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Research Article

Effect of Polyherbal and Allopolyherbal Formulation on Streptozotocin-Nicotinamide Induced Diabetic Nephropathy in Rats

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ABSTRACT

Objective: The objectives of the present study were to investigate the effect of polyherbal and allopolyherbal formulation on streptozotocin-nicotinamide induced diabetic nephropathy in rats.

Material and Methods: The polyherbal formulation was prepared by mixing the five holistic extracts of *Emblica officinalis, Gymnema sylvestre, Terminalia arjuna, Tinospora cordifolia and Zingiber officinale. The* extracts were obtained by supercritical fluid extraction (SFE) method. Diabetes mellitus (DM) was induced in rat by streptozotocin (STZ) 65mg/kg i.p. injected 15 min after nicotinamide (NAD) 110 mg/kg, i.p. The diabetic rats were treated with metformin, a polyherbal formulation (PHF) at three dose levels (100, 200 and 400 mg/kg, p.o.) and allopolyherbal formulation (APHF) at 200 mg/kg, p.o. The drugs were administered for 60th days after induction of DM. Blood glucose level (BGL) was measured on 0, 15th, 30th, 45th, 60th days of study whereas glycated hemoglobin (Hb_{A1C}), the plasma insulin level was measured at the end of the study. Various parameters of renal function tests, such as serum creatinine, urea, uric acid, total protein and albumin and blood urea nitrogen (BUN) and markers of oxidative stress such as renal malondialdehyde (MDA) and glutathione (GSH) level, superoxide dismutase (SOD) and catalase (CAT) activities were measured at the end of the study. After 60 days treatment, urine creatinine, urea, uric acid, albumin, urine volume and kidney weight were measured and histopathological examination was also carried out. Result and Discussion: At the end of the study, the diabetic control rats were showed significant increase in BGL, HbA1c and urine volume while treatment of diabetic rats with PHF and APHF was showing a significant decrease in BGL, HbA1c, urine volume. Diabetic rats showed a significant reduction in renal function, which was reflected by an increase in serum creatinine, urea, uric acid and BUN and urine albumin while a decrease in serum total protein and albumin and urine creatinine, urea and uric acid. In addition, STZ-NAD caused renal tubular damage with a higher MDA level, depletion of SOD and CAT activity and GSH level. All the above parameters were significantly reversed with PHF and APHF treatment. Conclusion: This finding suggests that the treatment with PHF and APHF showed significant nephro-protective effect against STZ-NAD induced DN.

Keywords: Diabetic nephropathy, Streptozotocin, Nicotinamide, Polyherbal, Allopolyherbal, Renal function test

INTRODUCTION

Diabetes mellitus is characterized by hyperglycemia and long term complications affecting the kidneys, eyes, nerves and blood vessels. Diabetic nephropathy (DN) is one of the most serious complications in diabetes mellitus and has been the most common cause of end-stage renal disease (ESRD)[1]. ESRD due to diabetes has been estimated to be 30-47% of all incident cases worldwide[2]. Previous reports also suggest that 43% of the chronic renal disease (CRD) patients on dialysis have DN, 60% death cases of diabetes mellitus patients are due to DN, and death cases of diabetes mellitus patients due to renal failure are 17 times more as compared to non-diabetes mellitus patients[3]. A typical morphological change in the diabetic kidney involves an increase in kidney size and weight, increase glomerular volume, accumulation of extracellular matrix in glomeruli that correlates with the loss of renal function such as mesangial expansion, tubulointerstitial fibrosis, and irreversible deterioration[4,5]. DN is a pathological progression from hyper-filtration to micro-albuminurea then to macro-albuminurea and finally to renal failure[6]. A number of factors are important for the development of DN, including hyperglycemia, hypertension, oxidative stress, and inflammation, and have been shown to lead to histological changes[7]. Angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor antagonists are the main therapeutic agents presently seem to produce partial reduction in proteinuria and attenuate progression of CRD to ESRD. However, many patients do not respond to these agents and their progress to ESRD at an early stage. It was

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Table 1: Effect of PHF and APHF on kidney and body weight in STZ-NAD induced diabetic rats.

Groups	Treatment	Kidney weight (gm)	Body weight (gm) (0 day)	Body weight (gm) (60th days)
I	Normal Control	705.0±22.13[b]	193.16±3.96	297.66±7.79[b]
II	Diabetic Control	1179.0±18.79	191.5±4.72	153.5±3.88
III	Metformin 200mg/kg	808.33±28.39[b]	185.33±4.40	222.5±3.81[b]
IV	PHF-A 100mg/kg	914.66±16.23[b]	196.83±4.28	198.16±3.49[b]
V	PHF-B 200mg/kg	876.16±31.73[b]	189.33±2.64	207.0±7.78[b]
VI	PHF-C 400mg/kg	801.16±18.53[b]	192.5±3.83	214.66±8.32[b]
VII	APHF 200mg/kg	789.16±20.99 [b]	186.66±4.46	225.66±6.44[b]

Values are expressed as mean±SEM (n=6) in each group. Where, [C=]$P<0.05$, [b]=$P<0.01$, [a]=$P<0.001$, when compared to diabetic control groups. (One-way ANOVA followed by Dunnette's multiple comparison test) PHF=Polyherbal formulation, APHF (Allopolyherbal formulation) =PHF 100mg/kg+ Metformin 100mg/kg.

Table 2: Effect of PHF and APHF on BGL in STZ-NAD induced diabetic fasted rats.

Groups	Treatment	BGL at different time interval after treatment				
		Initial	15th days	30th days	45th days	60th days
I	Normal Control	76.16±3.47	85.00±3.22[b]	86.83±4.24[b]	83.5±4.46[b]	80.83±3.97[b]
II	Diabetic Control	219.5±5.34	223.33±8.02	246.66±3.33	276.66±4.41	304.33±9.46
III	Metformin 200mg/kg	217.16±5.96	183.33±4.41[b]	170.00±4.83[b]	158.33±3.41[b]	142.5±3.81[b]
IV	PHF-A 100mg/kg	217.6±5.32	199.33±6.25[c]	189.33±4.63[b]	170.83±6.63[b]	157.5±3.81[b]
V	PHF-B 200mg/kg	221.83±4.46	193.5±6.82[b]	179.16±5.38[b]	162.83±3.16[b]	150.33±5.04[b]
VI	PHF-C 400mg/kg	216.5±6.40	185.66±3.72[b]	169.5±4.64[b]	159.5±5.73[b]	145.83±6.63[b]
VII	APHF 200mg/kg	221.00±6.28	173.83±4.12[b]	165.00±4.65[b]	151.51±4.41[b]	136.66±3.33[b]

Values are expressed as mean±SEM (n=6) in each group. Where, [C=]$P<0.05$, [b]=$P<0.01$, [a]=$P<0.001$, when compared to diabetic control groups. (One-way ANOVA followed by Dunnette's multiple comparison test) PHF=Polyherbal formulation, (Allopolyherbal formulation) = PHF 100mg/kg+ Metformin 100mg/kg, BGL=Blood Glucose Level.

reported that there has been increasing evidence in the protective effect of PHF as potential adjuvant therapy to prevent or delay diabetic complication[1]. The concept of polyherbalism has been highlighted in *Sharangdhar Samhita*, an Ayurvedic literature dating back to 1300 AD. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity[8]. PHF enhance the therapeutic action and reduce the concentrations of single herbs thereby reducing adverse events. In the modern era, herbal formulations have gained greater importance than ever before, mainly due to their efficacy and easy availability as well as less side effects as compared to the synthetic drugs[9]. World Health Organization (WHO) also estimated that 80% of the world's people still rely mainly on traditional medicines for their health care. The subcontinent of India is well-known to be one of the major biodiversity centers with about 45,000 plant species. Amla *(Emblica officinalis)*[10,11], Gudmar *(Gymnema sylvestre)*[12,13], Arjuna *(Terminalia arjuna)*[14,15], Guduchi *(Tinospora cordifolia)*[16,17] and *Ginger (Zingiber officinale)*[18,19] are well-known herbs available throughout India and they are commonly used for the treatment of various diseases including diabetes mellitus and diabetic complications. All the extracts of herbs used for the study were obtained by supercritical fluid extraction (SFE) method and these extracts are called holistic extract. They are pure, highly powerful and extremely concentrated and free from any residues of chemical insecticides, pesticides & herbicides[20]. SFE allows the processing of plant material at low temperatures, hence limiting thermal degradation, and avoids the use of toxic solvents[21]. The main objective of the present study is to prepare a polyherbal formulation of holistic extracts and evaluate its nephro-protective activity in the STZ-NAD induced diabetic nephropathy in rats and to check PHF treatment could have a synergistic effect with metformin.

MATERIAL AND METHODS

Materials

Drugs and Chemicals

Streptozotocin (STZ) was procured from Chemvenio, LIC, Gulbarga, Karnataka, Nicotinamide (NAD) was procured from SDS Neutraceuticals, Karad and Metformin tablet was procured from USV Pharma. All other chemicals used in this study were of analytical grade.

Holistic Extracts

Table 3: Effect of PHF and APHF on serum parameters in STZ-NAD induced diabetic rats.

Grop	Treatment	Glycated hemoglobin (%)	Insulin (mIU/l plasma)	BUN (mg/dl)	Creatine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Total protein (g/dl)	Albumin(g/dl)
1	Normal saline	4.06 ±0.24[b]	15.27 ±0.78[b]	17.26 ±0.48[b]	0.53 ±0.026[b]	25.33 ±1.97[b]	2.94 ±0.12[b]	4.25 ±0.31 [b]	7.15 ±0.25[b]
2	Diabetic control	9.91 ±0.47	8.85 ±0.29	30.18 ±0.95	1.48 ±0.166	44.16 ±3.24	5.01 ±0.31	2.21 ±0.21	4.37 ±0.20
3	Metformn 200mg/kg	6.10 ±0.21[b]	13.15 ±0.45[b]	22.15 ±0.83[b]	0.65 ±0.029[b]	32.5 ±1.96[b]	3.58 ±0.16[b]	3.61 ±0.17[b]	6.21 ±0.12[b]
4	PHF-A 100mg/kg	7.36 ±0.22[b]	10.95 ±0.50[c]	25.28 ±0.77[b]	0.865 ±0.030[b]	35.66 ±1.76[c]	3.78 ±0.16[b]	3.11 ±0.17[c]	5.42 ±0.26[c]
5	PHF-B 200mg/kg	7.01 ±0.27[b]	12.17 ±0.37[b]	23.71 ±0.65[b]	0.75 ±0.019[b]	33.66 ±1.54[b]	3.64 ±0.08[b]	3.23 ±0.20[b]	5.65 ±0.30[b]
6	PHF-C 400mg/kg	6.45 ±0.26[b]	13.01 ±0.33[b]	22.00 ±0.89[b]	0.68 ±0.028[b]	30.16 ±2.34[b]	3.60 ±0.13[b]	3.58 ±0.14[b]	6.02 ±0.24[b]
7	APHF 200mg/kg	5.98 ±0.27[b]	14.05 ±0.28[b]	20.33 ±1.62[b]	0.625 ±0.028[b]	28.16 ±1.30[b]	3.17 ±0.16[b]	3.63 ±0.15[b]	6.47 ±0.16[b]

Values are expressed as mean±SEM (n=6) in each group. Where, [C=]$P<0.05$, [b]=$P<0.01$, [a]=$P<0.001$, when compared to diabetic control groups. (One-way ANOVA followed by Dunnette's multiple comparison test) PHF=Polyherbal formulation, (Allopolyherbal formulation) = PHF 100mg/kg+ Metformin 100mg/kg

All the extracts were obtained by supercritical fluid extraction (SFE) method and they are procured from Nisarga Biotech Pvt. Ltd, Satara as a gift sample.

Experimental Animals

Albino Wistar rats of either sex weighing 180-200g were procured from Shri Venkateshwara Enterprises, Banglore. All animals were maintained under standard laboratory conditions of temperature (22 ± 2°C) and humidity 50 ± 15% with 12 hours day: 12 hours night cycle. Rats had free access to water and rodent pellet diet (Hindustan Lever Ltd, Bangalore, India). Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The experimental protocol has been approved by the Institutional Animal Ethics Committee of the Satara College of Pharmacy, Satara and all the animal experiments were carried out according to CPCSEA guidelines[22].

METHODS

Preparation of Polyherbal Formulation

The polyherbal formulation was prepared by mixing all the five holistic extracts by taking an equal quantity of the individual extract and adding 1% CMC solution as a surfactant, continuously triturating till uniform suspension are formed. The quality of the finished product was evaluated as per the WHO guidelines for the quality control of herbal materials.

Acute Oral Toxicity of Polyherbal Formulation

Acute oral toxicity of the polyherbal formulation was carried out as per the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423. The principle involves a stepwise procedure with the use of a minimum number of animals per step to obtain sufficient information on the acute toxicity of the test substance to enable its classification. Healthy Wistar rats (3 animals/dose) of either sex were used for the experiment. Overnight fasted rats were orally fed with a polyherbal formulation in increasing dose levels of 5, 50, 300 and 2000 mg/kg body weight respectively. The animals were observed for their behavioral (alertness, restlessness, irritability and fearfulness), neurological (spontaneous activity, reactivity, touch response, pain response and gait) and autonomic (defecation and urination) profiles continuously for 24 h. Thereafter the animals were observed daily for 14 days for morbidity and mortality.

Induction of Diabetic Nephropathy

Type 2 DM was induced in overnight fasted adult albino Wistar rats by a single intraperitoneal injection of 65 mg/kg streptozotocin (STZ) 15 min after the i.p. administration of 110 mg/kg of nicotinamide (NAD). STZ was dissolved in citrate buffer (pH 4.5) and NAD was dissolved in normal saline. Hyperglycemia was confirmed by the elevated blood glucose levels (BGL), determined at 72 hrs and then on day 7 after injection. Animals with fasting BGL greater than 250 mg/dl were considered as diabetic and were used for DN studies[23].

Experimental Design

Experimental animals were randomly divided into seven groups, each consisting six animals.

Group I: Normal rats treated with vehicle (1% CMC solution, 10 ml/kg)

Group II: Diabetic rats treated with vehicle (1% CMC solution, 10 ml/kg)

Group III: Diabetic rats treated with Metformin (200 mg/kg)

Group IV: Diabetic rats treated with PHF-A (polyherbal formulation, 100 mg/kg)

Group V: Diabetic rats treated with PHF-B (polyherbal formulation, 200 mg/kg)

Group VI: Diabetic rats treated with PHF-C (polyherbal formulation, 400 mg/kg)

Group VII: Diabetic rats treated with APHF (PHF100 mg/kg plus Metformin 100 mg/kg)

Collection of Blood and Urine Samples

Table 4: Effect of PHF and APHF on urine volume and urine parameters in STZ-NAD induced diabetic rats.

Groups	Treatment	Urine creatinine (mg/dl)	Urine urea (mg/dl)	Urine uric acid (mg/dl)	Urine Albumin (mg/24 h)	Urine volume (ml)
I	Normal Control	75.66±4.7[b]	21.83±0.90[b]	22.00±2.04[b]	0.52±0.05[b]	10.25±0.62[b]
II	Diabetic Control	39.16±5.23	9.5±0.76	9.6±0.51	2.48±0.24	36.5±1.9
III	Metformin 200mg/kg	61.83±3.01[b]	17.16±1.07[b]	16.55±0.44[b]	1.28±0.18[b]	20.16±1.42[b]
IV	PHF-A 100mg/kg	53.16±3.42[c]	14.66±0.88[c]	13.51±0.81 [c]	1.6±0.11[b]	26.66±2.69[b]
V	PHF-B 200mg/kg	57.66±3.22[b]	15.33±1.43[b]	14.2±0.63[b]	1.41±0.09[b]	23.83±1.97[b]
VI	PHF-C 400mg/kg	60.83±3.53[b]	16.16±1.5[b]	16.04±0.35[b]	1.35±0.17[b]	21.83±1.64[b]
VII	APHF 200mg/kg	64.33±3.42[b]	18.5±1.2[b]	17.1±0.48[b]	1.05±0.12[b]	19.16±1.64[b]

Values are expressed as mean±sem (n=6) in each group. Where, [c]=p<0.05, [b]=p<0.01, [a]=p<0.001, when compared to diabetic control groups. (one-way anova followed by dunnette's multiple comparison test) PHF=polyherbal formulation, (allopolyherbal formulation) = PHF 100mg/kg+ metformin 100mg/kg.

All the aforementioned treatments were started at 1 week after injection of STZ-NAD. All the treatments were given once daily to the respective group of animals for 60 days. After completion of treatment, the animals were kept for 24 hours in metabolic cages for urine collection. Volume of urine noted and the samples were used to analyze the urine creatinine, urea, uric acid, and albumin levels. The urine samples were analyzed by using urine analyzer (Urilyser-Aspen). Finally, blood samples were collected through cardiac puncture under anesthesia. The blood samples were used to analyze the blood glucose, HbA1c, insulin and serum creatinine, urea, and uric acid, total protein and albumin. The blood samples were analyzed by using automated random access clinical chemistry analyzer (TRANSASIA-EM200).

Estimation of Biomarkers of Oxidative Stress

The kidney was removed and kept on precooked (autoclaved) inverted Petri dish in cold conditions with ice cubes. The tissues were cross chopped with a surgical scalpel into fine slices in chilled 0.25 M sucrose, quickly blotted on filter paper. They were minced and homogenized in 10 mM Tris-HCl buffer, pH 7.4 with 25 strokes of tight teflon pestle of glass homogenizer (Sigma-Aldrich -Automatic Tissue processor) at a speed of 10,000 × g at 0°C. The clear supernatant obtained was used for the assay of lipid peroxidation (MDA) content, endogenous anti-peroxidative enzymes such as SOD, CAT and GSH[1].

Histopathological Examination

After completion of treatment the rats were sacrificed by ether anesthesia and the kidneys were rapidly dissected out and washed immediately with saline. The weights of kidneys were taken. The kidneys were fixed in 10% formalin and given to Disha Diagnostic Center, Satara for histopathological exam. These organs were embedded in paraffin. Then the slicing of organs was carried out by using Microtome machine (Howerlab-Spencer type). Then they were stained with hematoxylin and eosin and observed for histopathological changes under microscope[24].

Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM), statistical difference was tested by using one-way analysis of variance (ANOVA) followed by Dunnette's multiple comparison tests. A difference in the mean P<0.05 was considered as statistically significant.

RESULTS

Effect of PHF and APHF on kidney and body weight in STZ-NAD induced diabetic rats.

There was a significant (P<0.01) increase in kidney weight and significant (P<0.01) decrease in body weight after the 8th week in diabetic control rats as compared to normal control rats, whereas the treatment with metformin, PHF-A, PHF-B, PHF-C and APHF showed a significant (P<0.01) reduction in kidney weight and significant (P<0.01) increase in body weight as compared to diabetic control rats. [Table 1].

Effect of PHF and APHF on BGL in STZ-NAD induced diabetic fasted rats.

After daily administration of the metformin, PHF-A, PHF-B, PHF-C and APHF for 60 days significantly (P<0.01) decreased BGL in diabetic rats compared to untreated diabetic rats. Treatment with metformin, PHF-A, PHF-B, PHF-C and APHF showed significant (P<0.01) reduction in BGL by 34.38%, 27.61%, 32.23%, 32.48% and 38.16% respectively, when compared with the diabetic control group. BGL was measured randomly at 0, 15th, 30th, 45th and 60th days of study. BGL in diabetic rats was raised nearly 2 to 2.5 fold as compared to normal control rats. The raised levels of BGL declined sharply after oral administration of metformin, PHF-A, PHF-B, PHF-C and APHF. When comparisons were made between '0' day and 60thday of treated groups, there was highly statistically significant (P<0.01) reduced in BGL. As far as the relative efficacy is concerned, APHF (200 mg/kg) produced more significant (P<0.01) anti-hyperglycemic activity than metformin, PHF-A, PHF-B and PHF-C treated rats. [Table 2]

Effect of PHF and APHF on serum parameters in STZ-NAD induced diabetic rats.

In the diabetic control rats, HbA1c, BUN, creatinine, urea, uric acid level were significantly (P<0.01) increased and insulin, total protein and albumin level were significant (P<0.01) decrease when compared to normal control rats. The diabetic rats treated with metformin, PHF-A, PHF-B, PHF-C and APHF showed a significant (P<0.01) reduction in HbA1c, BUN, creatinine, urea, uric acid level, whereas significantly (P<0.01) increase in

Table 5: Effect of PHF and APHF on markers of oxidative stress in renal tissue in STZ-NAD induced diabetic rats.

Groups	Treatment	MDA (nm of MDA/g of tissue)	SOD (U/g of tissue)	CAT (µm of H_2O_2 consumed/g of tissue)	GSH (µg/g of tissue)
I	Normal Control	40.00±2.99[b]	133.33±4.59[b]	452.0±8.25[b]	62.88±3.63[b]
II	Diabetic Control	126.5±3.99	58.33±3.65	153.2±5.53	18.33±1.20
III	Metformin 200mg/kg	85.16±3.36[b]	103.16±4.81[b]	305.6±7.40[b]	40.0±3.20[b]
IV	PHF-A 100mg/kg	98.66±5.92[b]	79.83±2.70[b]	246.4±5.15[b]	31.16±1.5[b]
V	PHF-B 200mg/kg	97.33±3.80[b]	83.66±3.64 [b]	270.4±4.61[b]	36.0±2.94[b]
VI	PHF-C 400mg/kg	87.33±2.98[b]	96.66±3.99[b]	305.0±8.71[b]	39.66±2.36[b]
VII	APHF 200mg/kg	74.66±4.03[b]	109.83±4.70[b]	309.0±8.47[b]	33.71±5.05[b]

Values are expressed as mean±SEM (n=6) in each group. Where, [C=]$P<0.05$, [b]=$P<0.01$, [a]=$P<0.001$, when compared to diabetic control groups. (One-way ANOVA followed by Dunnette's multiple comparison test), SEM=Standard error of mean, MDA=Malondialdehyde, SOD=Superoxide dismutase, CAT=Catalase, GSH=Glutathione, PHF=Polyherbal formulation, APHF (PHF 100mg/kg+ Metformin 100mg/kg).

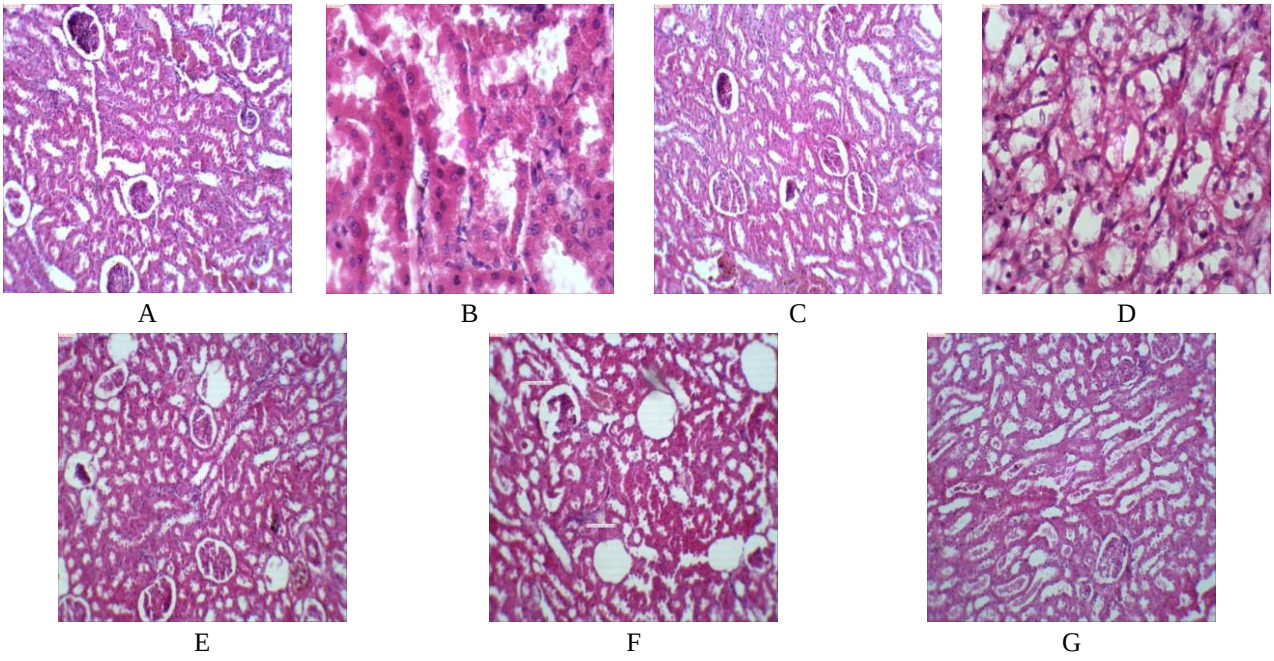

Figure 1: Light microscopy of renal tissues from rats, A: Normal Control, B: Diabetic Control, C: Metformin 200mg/kg, D: PHF-A 100mg/kg, E: PHF-B 200mg/kg, F: PHF-C 400mg/kg, G: APHF 200mg/kg, PHF=Polyherbal formulation, APHF (PHF 100mg/kg+ Metformin 100mg/kg).

insulin, total protein and albumin level as compared to diabetic control rats. However, the treatment with APHF showed more significant (P<0.01) effect on all these serum parameters as compared to metformin, PHF-A, PHF-B and PHF-C treated rats. [Table 3]

Effect of PHF and APHF on urine volume and urine parameters in STZ-NAD induced diabetic rats.

In diabetic control rats urine creatinine, urea, uric acid was significant ($P<0.01$) decreased and urine volume and urine albumin were significant ($P<0.01$) increased when compared to the normal control rats. When diabetic rats treated with metformin, PHF-A, PHF-B, PHF-C and APHF showed a significant increased in urine creatinine, urea, uric acid and significant ($P<0.01$) reduction in urine volume and urine albumin as compared to diabetic control rats, however APHF showed more significant ($P<0.01$) effects as compared to metformin, PHF-A, PHF-B and PHF-C treated rats. [Table 4].

Effect of PHF and APHF on markers of oxidative stress in renal tissue in STZ-NAD induced diabetic rats.

The content of MDA, the end product of lipid peroxidation and marker of oxidative stress was significant ($P<0.01$) increased in renal tissue of diabetic control rats as compared to normal control rats after 60 days of the study. There was a significant ($P<0.01$) decrease in the levels of GSH, an endogenous antioxidant and antiperoxidative enzymes (SOD and CAT) in renal tissue as compared to normal control rats. The treatment of diabetic rats with metformin, PHF-A, PHF-B, PHF-C, APHF showed a significant ($P<0.01$) decrease in the levels of MDA and significant ($P<0.01$) increase in GSH level and SOD and CAT activities as compared to diabetic control rats. However APHF showed more antioxidant activity as compared with metformin, PHF-A, PHF-B and PHF-C group rats. [Table 5].

Effect of PHF and APHF on renal tissue in STZ-NAD induced diabetic nephropathy in rat.

Renal biopsy of normal control rats showed normal tissue cortex containing several glomeruli, normal capsular space, normal interstitial and normal tubules. The architecture of the kidney was disturbed in diabetic control rats as compared to normal control rats. Renal biopsy of diabetic rats showed glomerulosclerosis, tubular vacuolization, interstitial fibrosis, and glomeruli basement membrane were thickened and there was an increase in capsular space. The treatment with metformin in diabetic nephropathy rat, renal biopsy shows a renal cortex containing normal glomeruli without the obliteration of glomerular vessels and moderate tubular vacuolization and thickening of glomerular basement membrane. The treatment with polyherbal formulation showed renal cortex containing glomeruli, which are normal in number and size, mild to moderate glomerular necrosis, interstitial fibrosis, tubular vacuolization, as far as efficacy is concerned, a polyherbal formulation showed dose dependant renal protection. However, the treatment with allopolyherbal formulation showed a mild tubular swelling, interstitial fibrosis and thickening of glomerular basement membrane with absence of glomerulosclerosis. [Figure 1.] *Effect of PHF and APHF on renal tissue in STZ-NAD induced diabetic nephropathy in rat.*

DISCUSSION

Diabetic nephropathy is a major micro-vascular complication of both type 1 and type 2 diabetes[25]. DN in type 2 diabetes is the most common cause of end-stage renal disease and one of the leading causes of morbidity and mortality worldwide[1]. Preventing the progression of DN has been a challenge in biomedical research. Administration of STZ-NAD in Wistar rats produced marked and sustained increase in blood glucose level. STZ cause diabetes by the rapid depletion of β-cells of pancreas. Moreover, when administered along with nicotinamide, it causes minor damage to pancreatic β-cells[3]. Increased levels of serum glucose, creatinine, urea, uric acid and BUN are the markers of DN**.** Diabetes induced in rats is associated with the characteristic loss of body weight, which is due to increased muscle wasting and due to loss of tissue proteins[26,27]. Diabetic rats treated with the PHF and APHF showed an increase in body weight when compared to the untreated diabetic rats, which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis and glycogenolysis[28]. The kidney weight of the diabetic rats increased significantly due to renal enlargement, which is one of the key features occurring during initial changes by DN. In earlier stages of diabetic nephropathy, hypertrophy and hyper-functioning of the kidneys with a typical increase in kidney size and glomerular filtration rate is observed[5]. Diabetic rats treated with the PHF and APHF showed significant decrease in kidney weight, when compared to the untreated diabetic rats, which may be due to its nephro-protective effect. In this study, the BGL was significantly increased in the STZ-NAD induced diabetic nephropathy in rats[3], however PHF and APHF treatment significantly reduced the blood glucose levels. These results showed that PHF and APHF reduced BGL might provide the first step for the protective effects against the STZ-NAD induced diabetic nephropathy. The significantly increased levels of $HbA1_C$, BUN, serum creatinine, serum urea and serum uric acid and significantly decrease levels of serum total protein and serum albumin levels in the STZ-NAD induced diabetic nephropathy in rats. In the present study, administration of PHF and APHF has showed significant reduction in elevated level of $HbA1_C$, BUN, serum creatinine, serum urea and serum uric acid and significant increase in reduced levels of serum total protein and serum albumin as compared to diabetic control rats. Diabetes is known to trigger reactive oxygen species (ROS) in kidney, retina, and heart[25]. Oxidative stress induced by the hyperglycemic milieu drives the development of complications including diabetic nephropathy[29]. Several earlier investigations have confirmed the role of oxidative stress in developing diabetes mediated disorders, possibly via the formation of free radicals[30]. Several studies have shown that oxidative stress plays a major role in the pathogenic pathway of diabetic injuries. The free radicals such as superoxide and lipid peroxidation product like MDA can induce cell and tissue damage[1]. Administration of antioxidant herbs such as *Emblica officinalis*[11], *Tinospora cordifolia*[16] *Zingiber officinale*[19] and antiperoxidative like SOD, CAT and GSH protect the cells and tissue against oxidative stress mediated injuries[31] and in this way treatment with PHF and APHF reduced the development of DN. The diabetic control rats showed renal oxidative stress by a decrease in SOD, CAT and GSH due to diabetes induced hyperglycemia[25]. Treatment of diabetic rats with PHF and APHF reduced renal oxidative stress, indicating the antioxidant potential of polyherbal formulation[25]. Treatment with PHF and APHF increased antioxidant potential of SOD, CAT and GSH in STZ-NAD induced diabetic nephropathy in rats. However, co-administration of PHF and metformin has a more beneficial effect than when administered singly. In the present study, renal of normal rats showed normal morphology of glomerular and tubular architecture. Renal of diabetic rats showed glomerular damage and severe destruction of tubules occurs due to factors such as glomerular hyperplasia, tubular hypertrophy and interstitial expansion[3]. Treatment with PHF and APHF prevent glomerular necrosis, tubular swellings, glomerular and renal fibrosis.

CONCLUSION

These results indicate that the treatment with PHF and APHF showed significant nephro-protective effect against STZ-NAD induced DN. However, concomitant administration of both showed a better nephro-protective effect than Polyherbal formulation or metformin alone treatment by virtue of amelioration of lipid peroxidation as well as due to improvement of renal function. Finally, it was concluded that adjuvant therapy of PHF with

metformin might prevent or delay the diabetic nephropathy.

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Conflict of Interest

We declare that we have no conflict of interest.

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