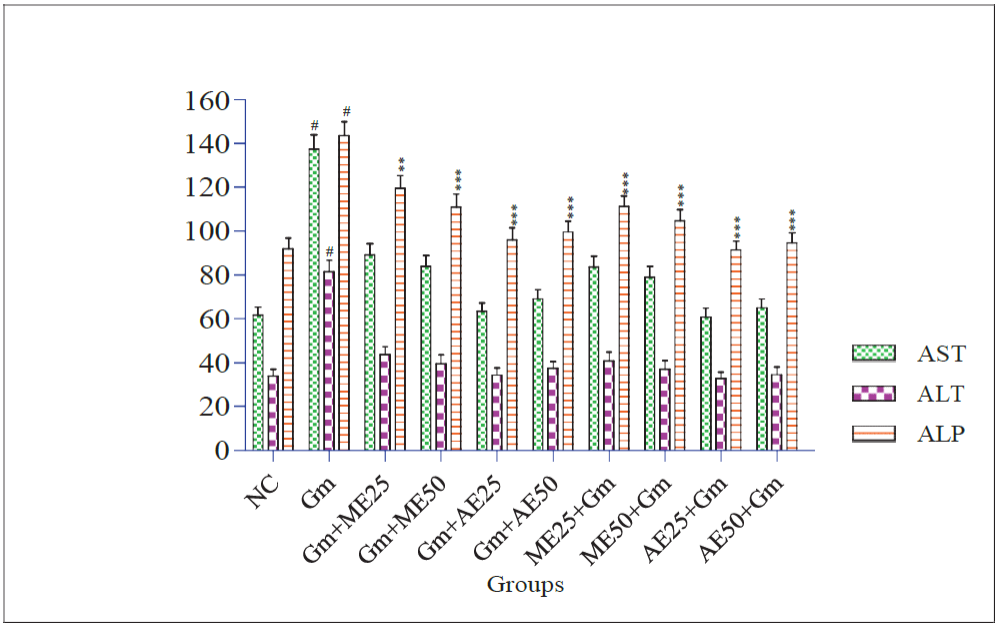
Table 16: The effect of the polyherbal combination on LFT (liver function test)

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

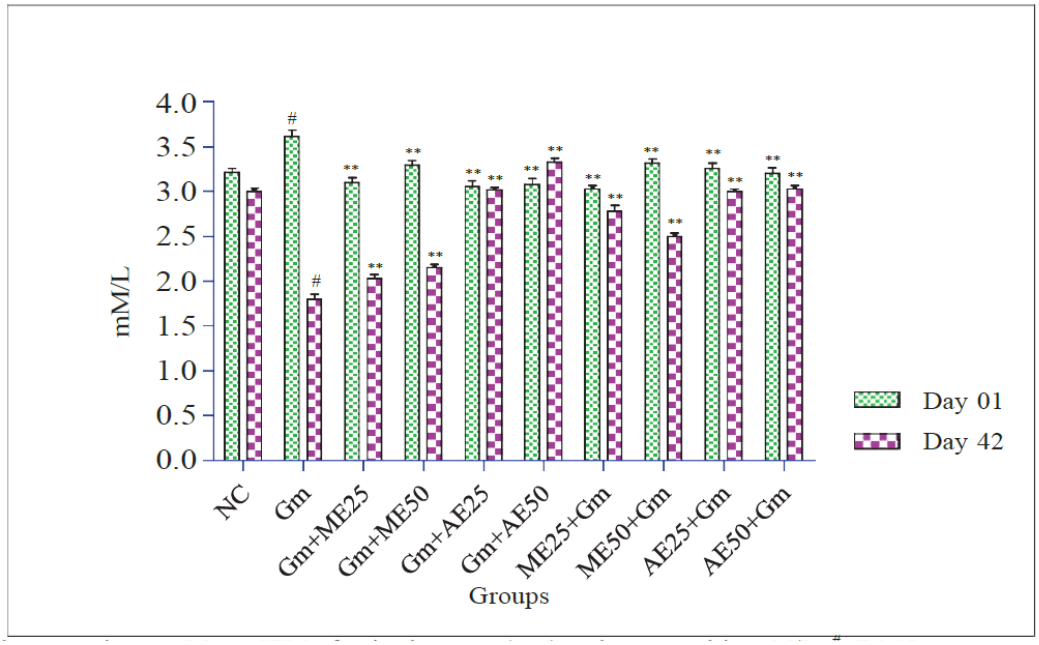
LFT=Liver Function Test, AST=Aspartate Aminotransferase, ALT=Alanine Aminotransferase, ALP=Alkaline Phosphatase.

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



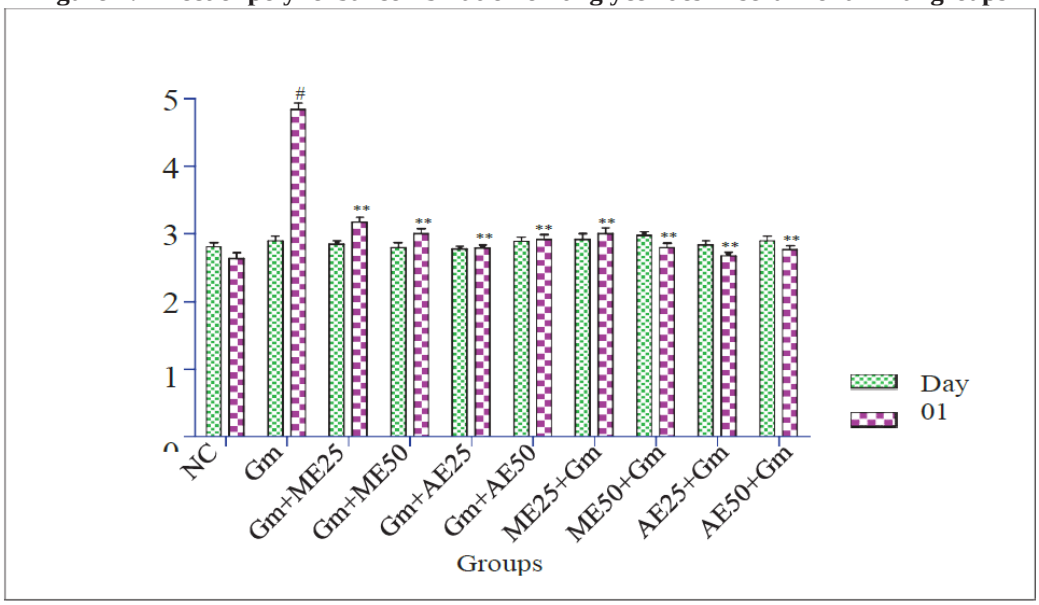
Values are given as Mean±SEM of animal groups (n=6) and expressed in IU/L. $^{\#}p0.05$ statistical significance against normal control and $^{***}p0.001$ statistical significance against disease control.

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



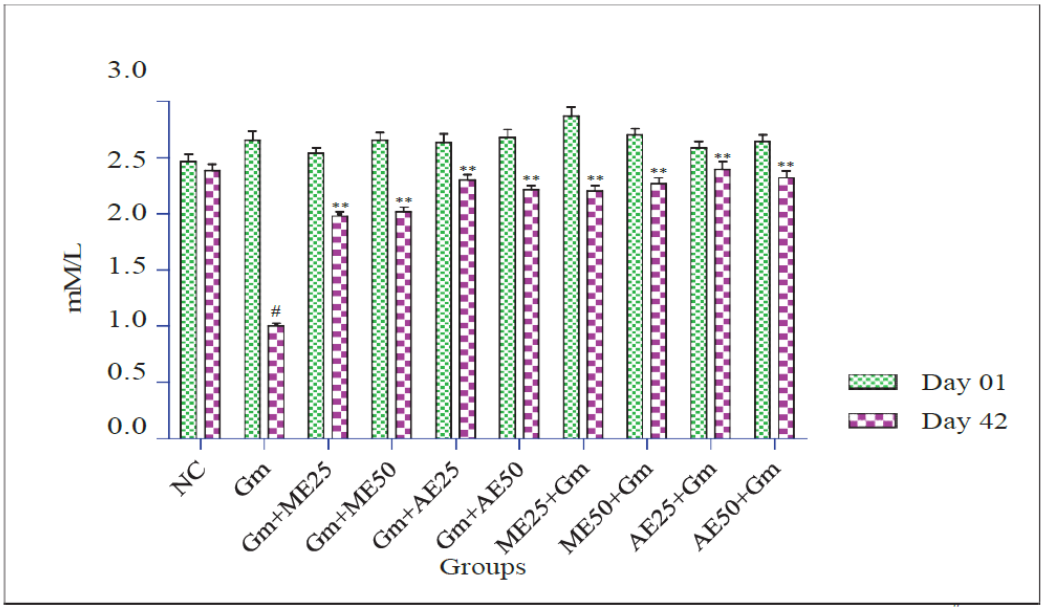
values are given as Mean±SEM of animal groups (n=6) and expressed in mM/L. $^{\#}p\pm0.05$ statistical significance against normal control and $^{**}p\pm0.05$ statistical significance against disease control.

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) in serum



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 17: 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

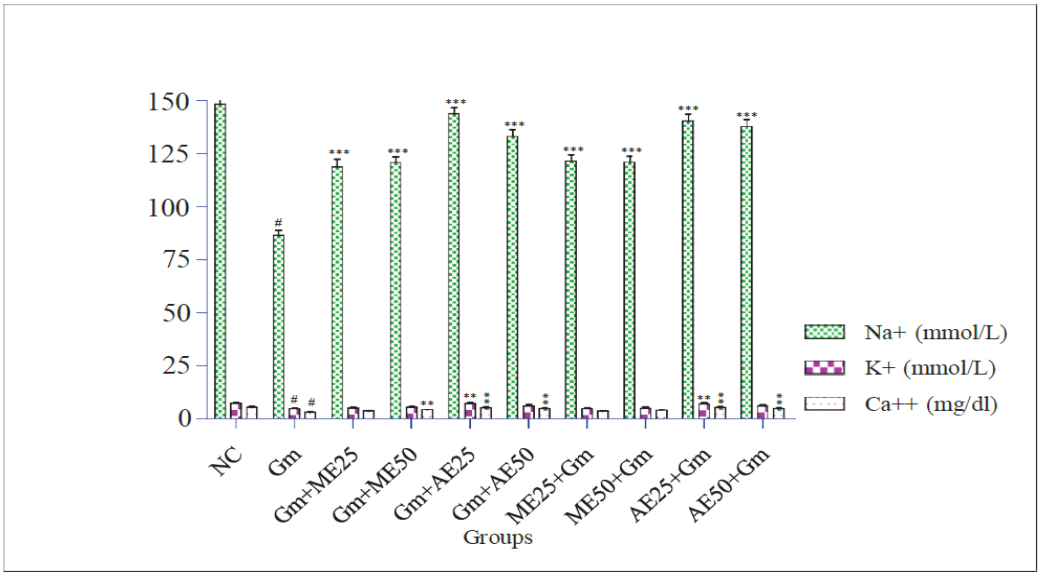
Electrolyte concentration (Na^+, K^+ and Ca^{++}) in serum and urine sample of animal groups:

Table 18: The effect of the polyherbal combination on electrolyte concentration

Groups	Electrolyte concentrations in serum			Electrolyte concentrations in urine		
	Na^+ mmol/ L	K^+ mmol/L	Ca^{++} mg/dl	Na^+ mmol/ L	K^+ mmol/L	Ca^{++} mg/dl
NC	148.10±3.11	07.02±0.51	05.48±0.37	68.00±2.18	83.40±3.02	0.82±0.23
DC	086.30±2.45#	04.68±0.42#	02.89±0.29#	157.03±3.66#	137.51±3.11#	3.01±0.42#
Gm+ME25	118.52±3.70***	05.03±0.45	03.50±0.27	103.50±2.58***	115.30±2.85***	2.42±0.35
Gm+ME50	120.63±2.65***	05.43±0.37	04.21±0.19**	107.32±2.62***	107.30±3.00***	2.01±0.25
Gm+AE25	143.64±3.00***	06.88±0.60**	05.20±0.42***	71.43±2.30***	88.20±2.42***	0.87±0.31**
Gm+AE50	132.89±3.24***	05.99±0.54	04.86±0.28***	83.01±2.41***	97.12±2.08***	0.93±0.42**
ME25+Gm	121.37±2.86***	04.73±0.46	03.39±0.24	97.02±2.50***	111.29±3.14***	1.90±0.50
ME50+Gm	120.80±2.79***	05.00±0.57	04.01±0.23	101.18±2.38***	103.83±2.63***	1.78±0.45
AE25+Gm	140.08±3.36***	06.78±0.60**	05.28±0.45***	69.00±2.05***	85.28±2.12***	0.85±0.35**
AE50+Gm	137.58±3.17***	05.98±0.51	04.90±0.27***	76.40±2.34***	93.70±2.07***	0.92±0.26**

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

Figure 9: Effect of polyherbal combination on electrolyte concentrations in serum



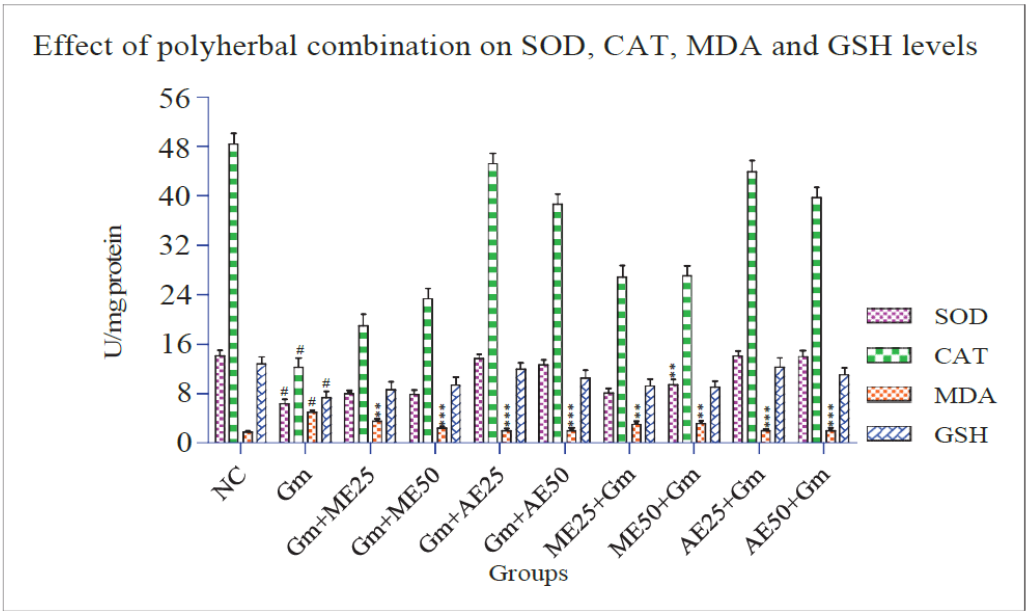
Values are given as Mean±SEM of animal groups (n=6) and expressed in mmol/L & mg/dl. #p±0.05 statistical significance against normal control; **p±0.05 and ***p±0.001 statistical significance against disease control

Table 19: Effect of polyherbal combination on antioxidant parameters

Groups	SOD (U/mg.protein)	CAT (U/mg.protein)	MDA (U/mg.protein)	GSH (U/mg.protein)
NC	14.16 ± 0.81	48.40 ± 1.77	1.80 ± 0.18	12.84 ± 1.08
DC	06.37 ± 0.67$^{\#}$	12.17 ± 1.53$^{\#}$	5.03 ± 0.26$^{\#}$	07.30 ± 1.02$^{\#}$
Gm+ME25	08.02 ± 0.45	19.00 ± 1.85	3.54 ± 0.35**	08.62 ± 1.28
Gm+ME50	07.86 ± 0.67	23.32 ± 1.69***	2.39 ± 0.22***	09.34 ± 1.25
Gm+AE25	13.61 ± 0.80***	45.20 ± 1.72***	1.98 ± 0.37***	11.88 ± 1.06
Gm+AE50	12.68 ± 0.74***	38.61 ± 1.68***	2.01 ± 0.41***	10.45 ± 1.33
ME25+Gm	08.13 ± 0.66	26.89 ± 1.84***	3.02 ± 0.50**	09.20 ± 1.07
ME50+Gm	09.48 ± 0.75**	27.03 ± 1.63***	3.18 ± 0.38**	09.00 ± 1.02
AE25+Gm	14.08 ± 0.76***	43.98 ± 1.72***	1.98 ± 0.25***	12.20 ± 1.54**
AE50+Gm	13.98 ± 0.91***	39.70 ± 1.67***	2.00 ± 0.41***	11.00 ± 1.06

Values are given as Mean±SEM of animal groups (n=6) and expressed in U/mg of protein. #p±0.05 statistical significance against NC; **p±0.05 and ***p±0.001 statistical significance against DC.

Figure10: Effect of polyherbal combination on SOD, CAT, MDA and GSH levels



Values are given as Mean±SEM of animal groups (n=6) and expressed in U/mg of protein. $^{\#}p\pm0.05$ statistical significance against NC; $^{**}p\pm0.05$ and $^{***}p\ 0.001$ statistical significance against DC.

Antioxidant parameters (LPO of kidney tissue and NO scavenging activity) of animal groups

Table 20: Effect of polyherbal combination on LPO of kidney tissue and NO scavenging activity of animal groups

Groups	LPO of kidney tissue (µmol/mg.protein)	NO scavenging activity (µmol/mg.protein)
NC	84.86 ± 2.00	15.30 ± 1.02
DC	103.20 ± 2.12$^{\#}$	25.50 ± 1.00$^{\#}$
Gm+ME25	97.24 ± 2.30	21.90 ± 1.82
Gm+ME50	93.75 ± 1.90**	22.04 ± 2.20
Gm+AE25	84.74 ± 1.87***	14.17 ± 2.01**
Gm+AE50	83.26 ± 1.62***	16.00 ± 2.06**
ME25+Gm	95.01 ± 2.10**	21.24 ± 2.25
ME50+Gm	89.32 ± 2.00***	20.00 ± 2.22
AE25+Gm	85.05 ± 1.77***	14.89 ± 2.06**
AE50+Gm	84.27 ± 1.80***	15.77 ± 2.17**

Values are given as Mean±SEM of animal groups (n=6) and expressed in µmol/mg of protein. #p±0.05 statistical significance against normal control; **p±0.05 and ***p±0.001 statistical significance against disease control. LPO=Lipid Peroxidation; NO=Nitric Oxide

POLYHERBAL FORMULATION AND EVALUATION:

Characterization of extract powder:

The micromeritic properties of polyherbal aqueous root extracts powder used for the preparation of dispersible tablets, because of it shows the pharmacological effect as designed for the glomerulonephritis activity in comparison to methanol extract.

Table 21: 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 22: 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 23: 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:

The disintegration time of dispersible tablets were evaluated by using two basket rack assembly USP apparatus.

Dispersion time:

The dispersion time of polyherbal dispersible tablets was observed by placing 2 tablets in 100 ml of water in a beaker with gently stirred until the completely dispersed.

Table 24: Disintegration and dispersion time of the polyherbal dispersibletablet

Formulation Code	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

The stability study of polyherbal dispersible tablets carried out for three months at different IP standard condition acceptable for pharmaceutical preparation.

Table 25: 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

SUMMARY AND CONCLUSION:

The study revealed that the methanol and aqueous root extracts of polyherbal combination possess several potent phytoconstituents observed in the methanol extract showed the presence of carbohydrates, steroids, glycosides, coumarins, saponins, flavonoids, alkaloids, tannins and phenols approximately in all plants. Whereas proteins and anthraquinones were reported in few plants and none of the plant showed the presence of lipids. The aqueous extracts also showed the presence of carbohydrates, steroids, glycosides, saponins, flavonoids and tannins approximately in all plants. Whereas, coumarins, phenols and anthraquinones reported in few plants only and none of the plat showed the presence of proteins, lipids and alkaloids. 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, 2017[239], 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 Gajjala, 2015. 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

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Conflicts of interest:

Authors have no conflicts of interest to declare.

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