Effect of poly-herbal formulation in experimentally induced diabetic nephropathy in rats

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Abstract

Introduction: Failure of kidney due to diabetes mellitus (DM) is a major outcome affecting around 25% of patients. In present times treatments related to diabetic kidney disease does not serves to its best with complications. Thus rationale for ongoing study was to scout the benefits of polyherbal formulation (PHF) in the disease condition.

Objectives: Study was to understand the hydroalcoholic extract of *Withania somnifera* (leaves), *Juniperus communis* (barries) and *Tinespora cordifolia* (stem) for Kidney protective effect in streptozotocin (STZ) and Nicotinamide (NA) induced diabetic nephropathy (DN) in rats.

***Materials and Methods*:** Male diabetes Wistar species were grouped in five, Normal control (NC), Disease control (DC), standard control (Metformine 70 mg/kg, p.o), T1 (Polyherbal formulation 100 mg/kg, p.o.) and T2 (Polyherbal formulation 200 mg/kg, p.o). the regime was or 6 weeks and biochemical investigations were carried out followed by histopathology of the kidney.

***Results*:** Animals without treatments were severely hyperglycemia with renal function. Animals of standard and Polyherbal formulation (PHF) groups developed significantly lower plasma glucose levels, and normal dropping volumes indicating a significant loop filtration rate. Decrease in proteinuria, creatinine and Bilirubin levels were observed as that of untreated animals.

***Conclusion*:** On administration with PHF the effect was beneficial on DN and thus, improvement of renal function parameters was observed.

Keywords: Diabetic kidney malfunction, Anti-oxidant, Serum creatinine, natural, PHF (Polyherbal formulation).

Introduction

Diabetes mellitus generally characterized as hyperglycemia with a long-term association affecting body organs like kidneys, eyes, nerves, blood vessels etc. Diabetic nephropathy Owing to its complications it becomes a serious factor causing to an end stage renal disease (ESRD)[1]. Worldwide 30-47% cases of ESRD are estimated due to diabetes mellitus [2, 3]. Researchers have claimed that 60% death cases are seen in DN. Patients with chronic renal disease who are on dialysis develop DN and due to which deaths are seen in 43% of patients. This is at an alarming state as compared with non diabetic patients which accounts to 17 times of those with diabetic patients [4].

Prevention of diabetic complications, can be an alternative use of herbs or natrula origins which are either used in single or as combinations for synergistic effects [5]. Still, prevailing herbal formulations are used for the remedy but the effects are not desired [6]. Withania somnifera (leaves), Tinospora cordifolia (stem) and Juniperus communis (barrirs) shows antidiabetic, hyperlipidemic,

anti-inflammatory and free radical moieties [7-13]. So these plants are selected for the preparation of polyherbal formulation and no scientific validations are are available of its effect of their polyherbal formulation in DN. Hence a scientifically validated investigation has been done for its assessment on streptozotocin (STZ) and Nicotinamide (NM) induced DN in rats.

Table 1: Plant profile.

Herb Name	Part Used	Uses
Juniperus communis (Common Juniper)	Berry	Anti-diabetic, Anti-microbial, Anti-inflammatory
Tinospora cordifolia (Guduchi)	Stem	Anti-inflammatory, Anti-Oxidant
Withania somnifera (Ashwagandha)	Leaves	Anti-oxidant, Anti-hyperglycemic, Hypolipidemic, Anti inflammatory

Materials and Methods

Protocol Approval: Healthy wistar rats (male) from 200-250g were used for the stud and they were approved by Institutional Animal Ethics Committee (IAEC of Parul Institute of Pharmacy and Research with protocol number : PIPR 984/2021/05).

Animals were acclimatized in polypropylene cages with standard laboratory conditions followed by CPCSEA guidelines.

Collection and authentication of plant material: *Juniperus communis* barries were purchased online from amazon. *Withania somnifera* and *Tinospora cordifolia* were obtained from herbal Garden of Parul University, Vadodara. All plants were authenticated by Parul Institute of Ayurveda, Parul University, Vadodara, Gujarat and a voucher specimen is preserved.

Preparation of extract: Equal quantity of 500 gm plant parts were weighed accurately and were extracted with 50% hydroalcoholic solvent for 72hrs. After 72hrs the extracts were cooled and filtered. The filtrate was concentrated over rotary evaporator to its 1/3rd volume. After concentrating to 1/3rd volume it was transferred to water bath at a temperature $60^{O} \pm 2^{O}C$ for further concentration. The final product was weighed and dried in desiccators for further use.

Acute toxicity: OECD-425 were followed for acute toxicity study. As medicinal plants were found to be very safe in previous studies dose of 2000 mg/kg was given. Each phase three animals were used. PHF at doseof 2000 mg/kg of bodyweight orally was given to each animal. Animals were observed every 30 minutes till 24 hours to 14 days. No signs of toxicity, morbidity was observed. Later 5000 mg/kg of bodyweight was given and observed. No abnormal behavior and death were seen. Hence, dose of 100 mg/kg as lower dose and 200 mg/kg as higher dose were taken up for the study.

Induction: Rats fasted with 8 hours before administration of Stereptozotocine (STZ) and Nicotinamide (NA). Stereptozotocine in citrate buffer (pH: 4.5) and NA was prepared in distilled water. NA (110 mg/kg, i.p) is administered 15 minutes before the administration of STZ (50 mg/kg, i.p). Biochemical analysis were performed with a strip detection method. Animal level less than 250 mg/dL were taken up for experiment[14,15].

Experimental design

Table 1: Experimental study Design.

Group no.	Name of drug to be administered	No. of animal
Normal control	Water	6
Disease Control	Streptozotocin (50mg/kg-i.p)+Nicotinamide (110mg/kg-i.p)	6
Standard	Streptozotocin (50mg/kg-i.p)+Nicotinamide (110mg/kg-i.p)+Metformin (70mg/kg-p.o)	6
Test-1	Streptozotocin (50mg/kg-i.p)+Nicotinamide (110mg/kg-i.p)+Low dose PHF (p.o)	6
Test-2	Streptozotocin (50mg/kg-i.p)+Nicotinamide (110mg/kg-i.p)+High dose PHF (p.o)	6

Estimation of blood glucose: They were determined by using DR Morphen Glucometer BG-03 at 0 to 6 weeks after commencing the treatment regime.

Analysis of Urinary protein concentration: Animals of 6 weeks kept in metallic cages for collection of urine. Urine volume was at 12 hr was taken. Its protein concentration was observed by sulfosalicylic acid method[16]. Graph at absorbance of 500 nm was employed in Table 2. 1.25 ml placed in all tubes and desired quantity of reagent was added and observed at 5 minutes in 500 nm recoreded in Table 2.

Blood Glucose Level (mg/dl) (Mean ± SEM)					
Week	Normal Control (NC)	Disease Control (DC)	Standard (Metformine 70 mg/kg)	Test I (100 mg/kg)	Test II (2mm mg/kg)
0	87 ±1.59	301.5 ±3.12****	312.5 ±5.70****	310.5 ±7.05****	296.5 ±4.20****
2	90 ±1.46	314.6±4.16****	188.8±3.11****	256.1±4.12**	225.3±4.28***
4	90.66 ±1.42	312.5±3.59****	178±1.98*****	288±4.78***	203.8±7.16***
6	88.66 ±1.94	339.5±7.58****	165±4.07*****	190.8±3.38***	172.1±3.40***

Table 2: Perparation of standard curve of sulfosalisaylic acid.

Test tube no.	Volume of albumin 10 mg/ ml solution added (ml)	0.9% NaCl (ml)	Final protein concentration (g/L)
1	0.05	9.95	0.05
2	0.1	9.0	0.1
3	0.2	9.8	0.2
4	0.5	9.5	0.5
5	1.0	9.0	1.0

For analysis, desired filtered urine (1.25 mL) was treated with reagent solution in the reaction tube. Blank with 3.75 mL of 0.9% NaCl solution was taken and measured at 500 nm..

Determination of BUN and serum creatinine levels: Samples of 6 weeks from overnight fasted rats wre sent to the Central Laboratory of Parul sevashram hospital, Waghodia, Vadodara for the estimation of BUN and serum creatnine.

Kidney weight: Animals were sacrificed after 8 weeks and kidneys were isolated, weighed using analytical weighing balance.

Histopathology of kidney: The isolated organs were studied for histopathological findings.

Statistical analysis: One-way ANOVA with Dunnett's multiple comparisons test, as mean ± standard error of mean (SEM) was done

Result

Blood glucose levels: Diabetic animals were elevated with PGL as compared to disease control. Standard and PHF resulted in lower PGL (Table 4).

Table 3: Blood glucose level.

Values are as Mean± SEM (n=6), where ***p<0.0001 compared to normal group.

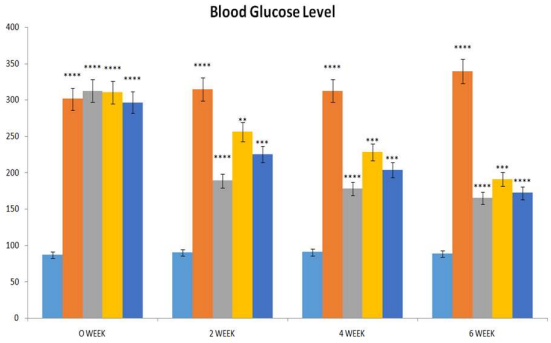


Figure 1 Blood glucose level.
****p<0.0001, ***p<0.0001, **p<0.001, *p<0.01

Serum creatinine

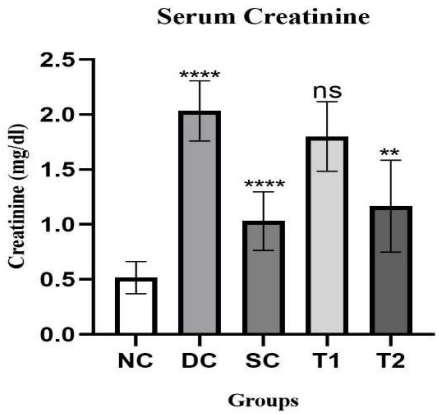
Table 4: Serum creatinine

Group	Treatment	Serum Creatinine Level (mg/dL) (Mean ± SEM)
1	Normal Control	0.55±0.07
2	Disease Control	1.9±0.13****
3	STD (Metformine 70 mg/kg)	0.9±0.10****
4	Test I (100 mg/kg)	1.8±0.15ns
5	Test II (200 mg/kg)	1.35±0.20**

Values are expressed as Mean± SEM (n=6), where p<0.0001 when compared to normal group.

*Significantly different from disease control, ****(p<0.0001), **(p<0.001), ns= non significant.

Figure 2 Serum creatinine



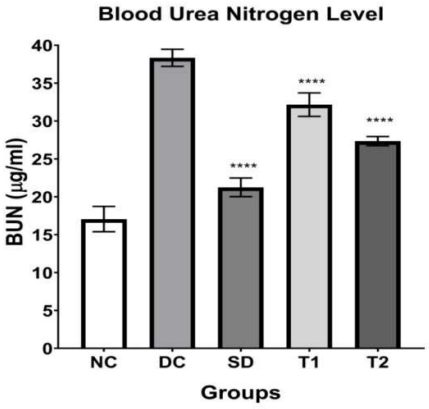
****p<0.0001, **p<0.001, ns= Non significant

Blood urea nitrogen (BUN)

Table 5: Blood urea nitrogen.

Group	Treatment	Blood Urea Nitrogen (mg/dL) (Mean ± SEM)
1	Normal Control	17.0±0.8
2	Disease Control	38.35±0.56
3	STD (Metformine 70 mg/kg)	21.25±0.50***
4	Test I (100 mg/kg)	32.16±0.77***
5	Test II (200 mg/kg)	27.35±0.30***

Values are expressed as Mean± SEM (n=6), where p< 0.0001when compared to normal group.



*Significantly different from disease control, ***(p<0.0001).

Figure 3 Blood urea nitrogen

****p<0.0001

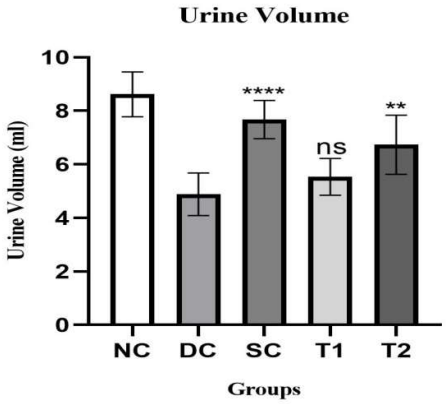
Urine volume

Table 6: Urine volume.

Group	Treatment	Urine Volume (ml) (Mean ± SEM)
1	Normal Control	8.61±0.41
2	Disease Control	4.88±0.39
3	STD (Metformine 70 mg/kg)	7.67±0.29****
4	Test I (100 mg/kg)	5.53±0.34ns
5	Test II (200 mg/kg)	6.73±0.55**

Values are expressed as Mean± SEM (n=6), where p<0.0001 when compared to normal group.
*Significantly different from disease control, ****(p<0.0001), **(p<0.001), ns= non significant.

Figure 4 Urine volume



****p<0.00001, **p<0.001, ns= Non significant

Urine protein

Table 7: Urine protein

Group	Treatment	Urine Protein (mg/day) (Mean ± SEM)
1	Normal Control	0.65±0.17
2	Disease Control	8.14±0.48****
3	STD (Metformine 70 mg/kg)	4.84±0.36***
4	Test I (100 mg/kg)	7.85±0.32ns
5	Test II (200 mg/kg)	6.54±0.24***

Values are expressed as Mean± SEM (n=6), where p<0.0001 when compared to normal group.
*Significantly different from disease control, ***(p<0.0001), ns= non significant.

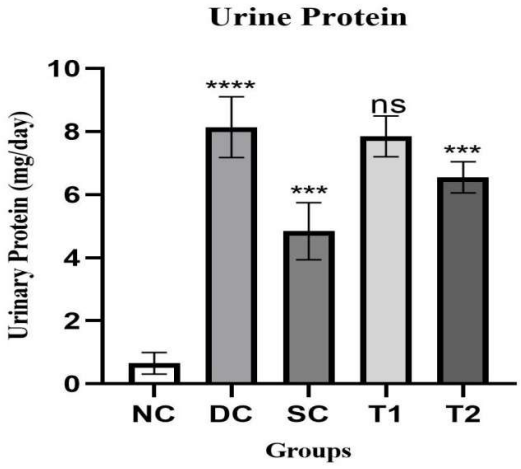


Figure 5 urine protein

****p<0.0001, ***p<0.0001, ns= Non significant

Kidney weight

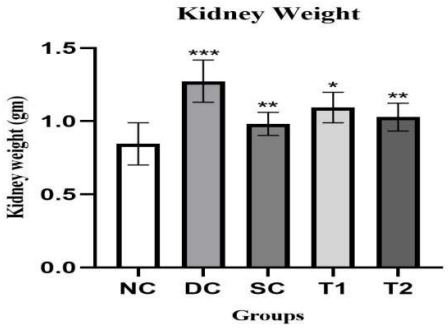
Table 8: Kidney weight.

Group	Treatment	Kidney weight (g) (Mean ± SEM)
1	Normal Control	0.84±0.07
2	Disease Control	1.27±0.07***
3	STD (Metformine 70 mg/kg)	0.98±0.03**
4	Test I (100 mg/kg)	1.09±0.05*
5	Test II (200 mg/kg)	1.02±0.04**

Values are expressed as Mean ± SEM (n=6), where $p<0.0001$ when compared to normal group

*Significantly different from disease control, ***($p<0.0001$), **($p<0.001$), *($p<0.01$).

Figure 6 Kidney weight



***p<0.0001, **p<0.001, *p<0.01

Histopathological study:

Histopathological study: Absence of lesions were observed in organ of control animals. Changes were observed in the test dose after scarifying animals in 6th Week and nephropathy was observed by tubular lumen deposits with increased basophilic staining of epithelium. Significance was about the area that were damaged due to diabetes. The area involved around 70-80% of tissue as grade 5 and subsequently 40 % in grade 3. Figure 7-11 are respectively of Normal control, Disease control, Standard control, Test I and Test II.

Figure 7: Normal control.

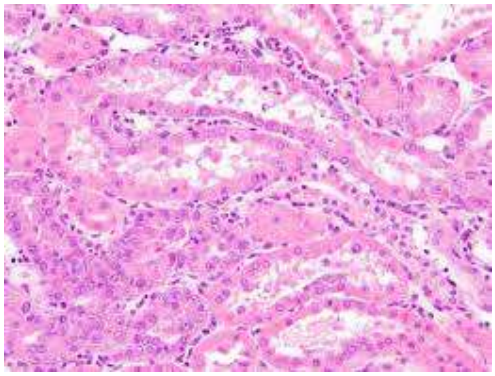


Figure 8: Disease control.

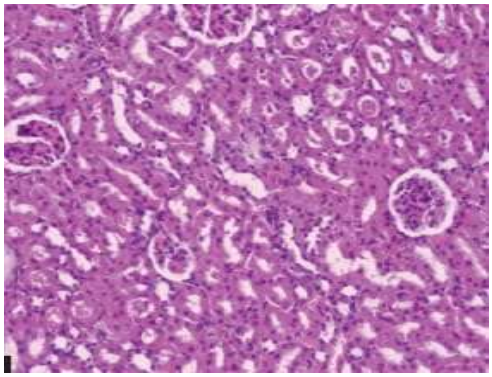


Figure 9: Standard control (Metformine -70 mg/kg).

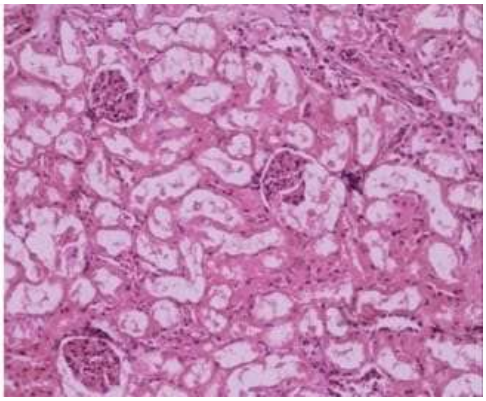


Figure 10: Test-I (100 mg/kg).

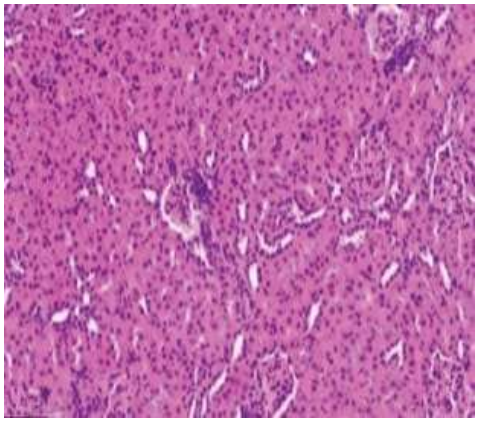
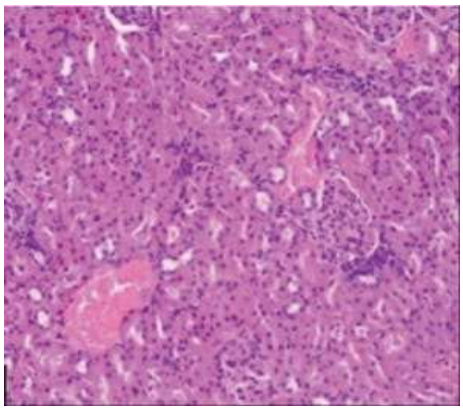


Figure 11: Test-I (200 mg/kg).



Discussion

Diabetes mellitus serves as a major disturbance in metabolism and energy homeostasis resulting in hyperphagia in animals [17]. Weight loss was observed in spite of food intake as the metabolism was faster as compared with PHF. The condition of polydipsia is common with hyperglycemia.

They have a vital importance in oxidative stress due electron transfer mechanism. [18]. However, Standard and PHF significantly had low sugar levels. Studies with *Withania somnifera*, *Juniperus communis* and *Tinospora cordifolia* had shown the gloucose lowering, anti-inflammatory and antioxidant effects [7-13]. However, our PHF of these plants is novel to be significantly effective in STZ+ NM induced Diabetic Nephropathy.

This model was found to be effective as comparative better than other existing models which regulates the biochemical levels and also enhances protection to the organs. [19, 20]. Conditions overlaying protein with macroalbuminuria leads the necrosis of kidney. [21]. PHF treated ratswere found with lower proteinuria as compare to rats indicating the antioxidant mechanism. Markers like serum creatinine and Bun were found to be lesser when treated with PHF. [22].

Conclusion

The result shows that treatment with the poly-herbal formulation showed a significant result in diabetic nephropathy rats. This formulation at its initial stage was very promising in reducing the levels of Blood glucose, BUN, Serum creatinine, Urine protein, Urine Volume and Kidney weight as compared with synthetic medicine, which possess various side effects and many other complications. Being a herbal formulation, the synergistic effects are more and better. This PHF can be taken up further in detail to study about the various effects in the molecular pattern, which will be beneficial to the humankind, and clinical trial can be one of the processes. Hence, it can be concluded that PHF are always better in terms of safety and efficacy.

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