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PHARMACOLOGICAL POTENTIAL OF POLYHERBAL COMBINATION FOR THE TREATMENT OF GLOMERULONEPHRITIS IN ALBINO RATS

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ABSTRACT: The polyherbal combination was prepared by using aqueous and methanol root extracts of *A. officinalis, B. diffusa, C. papaya, C. fistula, C. intybus, F. hispida, F. indica, C. nurvala, S. virgaurea* and *V. negundo* in equal proportion for the dose of 25 mg/kg and 50 mg/kg body weight. Albino Wistar rats were selected for the study; the serum uric acid, urea, creatinine, BUN, and albumin level were measured using standard procedures. The elevated parameters in serum shows the statistically significant ($^{**}p<0.05$) improvement with a low dose of aqueous extract combination *i.e.*, hemoglobin (11.15 g/dl), CBC (5.93 $\times 10^3$/µl), total bilirubin (0.61 mg/dl), total protein (09.85 mg/dl), AST (63.41 IU/L) & ALT (34.37 IU/L), uric acid (1.68 mg/dl), urea (57.06 mg/dl), creatinine (1.08 mg/dl), BUN (41.50 mg/dl) and albumin (40.84 mg/dl) in serum and urine. It was also reported as 24h urinary protein (2.96 mg/24h), albumin (09.23 mg/dl), urea (2.80 mg/dl), creatinine (38.28 mg/dl) and uric acid (126.08 mg/dl) shows significant improvement against disease control at the end of the study. Histological changes show that the gentamicin significant damaged tubular necrosis, epithelial cells, glomerular damage, and congestions of the nephron, which was completely reversed with aqueous extract polyherbal combination. Finally, it was concluded that the low dose (25 mg/kg) of aqueous extract has immense potential as compare to methanol extract and possess protective with therapeutic properties in the treatment of glomerulonephritis.

INTRODUCTION: Glomerulonephritis (GN) is a variety of immune-mediated disorders that cause inflammation within the glomerulus, and also the different filtering unit of the urinary organ has been described as the most prevalent causes of end-stage renal disease (ESRD) [1, 2]. Currently, GN becomes an intricate condition encompassing a diversity of individual disorders, associated with considerable morbidity and mortality [3].

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It often consequences in dialysis-requiring end-stage renal disease (ESRD), hospitalization, disability due to treatment with potent immuno-suppressive agents or underlying pathophysiologic processes. Glomerular diseases are the major cause of chronic kidney disease (CKD) in many developing countries has been associated with a significant socioeconomic burden [4].

In the United States, CKD is slightly more common among females than males, with 17% of females diagnosed with CKD in the period from 2011-2016, compared to just 14% of males. It occurs more frequently among older individuals, the recurrent cases found in patient ≥ 60 years [5]. The worsen condition associated with poorly characterized molecular risks in patients with normal renal

function and may poorly describe genetic risks in donors who are biologically related to living donor kidney transplant recipients with GN-ESRD, making pre-donation screening challenge [6]. The persistent proteinuria and hematuria developed after manifestations of GN, and it may progress rapidly to ESRD even after interventions are implemented [7]. However, it was additionally ascertained that the patients with primary GN were additional possible to progress end-stage renal disease (ESRD) than patients with GN because of systemic immunologic illness [8]. At present majority of basic and clinical research has been carried out on the medicinal plants and their herbal formulations, with the state of the art methods in several medical institutions or universities [9].

Traditional medication system principally herbal medicine considered as a major healthcare provider around the world mostly in rural and remote areas. A large number of populations depend on such medicine for their primary healthcare usually in underdeveloped or developing countries [10]. The medicinal plants also provide a rich source of antioxidants that are known to prevent or delay such type of disease condition [11]. The medicinal plants also contain other beneficial compounds or ingredients which can be used for food and medicinal purposes. Hence, the global knowledge about Ayurveda and Indian herbals hopefully enhance by information on the evidence-base of the plants [12]. The current research work is based on the development of potent polyherbal combination by using polyherbal plants' root extracts possessing anti-nephrotic activity [13].

MATERIALS AND METHODS:

Collection and Authentication of Plants: The fresh roots of all selected herbal plants were purchased from Khari Baoli, New Delhi, India. Fresh and shade dried roots of all selected plants have been collected consequently and authenticated by Botanical Survey of India, the Northern regional center 192, Kaulagarh Road, Dehradun-248195 with Boucher No: BSI/NRC/Tech.(Ident.)/2012-13/755.

Extraction of Selected Polyherbal Plants: The selected plant's part was carried out for aqueous and methanol extraction based on the published standard method as follows;

Aqueous Extract (H_2O): The collected herbal plant roots were separately cleaned, dried under shade and powdered by a mechanical grinder. The powdered roots of each plant (1000 g) were poured in distilled water until the complete exhaustion for 48 h through cold maceration. The extracts were separately filtered by using Whatman filter paper, and concentrated by rotary evaporator at a temperature of 40 °C and dried using a freeze drier to get extract powder. The yield was found to be between 18-20% (w/w) respectively and stored in a closed container [14].

Methanol Extract (MeOH): The collected herbal plant roots were separately cleaned, dried under shade and powdered by a mechanical grinder. The powdered roots of each selected plants (1000 g) were poured in methanol until the complete exhaustion for 48 h through cold maceration. The solvent was evaporated by using a vacuum rotary evaporator at 40 °C to get the concentrated extract and then finally dried using a freeze drier. The yield was found to be between 18-20% (w/w) respectively and absolute powder stored in a closed container [15].

Preliminary Phytochemical Screening: Qualitative screening of various extracts of polyherbal plants was performed for the identification of various classes of active chemical constituents by using standard published methods. The phytochemical screening of the aqueous and methanolic extracts of the plants used in polyherbal combination analyzed for the presence/absence of carbohydrates, lipids, protein, steroids, glycosides, saponins, flavonoids, alkaloids, tannins, phenolics, anthraquinones, terpenoids [16, 17, 18].

Composition of Polyherbal Combination: The compositions of plants selected for the preparation of a potent polyherbal combination for the treatment of glomerulonephritis are mentioned in **Table 1**.

Experimental Animal: Albino Wistar rats (150-200 g) of either sex were procured from the animal house of Bhimtal Campus, Kumaun University, Nainital, India and followed the institute animal ethical clearance (Approval No.: KUDOPS/17).

All rats were kept in polypropylene cages at 25 ± 5 °C room temperature under 12 h dark-light cycles

and fed with standard pellet diet with free access of purified water *ad libitum*. All the animals were acclimatized for laboratory condition as per CPCSEA guideline for a week before use.

TABLE 1: COMPOSITION OF POLYHERBAL COMBINATION

S. no.	Botanical name	Family	Quantity
1	*Angelica officinalis* Linn.	Umbelliferae	25mg
2	*Boerhavia diffusa* Linn.	Nyctaginaceae	25mg
3	*Carica papaya* Linn.	Caricaceae	25mg
4	*Cassia fistula* Linn.	Fabaceae	25mg
5	*Cichorium intybus* Linn.	Asteraceae	25mg
6	*Ficus hispida* Linn.	Moraceae	25mg
7	*Fumaria indica* Linn.	Fumariaceae	25mg
8	*Crataeva nurvala* Linn.	Capparidaceae	25mg
9	*Solidago virgaurea* Linn.	Asteraceae	25mg
10	*Vitex negundo* Linn.	Verbenaceae	25mg

Acute Toxicity Study: The prepared polyherbal root extract combination was subjected for acute oral toxicity study as per the OECD test guideline 423. The protocol was approved before examination through the Institutional Animal Ethics Committee (IAEC Approval number – KUDOPS/17, none of the mortality and no signs of toxicity found after administration at dose 2000 mg/kg in the rats. Hence, the lowest dose of this extract combination selected for pharmacological screening on glomerulonephritis rats [19].

Experimental Design: All Wistar rats were divided into 10 groups having 6 animals in each. The study was designed and conducted for 42 days.

Group-I served as normal control (saline 0.9% NaCl), Group-II as disease control (Gentamicin 100 mg/kg/day for 8 days), and continued with free access to water & diet. The rest of the animals were divided into two major treatment groups, *i.e.* therapeutic and prophylactic groups.

Therapeutic Groups: At first rats were administered with gentamicin 100 mg/kg/d for 8 days of study, after that divided into four different treatment groups such as; Group-III administered as gentamicin 100 mg/kg/day for 8 days and then methanol extract of the formulation (25 mg/kg/day), Group-IV served as gentamicin 100 mg/kg/day for 8 days and then methanol extract of formulation (50 mg/kg/day), Group-V as gentamicin 100 mg/kg/day for 8 days and then aqueous extract of formulation (25 mg/kg/day), and Group-VI as gentamicin 100 mg/kg/day for 8 days and then aqueous extract of formulation (50 mg/kg/day) for rest 34 days.

Prophylactic Groups: At first rats were administered with extracts every day for 34 days then gentamicin 100 mg/kg/d for rest 8 days and the division of groups was as follows; Group-VII administered with methanol extract of formulation (25 mg/kg/day) for 34 days and then gentamicin 100 mg/kg/day, Group-VIII served as **a** methanol extract of formulation (50 mg/kg/day) for 34 days and then gentamicin 100 mg/kg/day, Group-IX administered with aqueous extract of formulation (25 mg/kg/day) for 34 days and then gentamicin 100 mg/kg/day, and Group-X served as aqueous extract of formulation (50 mg/kg/day) for 34 days and then gentamicin 100 mg/kg/day continued for rest 8 days of the study.

Investigational Parameters: Physical parameters such as body weight, kidney weight, and 24h urinary volume. Hematological parameters like hemoglobin, CBC, total bilirubin, total protein, AST, ALT, total cholesterol, triglycerides, LDL, and HDL. Biochemical parameters in serum sample such as; BUN, albumin, urea, creatinine, uric acid, and in urine sample, *i.e.*, urinary protein, albumin, urea, creatinine, uric acid was estimated in the experimental animal. Electrolyte concentrations like sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) were estimated in serum and the urine sample. Antioxidant enzymes such as SOD, CAT, MDA, GSH, LPO, and NO of kidney tissue were determined in kidney homogenate.

Histological Evaluation: The kidneys were removed and freshly collected to keep in 10% neutral formalin solution. The organ was processed and embedded in paraffin wax, and the narrow sections were taken using a microtome. The

excellent sections were stained with hematoxylin and eosin color, then the slide covered with a coverslip and fixed with a fixation solution for observation under the digital light microscope to capture clear kidney section.

Statistical Analysis: The data sets acquired from animal trials were communicated as mean ± S.E.M. The statistically significant difference between experimental groups were assessed by one-way ANOVA followed by Bonferroni's multiple comparison tests plus factorial analysis, Student's t-test for the number of 6 animals.

Significance of data was expressed as $^{\#}p<0.05$ against normal control; $^{**}p<0.05$ and $^{***}p<0.001$ against disease control.

RESULTS:

Preliminary Phytochemical Screening: The phytochemical screening of polyherbal plants used in the study was performed in aqueous and methods solvent for extraction. The extracting values were obtained in the aqueous extract (18-20%) and the methanol extracts (18-20%), respectively. The aqueous and methanol extract was possessed different phytoconstituents, such as carbohydrates, proteins, lipids, steroids, glycosides, coumarins, saponins, flavonoids, alkaloids, tannins, phenols, anthraquinones, and terpenoids as major constituents.

Pharmacological Screening: The pharmacological screening for GN was conducted on experimental animals for hematological, differential cholesterol, biochemical, electrolyte concentration, antioxidant parameters and histological analysis.

Hematological Parameters: The hematological parameters of all groups were observed for hemoglobin, CBC, total bilirubin, total protein, AST, and ALT at the end of the study. The hemoglobin and CBC showed statistically non-significant (NS) changes as compared between the groups, but the animal treated with a low dose (25 mg/kg b.w.) of polyherbal combination shows a little improvement in comparison to disease control. The other parameters like total bilirubin, total protein, AST and ALT shows the surprising result at the end of the study. The rats treated with Gentamicin showed statistically significant ($^{\#}p<0.05$) changes in comparison to normal control and the treatment groups showed statistically significant ($^{**}p<0.05$ and $^{***}p<0.001$) difference in comparison to disease control but the rats treated with a low dose (25 mg/kg b.w.) of aqueous extract showed statistically significant ($^{***}p<0.001$) improvement against disease control as shown in **Table 2**.

Differential Cholesterol Levels: The differential cholesterol levels in the serum, *i.e.,* total cholesterol, triglycerides, LDL, and HDL for all groups were observed at the end of the study. The rats treated with gentamicin showed statistically significant ($^{\#}p<0.05$) decrease in cholesterol and LDL levels but in case of triglyceride and HDL it showed statistically significant ($^{\#}p<0.05$) increase in these levels as compared to normal control.

TABLE 2: EFFECT OF POLYHERBAL COMBINATION ON HEMATOLOGICAL PARAMETERS

Groups	Haemoglobin (g/dl)	CBC ($\times 10^3$/µl)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)	AST (IU/L)	ALT (IU/L)
I	13.11±1.86	6.80±2.15	0.58±0.04	09.50±0.93	61.82±3.56	33.76±3.25
II	10.80±1.64	5.00±1.36	1.38±0.06$^{\#}$	03.58±0.61$^{\#}$	137.30±6.83$^{\#}$	81.39±5.34$^{\#}$
III	11.18±1.67	5.83±1.58	0.77±0.05***	05.62±0.72	89.16±5.00***	43.76±3.48***
IV	11.13±1.57	6.00±2.00	0.85±0.07***	07.50±0.80**	83.98±4.83***	39.69±4.03***
V	12.88±1.54	6.98±2.16	0.61±0.04***	09.85±0.68***	63.41±3.89***	34.37±3.22***
VI	12.90±1.66	6.93±2.01	0.67±0.06***	08.67±0.82***	69.32±3.90***	37.45±3.18***
VII	11.15±1.37	5.93±1.51	0.75±0.05***	07.26±0.67**	83.72±4.83***	40.88±4.01***
VIII	11.16±1.47	6.11±2.02	0.80±0.07***	08.04±0.70***	79.12±4.76***	36.98±4.00***
IX	12.89±1.94	6.90±2.16	0.56±0.04***	09.52±0.63***	60.91±3.90***	32.79±3.02***
X	12.60±1.77	6.53±2.11	0.63±0.06***	09.01±0.72***	65.20±4.00***	34.65±3.23***

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

All the treatment groups showed statistically significant ($^{**}p<0.05$) improvement in these elevated levels as compared to disease control. The rats treated with a low dose (25 mg/kg) aqueous

extract combination showed well statistically significant ($^{**}p<0.05$) improvement compared to disease control and other treatment groups as shown in **Table 3**.

TABLE 3: EFFECT OF POLYHERBAL COMBINATION ON DIFFERENTIAL CHOLESTEROL LEVELS IN SERUM

Group	Total Cholesterol (mM/L)	Triglycerides (mM/L)	LDL (mM/L)	HDL (mM/L)
I	3.00±0.04	2.64±0.08	2.38±0.06	2.98±0.05
II	1.80±0.06$^{\#}$	4.84±0.10$^{\#}$	1.00±0.03$^{\#}$	4.88±0.12$^{\#}$
III	2.03±0.05**	3.18±0.07**	1.98±0.04**	3.58±0.06**
IV	2.15±0.04**	3.00±0.08**	2.02±0.04**	3.39±0.05**
V	3.02±0.03**	2.79±0.05**	2.30±0.05**	3.00±0.04**
VI	3.33±0.05**	2.92±0.07**	2.21±0.04**	3.26±0.05**
VII	2.78±0.07**	3.00±0.09**	2.20±0.05**	3.33±0.04**
VIII	2.50±0.04**	2.80±0.06**	2.27±0.05**	3.18±0.05**
IX	3.00±0.03**	2.68±0.05**	2.39±0.07**	2.90±0.03**
X	3.03±0.04**	2.77±0.06**	2.32±0.06**	2.99±0.04**

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

Biochemical Parameters in Serum: The biochemical parameters such as BUN, albumin, urea, creatinine, and uric acid in serum were reported at the end of the study. The rats treated with gentamicin showed statistically significant ($^{\#}p<0.05$) increase the levels of BUN, urea, creatinine, and uric acid but decrease the albumin level as normal control. The treatment groups showed statistically significant ($^{***}p<0.001$) improvement in these elevated parameters compared to disease control. While the rats treated with a low dose (25 mg/kg) of aqueous extract combination shows more statistically significant ($^{***}p<0.001$) improvement compared to disease control and other treatment groups as shown in **Table 4**.

TABLE 4: EFFECT OF POLYHERBAL COMBINATION ON BIOCHEMICAL PARAMETERS IN SERUM

Groups	BUN (mg/dl)	Albumin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
I	42.06±1.21	42.85±1.26	56.03±2.08	1.02±0.05	1.65±0.42
II	63.57±2.04$^{\#}$	12.54±0.67$^{\#}$	132.10±2.45$^{\#}$	6.25±0.47$^{\#}$	3.08±0.71$^{\#}$
III	52.16±1.36***	28.68±1.36***	109.23±2.03***	3.92±0.34**	1.98±0.53**
IV	50.60±1.24***	32.25±1.63***	103.07±1.91***	2.88±0.60***	1.87±0.38***
V	41.50±1.64***	40.84±2.00***	57.06±1.64***	1.08±0.45***	1.68±0.41***
VI	44.06±2.01***	37.59±1.96***	68.08±2.01***	1.25±0.64***	1.81±0.35***
VII	51.68±2.10***	33.87±1.89***	98.78±2.61***	2.32±0.34***	2.00±0.51***
VIII	52.96±2.05***	36.67±1.93***	96.03±2.05***	2.19±0.66***	1.94±0.43**
IX	40.07±1.05***	43.04±2.01***	54.85±2.12***	1.05±0.25***	1.63±0.33***
X	42.93±2.21***	41.09±2.06***	62.01±2.00***	1.21±0.44***	1.76±0.46***

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

Biochemical Parameters in Urine: The biochemical parameters of all groups were observed for 24 h urinary protein, albumin, urea, creatinine, and uric acid in the urine sample at the end of the study. The disease control group shows the statistically significant ($^{\#}p<0.05$) increase in the level of urinary protein and albumin but decreases the levels of urea, creatinine, and uric acid as compared to normal control. All treatment groups showed statistically significant ($^{***}p<0.001$) improvement in these values compared to diseases control, which was almost comparable as normal control, as shown in **Table 5**.

Electrolyte Concentrations: The effect of the polyherbal combination on electrolyte concentration in serum (Na^+, K^+ & Ca^{++}) and urine (Na^+, K^+ & Ca^{++}) sample of all experimental groups were observed and end of the study. The gentamicin-treated rats show statistically significant ($^{\#}p<0.05$) reduction in electrolyte concentration in the serum sample, but in case of the urine sample it was observed that statistically significant ($^{\#}p<0.05$) increase in electrolyte concentration as compared to normal control. The observed values of treatment groups showed significant improvement in both sample as

compared to disease control while the rats treated with a low dose (25 mg/kg) of aqueous extract combination showed statistically significant ($^{***}p<0.001$) improvement against disease control and other treatment groups as shown in **Table 6**.

TABLE 5: EFFECT OF POLYHERBAL COMBINATION ON BIOCHEMICAL PARAMETERS IN URINE

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

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

TABLE 6: EFFECT OF POLYHERBAL COMBINATION ON ELECTROLYTE CONCENTRATIONS

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
I	148.10±3.11	07.02±0.51	05.48±0.37	68.00±2.18	83.40±3.02	0.82±0.23
II	086.30±2.45$^{\#}$	04.68±0.42$^{\#}$	02.89±0.29$^{\#}$	157.03±3.66$^{\#}$	137.51±3.11$^{\#}$	3.01±0.42$^{\#}$
III	118.52±3.70***	05.03±0.45	03.50±0.27	103.50±2.58***	115.30±2.85***	2.42±0.35
IV	120.63±2.65***	05.43±0.37	04.21±0.19**	107.32±2.62***	107.30±3.00***	2.01±0.25
V	143.64±3.00***	06.88±0.60**	05.20±0.42***	71.43±2.30***	88.20±2.42***	0.87±0.31**
VI	132.89±3.24***	05.99±0.54	04.86±0.28***	83.01±2.41***	97.12±2.08***	0.93±0.42**
VII	121.37±2.86***	04.73±0.46	03.39±0.24	97.02±2.50***	111.29±3.14***	1.90±0.50
VIII	120.80±2.79***	05.00±0.57	04.01±0.23	101.18±2.38***	103.83±2.63***	1.78±0.45
IX	140.08±3.36***	06.78±0.60**	05.28±0.45***	69.00±2.05***	85.28±2.12***	0.85±0.35**
X	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). $^{\#}p<0.05$ statistical significance against normal control; $^{**}p<0.05$ and $^{***}p<0.001$ statistical significance against disease control.

Antioxidant Enzymes Activity: The average values of SOD were observed between 6.37 ± 0.67 U/mg.protein (disease control) to 14.16 ± 0.81 U/mg.protein (normal control) of the experimental groups, which showed statistically significant ($^{\#}p<0.05$) difference in SOD concentration.

All treatment groups showed considerable improvement in SOD, which was almost comparable to normal control groups. In case of CAT, the observed values varied between 12.17 ± 1.53 U/mg.protein (disease control) to 48.40 ± 1.77 U/mg.protein (normal control) of the experimental groups, which showed statistically significant ($^{\#}p<0.05$) reduction in catalase concentration. The other treatment groups showed statistically significant ($^{***}p<0.001$) improvement in CAT, which was almost comparable to normal control. The MDA level of kidney tissues was observed between 1.80 ± 0.18 U/mg. Protein (normal control) to 5.03 ± 0.26 U/mg.protein (disease control) of the experimental groups, which showed statistically significant ($^{\#}p<0.05$) increase in malondialdehyde concentration. The treatment group also shows the considerable improvement in the MDA level, which was almost comparable with normal control, but in case of GSH the kidney tissue level was observed between 7.30 ± 1.02 U/mg.protein (disease control) to 12.84 ± 1.08 U/mg.protein (normal control) of the experimental groups, which showed statistically significant ($^{\#}p<0.05$) reduction in GSH concentration as compared to the normal group while the treatment group show non-significant improvement compared to disease control as shown in **Fig. 1**.

The average value of LPO was observed between 84.27 ± 1.80 µmol/mg of protein (treatment group) to 103.20 ± 2.12 µmol/mg of protein (disease control) of the experimental groups, which showed

statistically significant ($^{\#}p<0.05$) difference in comparison with normal control (84.86 ± 2.00 µmol/mg of protein) and the other treatment groups values comparable to normal control and showed statistically significant ($^{**}p<0.05$ & $^{***}p<0.001$) difference compared to disease control. In case of NO scavenging activity, gentamicin treated group (25.30 ± 1.00 µmol/mg of protein) showed statistically significant ($^{\#}p<0.05$) difference in comparison with normal control (15.30 ± 1.02 µmol/mg of protein). The aqueous extract combination treated groups showed statistically significant ($^{**}p<0.05$) difference in comparison with the disease control group. While the methanol extract combination treated group showed non-significant changes compared to disease control, as shown in **Fig. 2**.

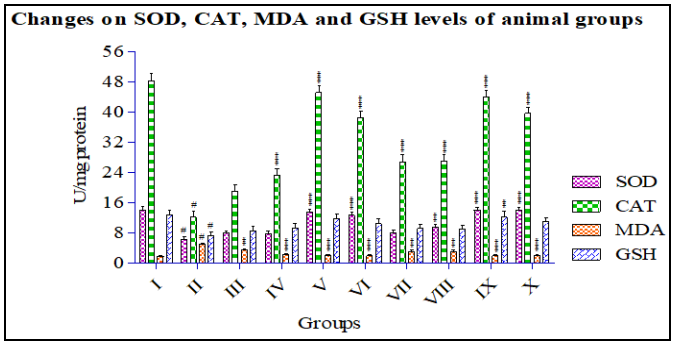

FIG. 1: EFFECT OF POLYHERBAL COMBINATION ON ANTIOXIDANT ENZYMES. Values are given as Mean ± SEM of animal groups (n=6). $^{\#}p<0.05$ statistical significance against normal control, $^{**}p<0.05$ and $^{***}p<0.001$ statistical significance against disease control. SOD=Superoxide dismutase; CAT = Catalase; MDA = Malondialdehyde; GSH = Glutathione

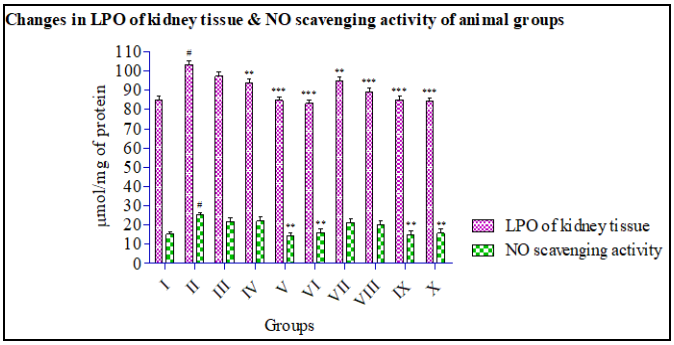

FIG. 2: EFFECT OF POLYHERBAL COMBINATION ON LPO OF KIDNEY TISSUE & NO SCAVENGING ACTIVITY. Values are given as Mean ± SEM of animal groups (n=6). $^{\#}p<0.05$ statistical significance against normal control, $^{**}p<0.05$ and $^{***}p<0.001$ statistical significance against disease control. LPO = Lipid peroxidation; NO = Nitric oxide

Histological Examination:

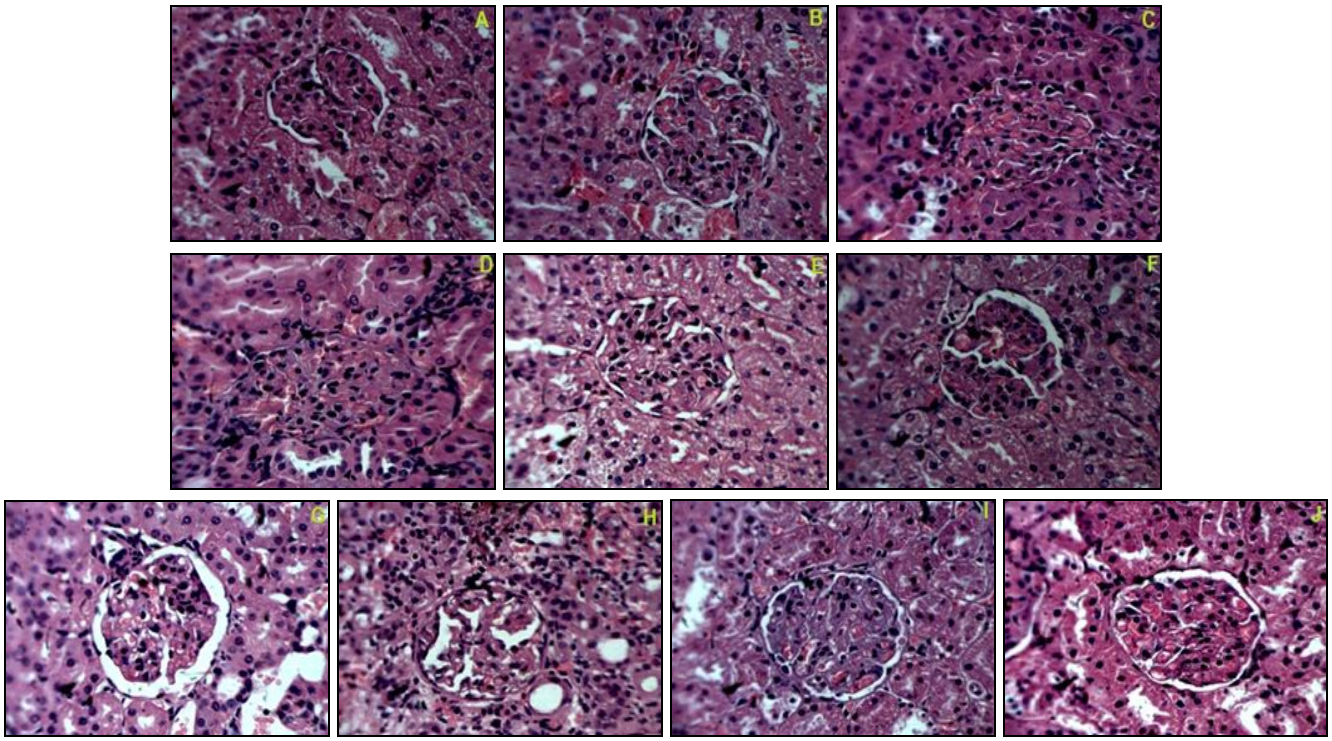

FIG. 3: THE LIGHT MICROSCOPIC IMAGE OF KIDNEY SECTION SHOWING CHANGES IN GLOMERULAR STRUCTURAL AS (A) Normal control group, (B) Gentamicin treated group, (C) Gentamicin + Methanol extract of combination 25mg treated group, (D) Gentamicin + Methanol extract of combination 50mg treated group, (E) Gentamicin + Aqueous Extract of combination 25mg treated group, (F) Gentamicin + Aqueous Extract of combination 50mg treated group, (G) Methanol extract of combination 25mg + Gentamicin treated group, (H) Methanol extract of combination 50mg + Gentamicin treated group, (I) Aqueous Extract of combination 25mg + Gentamicin treated group, (J) Gentamicin + Aqueous Extract of combination 50mg treated group which was stained hematoxylin and eosin (H&E), (H&E), observed at magnification 40x.

The histological features of the kidney of experimental groups were observed brush border cells, glomerular congestion, blood vessel, Bowman's space, tubular edema, necrosis and tubular casts with a disease control group of the animal, while all these parameters are not observed with the normal control group. The treatment group with a low dose (25 mg/kg) shows the significant disappearance of these elevated symptoms compared to the disease control. With the high dose (50 mg/kg), the elevated parameters were not completely suppressing as compare to the low dose of the treatment group, as shown in **Fig. 3**.

DISCUSSION: The phytoconstituents are playing a significant role in restoring of elevated biochemical parameters in the kidney, which affect the normal biological function. Carbohydrate is the primary source of energy, play an essential role to protect blood vessels in the case of chronic kidney disease; as we know the protein is a significant fuel for human and high-protein diet may worsen kidney function in people with kidney disease [20, 21]. The plants' protein plays a beneficial role in the maturation of nephron and protects the damage of glomerular cells of the kidney. It is well-known dyslipidemia contributes to glomerular and interstitial injury of the renal parenchyma, so lipids have significant implications for human renal disease. While, steroids play a crucial role in patients with IgA nephropathy, because it may prevent or delay loss of kidney function [22].

Regarding the kidney, glycosides maintain the cellular transport of sodium and potassium in a variety of tissues, because many drugs are infused into one renal artery, resulting from inhibition of renal tubular function manifested by a profound natriuresis, chlorosis, and diuresis. Coumarins and saponins possess a fantastic anti-inflammatory with antioxidant properties, which plays a protective role during acute kidney injury [23, 24]. The flavonoids reversed the increased expression of enzyme modulators and improved kidney function; while alkaloids protect rat's kidney cells against gentamicin-induced glomerulonephritis. Tannins and terpenoids are useful in chronic renal failure and preventing the further damages of the kidneys [25]. Verity of chemicals such as industrial chemicals, environmental pollutants that cause nephrotoxicity has been increased day by day. These nephrotoxicants can produce various symptoms such as acute renal failure, chronic renal failure, nephrotic syndrome, and other renal disorders [26].

The administration of gentamicin causing selective accumulation in the renal cortex and proximal tubules in rat kidneys leads to inflammation, lesions of proximal tubules, apoptosis, and necrosis of tubular cells. The necrosis of kidney cells has accountable for reducing renal blood flow and the decrease in glomerular filtration rate. The accumulation of Gentamicin is also responsible for the thickening of GBM that plays a critical role in mesangial cell contraction and increased protein content in the ultrafiltrate which contributes to ↓GFR and massive proteinuria manage the Glomerulonephritis.

The polyherbal combination possesses the potent phytoconstituents that responsible for suppressing the elevated factors, which induced after administration of gentamicin in the rats. The herbs used in the study have potent anti-inflammatory activity and individually play a crucial role such as *Angelica officinalis* responsible for urinary retention, *Boerhavia diffusa* act as an excellent diuretic, *Carica papaya* act as immunomodulators and recover kidney tissues, *Cassia fistula* protects deoxyribose from damage, *Cichorium intybus* has a good immunomodulatory action and modulate kidney function, *Ficus hispida* inhibits insulinase activity of kidney, *Fumaria indica* plays a crucial role in the 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.

The therapeutic and prophylactic treatment with the low dose (25 mg/kg) of polyherbal aqueous extract provides significant protection against Gentamicin-induced GN, with maintaining the elevated level of sodium, potassium, and calcium in the plasma and the urine sample. The antioxidant effect associated with the polyherbal combination possessing a potent phytoconstituents on administration produces regular SOD, CAT, and GSH activity to be entirely restored as normal cells [27].

The decreased level of antioxidant enzyme moderately explains the mechanism of nephrotoxicity induced by gentamicin. The accumulation of aminoglycoside in the excretory organ and bind covalently with renal protein also play a critical role in inducing GN [28]. The NO scavenging activity and lipid peroxidation (LPO) of kidney tissue is the ultimate measures related to free radicals generation of reactive oxygen species extremely expected in the induction of GN [29, 30].

CONCLUSION: The overall result observed from the study concluding that the treatment of aqueous extract of the polyherbal combination at a dose of 25 mg/kg orally administered has been significantly prevented all the elevated biological parameters and also improved gentamicin-induced renal damage as confirmed by histopathological examination that showing normal regain of tubular epithelial cells, glomeruli, and tubules.

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REFERENCES:

1. Gonzalez B, Guerra C and Morris D: Dendritic cells in infectious disease, hypersensitivity, and autoimmunity. International Journal of Interferon, Cytokine and Mediator Research 2010; 2: 137-47.
2. Tokodai K, Amada N and Kikuchi H: Outcomes of renal transplantation after end-stage renal disease due to diabetic nephropathy: a single-center experience. Transplantation Proceedings 2012; 44: 77-79.
3. Wetmore JB, Guo H, Liu J, Collins AJ and Gilbertson DT: The incidence, prevalence, and outcomes of glomerulonephritis derived from a large retrospective analysis. Kidney International 2016; 90: 853-60.
4. Eison TM, Ault BH and Jones DP: Post-streptococcal acute glomerulonephritis in children: clinical features and pathogenesis. Pediatric Nephrology 2011; 26: 165-80.
5. Broder A, Mowrey WB, Izmirly P and Costenbader KH: Validation of systemic Lupus Erythematosus diagnosis as the primary cause of renal failure in the United States Renal Data Systems. Arthritis Care Res (Hoboken) 2017; 69: 599-04.
6. Anjum S, Muzaale AD, Massie AB, Bae S, Luo X and Grams ME: Patterns of end-stage renal disease caused by diabetes, hypertension, and glomerulonephritis in live kidney donors. American Journal of Transplantation 2016; 16: 3540-47.
7. Hoy WE, White AV and Dowling A: Post-streptococcal glomerulonephritis is a strong risk factor for chronic kidney disease in later life. Kidney International 2012; 81: 1026-32.
8. Grogan AM, Franssen CFM and Vries CSD: The incidence of primary glomerulonephritis worldwide: a systematic review of the literature. Nephrology Dialysis Transplantation 2011; 26: 414-30.
9. Pandey MM, Rastogi S and Rawat AKS: Indian traditional Ayurvedic system of medicine and nutritional supplementation. Evidence-Based Complementary and Alternative Medicine 2013; 1-12.
10. Sen S and Chakraborty R: Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. Journal of Traditional and Complementary Medicine 2017; 7: 234-44.
11. Vaidya AD and Devasagayam TP: Current status of herbal drugs in India: an overview. Journal of Clinical Biochemistry and Nutrition 2007; 41: 1-11.
12. Shakya AK: Medicinal plants: Future source of new drugs. International Journal of Herbal Medicine 2016; 4: 59-64.
13. Mahima, Rahal A, Deb R, Latheef SK and Samad HA: Immunomodulatory and therapeutic potentials of herbal, traditional/indigenous and ethnoveterinary medicines. Pakistan Journal of Biological Sciences 2012; 15: 754-74.
14. Kandil O, Radwan NM and Hassan AB: Extracts and fractions of *Thymus capitatus* exhibit antimicrobial activities. Journal of Ethnopharmacology 1994; 44: 19-24.
15. Chhabra SC, Shao JF and Mshiu EN: Antifungal activity among traditionally used herbs in Tanzania. The Dar Medicinal Journal 1982; 9: 68-73.
16. Pandey AK, Ojha V, Yadav S and Sahu SK: Phytochemical evaluation and radical scavenging activity of *Bauhinia variegata*, *Saraca asoca* and *Terminalia arjuna* barks. Research Journal of Phytochemistry 2011; 5: 89-97.
17. Golwala DK and Patel LD: Pharmacognostical studies of *Bauhinia variegata* Linn. root. Journal of Young Pharmacists 2009; 1: 36-41.
18. Rajesh G, Padma MP and Usha G: Pharmacognostic and phytochemical screening of *Bauhinia variegata* Linn. Leaves. Journal of Pharmacy Research 2009; 2: 1196-98.
19. OECD guidelines for the testing of chemicals: Adopted on 17th December 2001; Section 4, test no. 423 acute oral toxicity - acute toxic class method 2001; 1-14.
20. Nazar CMJ: The significance of diet in chronic kidney disease. Journal of Nephropharmacology 2013; 2: 37-43.
21. Nisha R, Kannan SSR, Mariappan TK and Jagatha P: Biochemical evaluation of creatinine and urea in patients with renal failure undergoing hemodialysis. Journal of Clinical Pathology and Laboratory Medicine 2017; 1: 1-5.
22. Merlini G and Pozzi C: Mechanisms of renal damage in plasma cell dyscrasias: an overview. Contributions to Nephrology 2007; 153: 66-86.
23. Ivy JR and Bailey MA: Pressure natriuresis and the renal control of arterial blood pressure. The Journal of Physiology 2014; 592: 3955-67.
24. Gajjala PR, Sanati M and Jankowski J: Cellular and molecular mechanisms of chronic kidney disease with diabetes mellitus and cardiovascular diseases as its comorbidities. Frontiers in Immunology 2015; 6: 1-15.
25. Musabayane C: The effects of medicinal plants on renal function and blood pressure in diabetes mellitus. Cardiovascular Journal of Africa 2012; 23: 462-68.

26. Sharma RK, Rajani GP, Sharma V and Komala N: Effect of ethanolic and aqueous extracts of *B. variegata* Linn. on gentamicin-induced nephrotoxicity in rats. Indian Journal of Pharmaceutical Education and Res 2011; 45: 192-98.
27. Linares MV, Bellés M, Albina ML and Sirvent JJ: Assessment of the pro-oxidant activity of uranium in kidney and testis of rats. Toxicology Letters 2006; 167: 152-61.
28. Gurditta PM: Basketful benefits of Papaya. International Research Journal of Pharmacy 2011; 2: 6-12.
29. Somani SM, Husain K, Whitworth C and Trammell GL: Dose-dependent protection by lipoic acid against Cisplatin-induced nephrotoxicity in rats: antioxidant defense system. Pharmacology and Toxicology 2000; 86: 234-41.
30. Ghosh A and Sil PC: Anti-oxidative effect of a protein from *Cajanus indicus* L. against acetaminophen-induced hepato-nephrotoxicity. Biochemistry and Molecular Biology 2007; 40: 1039-49.

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