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CONJUGATE EFFECT OF *BRUHATYADI KASHAYA GHANA*, A POLY-HERBAL FORMULATION WITH METFORMIN AND ENALAPRIL IN STZ INDUCED DIABETIC NEPHROPATHY RATS: NEOTERIC APPROACH IN DIABETIC KIDNEY DISEASE

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ABSTRACT

Background: Diabetes along with hypertension is now the major cause of end stage renal failure worldwide, not only within the developed world but also increasing within the emerging world. There is increasing optimistic evidence that the progression of Diabetic Kidney Disease (DKD) and its associated mortality can be ameliorated by integrated approach with *Ayurveda* and Modern medicine if started at an early stage. **Objective:** The present study aimed to investigate efficacy and safety of *Bruhatyadi Kashaya Ghana*, a poly-herbal formulation in management of STZ induced diabetic nephropathy in rats. **Materials and Methods:** After acclimatization period of seven days diabetes was induced in overnight fasted rats by single intra-peritoneal injection of freshly prepared STZ (50mg/kg dissolved in 0.1 ml cold citrate buffer, (pH-4.5). Selected rats were divided into six groups each containing six animals. All groups except the diabetic control group were treated with vehicle / standard

(Metformin 225 mg/kg, Enalapril 3.2 mg/kg) *Bruhatyadi Kashaya Ghana* at three dose levels (200 mg/kg / 400 mg/kg and 800 mg/kg) in combination with standard (M+E), daily for 28 days by per oral route and was monitored on 7th, 14th & 28th days of study by carrying out serum and urine biochemical estimations. **Results:** Significant positive results were obtained in all observed parameters. The result of present study showed that concomitant administration of *Bruhatyadi Kashaya Ghana* in combination with standard allopathic medicines (M+E) not only exerted good glycemic control but also produced significant decrease in Diabetic kidney disease. **Conclusion:** We conclude that *Bruhatyadi Kashaya Ghana*, a poly- herbal formulation is found to be efficacious and safe in management of STZ induced diabetic nephropathy in rats.

Keywords: Diabetic Kidney Disease, *Brihatyadi Kashaya Ghana*, Streptozotocin (STZ)

INTRODUCTION

Diabetic kidney disease (DKD) is one of the major causes of morbidity and mortality in diabetes. The more mortality of diabetes occurs mainly in individuals with diabetes along with proteinuria and results not only from end-stage renal disease but also from cardiovascular disease, with the later being particularly common in patients with type 2 diabetes. [1,2] Diabetic kidney disease is a clinical syndrome characterized by persistent albuminuria (>300 mg/d or >200ug/min), progressive decline in the glomerular filtration rate and elevated arterial blood pressure. [3] By the 1950s, kidney disease was clearly recognized as a common complication of diabetes. It is an important complication of diabetes firstly as it is relatively common, affecting about one in four patients with IDDM and approximately 40% of patients with type 2 DM shows signs of CKD (Stage 1-5). [4] Secondly the proteinuria which is its hallmark is only one consequences of widespread damage to small and large blood vessels and is a marker for the cardiovascular disease which is common cause of death in these patients. Thirdly, there is increasingly convincing and optimistic evidence that the progression of nephropathy and its associated mortality can be ameliorated by early intervention. Thus, it is medically imperative to conduct research in this area for awareness, detection, prevention and integrated management approach to deal with it. [5]

Management of established diabetic kidney disease according to conventional science demands for effort to achieve good glycemic control and a normal blood pressure and to correct any other risk factors for CVD. [6] There is no specific targeted therapy for nephro-protection whereas only serious threats are being taken care of. In one of the Ayurvedic classics '*Sushruta Samhita*' '*Bruhatyadi Kashaya Ghana*' has been mentioned for all sorts of urinary system complaints. [7] It is being effectively used to treat diabetic kidney disease by practitioners since long. The present experimental study was conducted to investigate efficacy and safety of *Bruhatyadi Kashaya Ghana*, a poly-herbal formulation in management of STZ induced diabetic nephropathy in rat.

Materials and Methods:

Drugs and chemicals:

Standard drug Metformin (Okamet – 500mg Cipla ltd) and Enalapril (Enam 5 Dr Reddy's Lab Pvt. Ltd) were purchased from local medical shop. Streptozotocin (STZ) was procured from Sisco Research Lab Pvt. Ltd. (SRL). Test drug *Bruhatyadi Kashaya Ghana* (BKG)was prepared at Dr. G. D. Pol Foundation's YMT Ayurvedic College pharmacy observing strict standard operating process. Finished product was critically analyzed in accordance with ASU drug standards. All other chemicals and solvents were used of analytical grade purchased from SD chemicals and Pallav chemical from Mumbai. Bio-chemical estimation kits were procured from ERBA diagnostics.

Animals: Male Wistar rats were procured from Bharat serum and vaccines. The rats were housed in institute's animal house (CPCSEA registration no. 25/PO/ReBi/S/99/CPCSEA dtd. 10/03/1999) at room temperature 24°c ± 1°c, relative humidity of 65% ±

10%- and 12-hours light and dark cycle and food and water ad libitum. This experimentation was carried out according to CPCSEA guidelines.

Preparation of test Solution: 50 g/kg STZ solution was freshly prepared by dissolving 75 mg of STZ in 5 ml of 0.1 M cold citrate buffer, PH 4.5 to prepare solution of 1.5% w/v. Metformin solution was prepared by dissolving two marketed Metformin tablets in 20ml of distilled water to prepare a solution of 5%w/v. Enalapril solution: Enalapril solution was prepared by dissolving three marketed Enalapril tablets in 10 ml of distilled water to prepare a solution of 0.15% w/v. *Bruhatyadi Kashaya Ghana*, i.e. herbal preparation was prepared by dissolving 2.5 gm of *Bruhatyadi Kashaya Ghana* in 20ml of distilled water using 0.1% w/v CMC as a suspending agent to prepare a solution of 12.5% w/v.

Procedure for induction of diabetes: After acclimatization period of seven days diabetes was induced in overnight fasted rates by single intra-peritoneal injection of freshly prepared STZ (50 mg/kg dissolved in 0.1 M cold citrate buffer, pH 4.5).[8] The STZ treated animals were allowed to drink 20% glucose solution for 24 hrs to overcome initial drug induced hypoglycemic mortality. Three days after STZ injection blood samples were collected through retro orbital plexus and blood glucose levels were measured using ERBA diagnostic reagent kit by glucose oxidase peroxidase method. Animals with blood glucose level 250 mg/dl and above were selected for the study.

Experimental designs: Animals were divided into six groups consisting of six rats each. Group 1 (Vehicle control) was normal vehicle group; in group 2 (Diabetic Control) the rats were treated with STZ (50 mg/kg intra-peritoneal) and monitored for eight weeks to induce diabetic nephropathy. In group 3, the rats were treated with STZ (50mg/kg intra-peritoneal) and monitored for four weeks for induction of diabetic nephropathy and then were treated daily with standard drug combination Metformin (225 mg/kg per oral) and Enalapril (3.2 mg/kg per oral). In group 4, The rats were treated with STZ (50 mg/kg intra-peritoneal) and monitored for four weeks for induction of diabetic nephropathy and were treated daily with standard drugs (M+E) and *Bruhatyadi Kashaya Ghana* (200 mg/kg per oral) for next four weeks. In group 5, the rats were treated same as above and treated daily with standard drug (M+E) and *Bruhatyadi Kashaya Ghana* in dose of (400mg/kg per oral) for next four weeks. In group 6, the rats were treated same as above and treated daily with standard drug (M+E) and *Bruhatyadi Kashaya Ghana* in dose of (800 mg/kg per oral) for next four weeks.

Assessment and follow up: The above animals were monitored on 0^{th}, 7^{th}, 14^{th} and 28^{th} day by carrying out serum and urine biochemical estimations. At the end of treatment blood samples were collected from retroorbital plexus and were allowed to clot at room temperature for 30 mins and centrifuged (Remi 4CDx) at 3000 rpm for 10 minutes to obtain clear serum and aliquots were used for the respective analytical determination.

Blood and urine sampling and analysis: The determination of serum levels of glucose, creatinine, total protein albumin, BUN, was carried out by available regent kit (Erba Manheim).Commercial enzyme linked immunosorbent assay kit (Elab Science) was used to quantify TGF-ß levels by using microplate Reader E-EL-HO 110 (Biotek instrument). Urine was collected by placing the animal in metabolic cages for 24 hours. The 24 hrs collected urine was centrifuged at 1500 rpm for 10 min. Urinary volume, creatinine, albumin, glucose, total protein was estimated using commercial assay kits (Erba Diagnostics).

Estimation of Tissue Parameters: Homogenate of kidney tissue (10% w/v) was prepared in ice cold phosphate buffer (PH 7.4) and an aliquot was used for lipid peroxidase estimation as per reference by Taghizadenetal 2007. The remaining portions of homogenates were then centrifuged at 4000 rpm for 10 mins and the supernatant obtained was used for estimation. Superoxide dismutase (SOD) was evaluated as per referenced by Wagnet al 2011, Catalase (CAT) as per Elberry 2015 and Reduced gluthathione (GSH) as per Loeken 2004.

Statistical Analysis: Statistical analysis was performed by using Graph Pad in Stat statistical software. The values were expressed as mean ±SEM and analysis were done by one-way analysis of variance (ANOVA) Followed by Turkey's Kramer multiple tests for comparison.

Results:

Effect of *Bruhatyadi Kashaya Ghana* on blood glucose (mg/dl) level: Fasting blood glucose levels of all animals were within the normal range initially and STZ induction raised blood glucose level significantly with time in respect to vehicle control group. On 7th and 14th days of treatment, in (M+E), (M+E+BKG200), (M+E+ BKG400) and (M+E+ BKG800) group serum glucose levels were found to be significantly ($p<0.001$) decreased compared to that of diabetic control group respectively. Serum glucose levels in (M+E), (M+E+ BKG200), (M+E+BKG400) and (M+E+BKG800) groups were found to be significantly ($p < 0.001$) decreased by 33.75, 37.21, 40.43 and 39.91% as compared to 28th day of diabetic control group respectively. (Fig. 1)

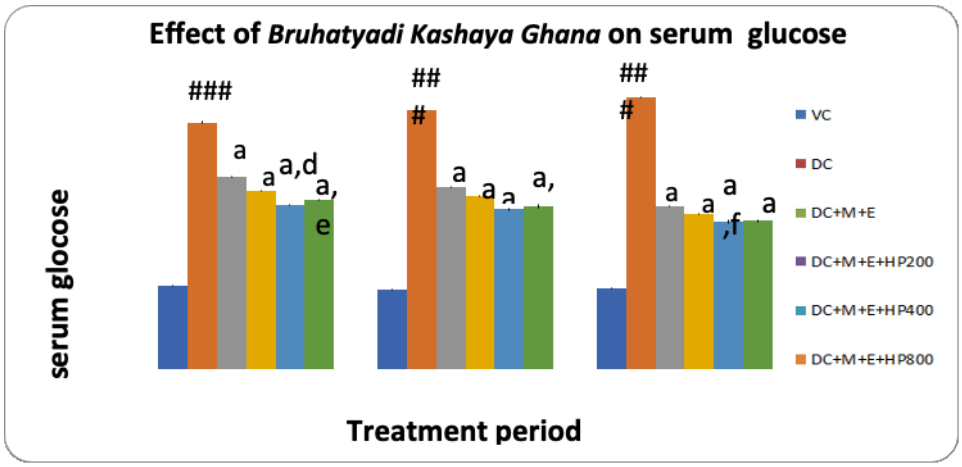

Fig. 1. Effect of *Bruhatyadi Kashaya Ghana* on blood glucose (mg/dl) level

Effect of *Bruhatyadi Kashaya Ghana* on Serum Albumin (mg/dl) level: On 7th day of treatment, serum Albumin levels were found to be significantly increased by 16.01($p<0.05$) and 25.76($p<0.001$) % in (M+E+BKG400) and (M+E+BKG800) groups as compared to 7th day of diabetic control group respectively. On 14th and 28th days, serum Albumin levels were found to be significantly ($p<0.001$) increased in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group as compared to 14th and 28th days of diabetic control group respectively. (Table 1)

Effect of *Bruhatyadi Kashaya Ghana* on Serum Total Protein (mg/dl) level: On 7th and 14th days of treatment, serum total protein levels were found to be significantly ($p<0.001$) increased in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) groups as compared to 7th and 14th days in diabetic control group respectively. On 28th day serum total protein levels were found to be significantly ($p<0.001$) increased by 69.98, 77.99, 96.05 and 86.68 % in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) groups as compared to 28th day diabetic control group respectively. (Table 1)

Table 1: Effect of *Bruhatyadi Kashaya Ghana* on and Serum Albumin and Serum Total Protein (mg/dl) level

GROUPS	**Serum Albumin(g/dl)**			**Serum Total Protein (g/dl)**		
	7th day	**14th day**	**28th day**	**7th day**	**14th day**	**28th day**
Vehicle Control	7.185 ± 0.043	7.183± 0.047	7.17 ± 0.047	6.188± 0.068	6.186 ± 0.065	6.181 ± 0.071
Diabetic Group	2.841$^{###}$± 0.0161	2.53$^{###}$± 0.0196	2.255$^{###}$± 0.0176	2.721$^{###}$± 0.161	2.525 $^{###}$± 0.133	2.381$^{###}$± 0.167
Treatment Standard (M+E)	3.195± 0.0077	3.83$^{(a)}$± 0.0185	4.04$^{(a)}$± 0.0259	3.438$^{(a)}$± 0.077	3.896$^{(a)}$± 0.081	3.9769$^{(a)}$± 0.080
Treatment (M+E+BKG 200)	3.21 ± 0.016	3.92$^{(a)}$± 0.053	4.321$^{(a)}$± 0.012	3.535$^{(a)}$± 0.077	4.103 $^{(a)}$± 0.081	4.238$^{(a)}$± 0.080
Treatment (M+E+ BKG 400)	3.296$^{(c)}$± 0.050	4.19$^{(a)}$± 0.013	4.683$^{(a,d)}$± 0.048	3.986$^{(a)}$± 0.028	4.518 $^{(a)}$± 0.031	4.668$^{(a,f)}$± 0.030
Treatment (M+E+ BKG 800)	3.573$^{(a)}$± 0.051	4.033$^{(a)}$± 0.038	4.396$^{(a)}$ ±0.042	3.971$^{(a)}$± 0.055	4.29$^{(a)}$± 0.051	4.445$^{(a)}$± 0.045

Effect of *Bruhatyadi Kashaya Ghana* on Blood Urea Nitrogen (BUN) (mg/dl) level: On 7th day and 14th days of treatment, BUN levels were found to be significantly ($p<0.001$) decreased in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group as compared to 7th and 14th days of diabetic control group respectively. On 28th day in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group BUN levels were found to be significantly ($p<0.001$) decreased by 51.85, 58.75, 61.61 and 60.76% as compared to 28th day diabetic control group respectively. (Table 2)

Effect of *Bruhatyadi Kashaya Ghana* on Serum Creatinine (mg/dl) level: On 7th and 14th days of treatment, serum Creatinine levels were found to be significantly ($p<0.001$) decreased in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group as compared to 7th 14th days of diabetic control group respectively. On 28th day serum Creatinine levels were found to be significantly ($p<0.001$) decreased by 78.49, 79.25, 80.026 and 80.04 % in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) groups as compared to 28th day diabetic control group respectively. (Table 2)

Table 2: Effect of *Bruhatyadi Kashaya Ghana* on Blood Urea Nitrogen (BUN) (mg/dl) and Serum Creatinine(mg/dl) level

	BLOOD UREA NITROGEN (BUN) (mg/dl)			SERUM CREATININE (mg/dl) LEVEL		
	7th day	14th day	28th day	7th day	14th day	28th day
Vehicle Control	17.373 ± 0.34	17.35 ± 0.26	17.41 ± 0.29	1.328 ± 0.025	1.34 ± 0.020	1.305 ± 0.0181
Diabetic Group	44.053$^{###}$ ±0.755	47.848$^{###}$ ±0.958	52.123$^{###}$ ±0.929	14.952$^{###}$ ± 0.195	16.798 $^{###}$ ± 0.217	19.14$^{###}$ ± 0.253
Treatment Standard (M+E)	33.65 ± 0.135	28.217 ± 0.234	25.095 ± 0.261	10.138$^{(a)}$ ± 0.105	6.83$^{(a)}$ ± 0.0654	4.117$^{(a)}$ ± 0.253
Treatment (M+E+BKG 200)	31.26 ± 0.33	27.67 ± 0.28	21.50$^{(a)}$ ± 0.20	8.385$^{(a,f)}$ ± 0.099	6.741$^{(a)}$ ± 0.077	3.97$^{(a)}$ ± 0.042
Treatment(M+E+ BKG 400)	30.82 ± 0.40	25.52$^{(a)}$ ± 0.29	20.01$^{(a,f)}$ ± 0.15	7.715$^{(a,d)}$ ± 0.108	6.201$^{(a)}$ ± 0.110	3.823$^{(a)}$ ± 0.054
Treatment (M+E+ BKG 800)	32.19 ± 0.50	25.23$^{(a)}$ ± 0.39	20.45$^{(a,f)}$ ± 0.26	7.325$^{(a,d)}$ ±0.126	6.381$^{(a)}$ ± 0.121	3.82$^{(a)}$ ± 0.057

Effect of *Bruhatyadi Kashaya Ghana* on Urinary Protein (mg/dl) level: There was no significant difference on 7th day treatment as compared 7th day diabetic control group. On 14thday of treatment,

Urinary protein levels were found to be significantly ($p<0.001$) decreased by 26.43, 28.35, 44.99 and 48.032 % in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group as compared to 14th day diabetic control group respectively. On 28th day in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group Urinary protein levels were found to be significantly ($p<0.001$) decreased by 43.95, 45.37, 58.62 and 59.43% as compared to 28th day diabetic control group respectively. **(Table 3)**

Effect of *Bruhatyadi Kashaya Ghana* on Urinary Albumin (g/dl) level: On 7th day of treatment, Urinary albumin levels were found to be significantly decreased by 14.25 ($P<0.01$), 18.51 ($P<0.001$), 30.15 ($P<0.001$), and 28.39 ($P<0.001$) % in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) groups as compared to 7th day of diabetic control group respectively. On 14th and 28th days, Urinary albumin levels were found to be significantly ($p<0.001$) decreased as compared to 14th and 28th days in diabetic control group respectively. **(Table 3)**

Table 3: Effect of *Bruhatyadi Kashaya Ghana* on Urinary Protein (mg/dl) level and Urinary Albumin (g/dl)

GROUPS	URINARY PROTEIN (g/dl)			URINARY ALBUMIN (g/dl)		
	7th day	14th day	28th day	7th day	14th day	28th day
Vehicle Control	0.683 ± 0.0148	0.686 ± 0.0137	0.683 ± 0.0151	0.275 ± 0.01003	0.278 ± 0.013	0.276 ± 0.013
Diabetic Group	2.106### ± 0.0283	2.338### ± 0.0175	2.83### ±0.0234	1.761### ±0.0179	2.138### ±0.0313	2.78### ± 0.0298
Treatment Standard (M+E)	2.1033 ± 0.0268	1.72(a) ± 0.0068	1.586 (a)± 0.0124	1.51(b) ± 0.0182	1.381(a) ± 0.0095	1.308(a)± 0.0083
Treatment (M+E+BKG 200)	2.076 ± 0.013	1.675 (a) ± 0.0226	1.546 (a) ± 0.02	1.435(a) ± 0.0091	1.378(a) ± 0.0086	1.32(a) ± 0.0098
Treatment (M+E+ BKG 400)	1.94 ± 0.021	1.286 (a,d) ± 0.0168	1.171 (a,d) ±0.0146	1.23(a,e) ± 0.015	0.938(a,d) ± 0.013	0.861(a,d) ± 0.011
Treatment (M+E+ BKG 800)	1.911 ± 0.0231	1.215 (a,d) ± 0.0086	1.148 (a,d) ±0.0084	1.261(a,e) ± 0.021	0.916(a,d) ± 0.014	0.845(a,d) ± 0.013

Effect of *Bruhatyadi Kashaya Ghana* on Urinary Creatinine (mg/dl) level:

No significant difference was found in urinary Creatinine level on 7th day of treatment in (M+E) and (M+E+BKG200) group as compared to 7th day diabetic control group. On 7thday in (M+E+BKG400) and (M+E+BKG800) group Urinary Creatinine levels were found to be significantly ($p<0.05$) decreased by 24.62 and 24.344 and % as compared to 7th day diabetic control group respectively. On 14th and 28th days Urinary Creatinine levels were found to be significantly ($p<0.001$) decreased in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group as compared to 14th and 28th days diabetic control group respectively.

3.9Effect of *Bruhatyadi Kashaya Ghana* on Creatinine Clearance (ml/min/kg) (CCR) level:

On 7th, 14th and 28thday diabetic control group CCR levels were found to be significantly ($p<0.001$) decreased by 40.74, 73.04 and 87.75% as compared to vehicle control group respectively. There was no significant difference in creatinine clearance level on 7th and 14th day of treatment as compared to 7th and 14th day of diabetic control group respectively. On 28th day in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group CCR levels were found to

be significantly ($p<0.001$) increased by 209.80, 249.67, 285.62 and 259.47% as compared to 28th day diabetic control group respectively.

Table 4: Effect of *Bruhatyadi Kashaya Ghana* on Urinary Creatinine (mg/dl) and Creatinine Clearance (ml/min/kg) levels:

GROUPS	URINARY CREATININE (mg/dl)			CREATININE CLEARANCE (ml/min/kg) (CCR)		
	7th day	14th day	28th day	7th day	14th day	28th day
Vehicle Control	44.86 ± 1.04	43.568 ± 1.09	44.05 ± 1.04	1.35 ± 0.05	1.358 ± 0.056	1.25 ± 20.44
Diabetic Group	25.213$^{###}$ ± 0.299	20.525$^{###}$ ± 0.244	16.44$^{###}$ ± 0.23	0.80$^{###}$ ± 0.016	0.366$^{###}$ ± 0.011	0.153$^{###}$ ± 0.107
Treatment Standard (M+E)	24.243 ± 0.218	37.945$^{(a)}$ ± 0.238	35.563$^{(a)}$ ± 0.311	0.307 ± 0.07509	0.392 ± 0.0656	0.474$^{(a)}$ ± 0.0154
Treatment (M+E+BKG 200)	25.55 ± 0.35	39.5(a) ± 0.36	40.06$^{(a)}$ ± 0.35	0.32 ± 0.0047	0.481 ± 0.007	0.535$^{(a)}$ ± 0.098
Treatment (M+E+ BKG 400)	32.39$^{(c,e)}$ ± 0.34	40.42$^{(a)}$ ± 0.20	41.82$^{(a,f)}$ ± 0.19	0.37 ± 0.0074	0.493 ± 0.0093	0.59$^{(a)}$ ± 0.099
Treatment (M+E+ BKG 800)	33.32$^{(b,e)}$ ± 0.55	39.64$^{(a)}$ ± 0.45	40.77$^{(a)}$ ± 0.437	0.37 ± 0.00846	0.495 ± 0.00845	0.55$^{(a)}$ ± 0.0931

3.10 Effect of *Bruhatyadi Kashaya Ghana* on Urinary Glucose (mg/dl) level: On 7th day in (M+E+BKG400) and (M+E+BKG800) group Urinary glucose levels were found to be significantly ($p<0.001$) decreased by 18.08 and 11.26% as compared to 7th day diabetic control group. On 14th day in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group Urinary glucose levels were found to be significantly ($p<0.001$) decreased by 44.22, 44.23, 46.79 and 47.58 % as compared to 14th day diabetic control group respectively. On 28th day in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group Urinary glucose levels were found to be significantly ($p<0.001$) decreased by 50.30, 50.31, 52.95 and 53.28% as compared to 28th day diabetic control group respectively. (Fig 2)

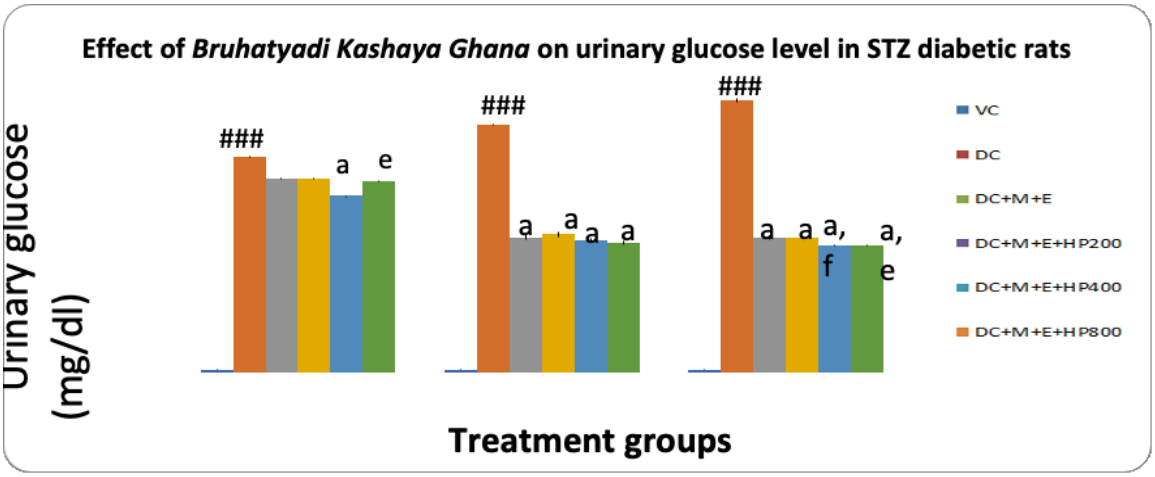

Fig 2: Effect of *Bruhatyadi Kashaya Ghana* on Urinary Glucose (mg/dl) level

Effect of *Bruhatyadi Kashaya Ghana* on Body Weight (gm.):

There was no significant difference in body weight levels on 7th day in (M+E), (M+E+BKG200) and (M+E+BKG400) group as compared to 7th day

diabetic control group. On 7th day (M+E+BKG800) group body weight was found to be significantly ($p<0.05$) decreased by 7.814 % as compared to 7th day diabetic control group. On 14th day in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group body weight were found to be significantly decreased by 12.60 ($p<0.05$), 24.36($p<0.001$), 26.05($p<0.001$) and 25.21 ($p<0.001$) % as compared to 14th day diabetic control group respectively. On 28th day in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) groups body weight were found to be significantly ($p<0.001$) decreased by 43.88, 32.041, 63.26 and 67.24 % as compared to 28th day diabetic control group respectively. (Table 5)

Effect of *Bruhatyadi Kashaya Ghana* on Serum TGF-B (Pg/Ml) level: Serum TGF-β level in diabetic control was found to be significantly ($p<0.001$) increased by 538.63% as compared to vehicle control group. Serum TGF-β level in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) found to be significantly ($p<0.01$) decreased by 42.33, 59.42, 79.71 and 75.55% as compared to diabetic control group respectively. Serum TGF-β level in (M+E+BKG400) and (M+E+BKG800) group was found to be significantly ($p<0.05$) decreased by 37.38 and 33.32% as compared to (M+E) group. (Table 5)

Table 5: Effect of *Bruhatyadi Kashaya Ghana* on Body Weight (gm.) and Serum TGF - Blevels

	BODY WEIGHT (gm.)			Serum TGF - B
GROUPS	7th day	14th day	28th day	
Vehicle Control	263.33 ± 1.571	260 ± 2.72	266.66 ± 1.542	544.44 ± 45.859
Diabetic Group	$213.33^{###}$ ± 1.778	$198.33^{###}$ ± 1.778	163.33### ± 2.239	$3477.77^{###}$ ± 150.63
Treatment Standard (M+E)	221.66 ± 0.07509	$223.33^{(c)}$ ± 2.078	$235^{(a)}$ ± 1.595	2005.56^{b} ± 115.101
Treatment (M+E+BKG 200)	233.33 ± 1.495	$246.66^{(a,f)}$ ± 1.242	$248.33^{(a)}$ ± 1.145	1411.11^{a} ± 70.613
Treatment (M+E+ BKG 400)	223.33 ± 1.571	$250^{(a,f)}$ ± 1.924	$266.66^{(a,e)}$ ± 2.484	$705.555^{a,f}$ ± 54.872
Treatment (M+E+ BKG 800)	$230^{(c)}$ ± 0.962	$248.33^{(a,f)}$ ± 1.145	$273.33^{(a,d)}$ ±1.842	$844.44^{a,f}$ ± 134.76

3.13 Effect of *Bruhatyadi Kashaya Ghana* on Kidney Index (Mg/G): Kidney index in diabetic control was found to be significantly ($p<0.001$) increased by 264.07% as compared to vehicle control group. Kidney index in (M+E), (M+E+BKG200), (M+E+BKG400) and (M+E+BKG800) group was found to be significantly ($p<0.01$) decreased by 56.002, 59.42, 66.11 and 67.26% as compared to diabetic control group respectively. Kidney index in (M+E+BKG400) and (M+E+BKG800) group was found to be significantly ($p<0.05$) decreased by 10.108 and 11.258% as compared to (M+E) group respectively. (Table 6)

Table 6: Effect of *Bruhatyadi Kashaya Ghana* on Kidney Index (Mg/G)

GROUPS	K. I. (mg/g)	% decrease
Vehicle Control	3.8 ± 0.0476	-
Diabetic Group	$13.835^{###}$ ± 0.08106	-264.07

Treatment Standard (M+E)	7.11[a] ± 0.152	48.60
Treatment (M+E+BKG 200)	6.087 [a] ± 0.0781	56.002
Treatment (M+E+ BKG 400)	4.688 [a, d] ± 0.0614	66.11
Treatment (M+E+ BKG 800)	4.529 [a, d] ± 0.0713	67.26

3.15 **Effect of *Bruhatyadi Kashaya Ghana* on Antioxidant Activity:**

Catalase in kidney was determined in terms of µ mole H2O2 consumed/ mg protein/min. The CAT levels were restored at dose levels (M+E+BKG400) and (M+E+BKG800) which was evident by significant ($p<0.0001$) increase in CAT levels as compared to diabetic control group. SOD in kidney was determined in terms of SOD units/ mg protein. There was significant increase in SOD units/ mg protein was found in (M+E) treated group when compared to diabetic control group. A significant increase in SOD units/ mg protein was observed in (M+E+BKG200) treated group when compared to diabetic control group respectively. SOD levels were returned to the normal values at the dose level (M+E+BKG400) and (M+E+BKG800) which was evident by significant ($P<0.001$) increase in SOD units/ mg protein as compared to the diabetic control group.

GSH in kidney was determined in terms of GSH µmoles/ mg protein. A significant ($p<0.05$) increase in GSH µmoles/mg protein was found in (M+E+BKG400) and (M+E+BKG800) treated group when compared to diabetic control group respectively.

No significant decrease in LPO units/ µmoles/ mg protein activity was found in (M+E) treated group when compared to diabetic control group. A significant ($p<0.0001$) decrease in LPO units/ µmoles/ mg protein was observed in groups treated with (M+E+BKG400) and (M+E+BKG800) treated group when compared to diabetic control group respectively. (Table 7)

Table 7: Effect of *Bruhatyadi Kashaya Ghana* on Antioxidant Activity

GROUPS	CATALASE (CAT) µ moles /mg protein/min	SUPEROXIDE DIMUTASE (SOD) unit/mg protein	LIPID PEROXIDATION unit/µmoles/mg protein	REDUCED GLUTATHIONE µmoles/mg protein
Vehicle Control	24.8069 ±0.237	30.976 ± 0.799	86.046 ± 5.632	32.935 ± 1.533
Diabetic Group	2.5225[###] ± 0.181	14.635### ± 0.285	191.439[###] ± 8.915	18.3008[##] ± 0.324
Treatment Standard (M+E)	7.514[a] ± 0.211	17.869 [a] ± 0.449	172.676 ± 5.925	20.079 ± 0.555
Treatment (M+E+BKG 200)	12.376 [a, e] ± 0.257	23.626 [a] ± 0.507	72.057 [a, d] ± 2.409	25.462 [c] ± 0.664
Treatment (M+E+ BKG 400)	20.282 [a, d] ± 0.405	27.066 [a, e] ± 0.540	79.87 [a, d] ± 3.716	29.748 [c]± 0.811
Treatment (M+E+ BKG 800)	21.977 [a, d] ± 0.440	26.885 [a, e] ± 0.811	81.275 [a, e] ± 0.937	30.537 [c, f] ± 1.012

DISCUSSION

Optimal care for patients with DKD is complex and best managed using comprehensive multifactorial risk reduction strategies. [9,10] Various research in DKD have analyzed use of ACE inhibitors, RAAS blockers and anti-lipid medicines in management of DKD. Recently there has been intense focus on whether combination of these agents could further improve outcomes in DKD. There is also an optimistic thought that timely and effective glycemic control may have a positive effect on the prevention of diabetic kidney disease. Considering these there is a current trend of using Enalapril and OHA in combination for management of DKD. Hence in present study it has been considered as a standard control group for comparison of trial drug preparation. In spite of

targeting multiple risk factors concomitantly with comprehensive care by modern medicine yet assured outcome remains suboptimal in care of DKD. Hence scope of traditional herbs remains only ray of hope in its management. *Bruhatyadi Kashaya* is mentioned in classical Ayurvedic text '*Sushruta Samhita*' for all sorts of Urinary system disorders. It is further reinforced with *Tribulus terrestris (Gokshura)* which is well known diuretic as cited in recent Ayurvedic compendia '*Sahastrayoga'* to potentiate its effect. This reference was considered for preparation of trial drug '*Bruhatyadi Kashaya Ghana*'. It is being effectively used by practitioners for treating kidney disorders. Hence the aim of this study was to probe the synergetic beneficial effect of trial drug preparation and to determine the optimal dose for administration. Herbs being the storehouse of many therapeutically potent phytoconstituents act via array of pharmacological targets and hence can be used efficiently to synergize effects of allopathic medicines which generally act on only one specific target .Herbal medicines also offer and additional advantage of overcoming untoward side effects of chemical molecules if used in combination with allopathic medicines. STZ is widely employed to induce experimental diabetes. The persistent hyperglycemia occurring during diabetes is responsible for development of Diabetic Nephropathy. [(11)] In present study induction of diabetic nephropathy was evident from elevated levels of urinary protein, albumin, and glucose and urine volume. Biochemical parameters like BUN, Sr. Creatinine, Sr. Glucose, TGF ß were also affirming same. Whereas levels of serum total protein, albumin, urinary creatinine & creatinine clearance were decreased in diabetic control group. STZ induced diabetes is characterized by severe loss of body weight and increase in kidney weight. [(12,13)] Treatment with M+E+HP produced increase in body weight as well as prevented increase whole kidney weight. The commonest symptom of diabetes mellitus is polyuria. Progression in kidney malfunctioning was indicated in this study with graded increase in urine volume over on experimental period in diabetic controls rats which was decreased on treatment with M+E+HP. In this study, successful induction of diabetes by STZ was evident from observed hyperglycemia over experimental period in diabetic control group. [(14)] Treatment with M+E+HP exhibited more significant glycemic control as compared to standard treatment over an experimental period suggesting potentiation of antihyperglycemic effect of modern medicine. This could be considered as contribution in delaying the progression of Nephropathy. The elevated serum levels of urea and creatinine accompanied with diabetic hyperglycemia are significant markers of renal dysfunction which ultimately reflects as decline in glomerular filtration rate. The present study exhibited rise in levels of these parameters in diabetic rats. However, on treatment with M+E+HP this rise in level of serum urea and creatinine were attenuated indicating rise in glomerular filtration rate. Improvement in glomerular filtration rate and renal functioning was evident by increased creatinine clearance also[3]. Elevated levels of urinary proteins and declined levels of serum proteins observed in STZ induced diabetic rats were reversed significantly on treatment with M+E+HP. Thus, these results ensure restoration of glomerular filtration capacity of nephrons indicating nephroprotective effect. Persistent albuminuria is hallmark of Diabetic Kidney disease. [(15)] In present study the excretion of albumin in urine was increased with simultaneous decrease in the levels of serum albumin in diabetic control rats probably owing to increased permeability of filtration membrane as a cascade of disease. This albuminuria was found to be abated on treatment with M+E+HP which reinforces earlier postulation as study drug manifesting nephro-protective effect. There is increasing evidence that in certain pathologic states, especially chronic diseases the increased production and or ineffective scavenging of reactive oxygen species (ROS) may play a critical role. High reactivity of ROS determines chemical changes in virtually all cellular components leading to lipid peroxidation. Production of ROS and disturbed capacity of antioxidant defense in diabetic subjects have been reported. [(16)] It has been suggested that enhanced production of free radicals and oxidative stress is

central event to the development of diabetic complications. In diabetes, deprivation of tissues from supply of glucose leads to oxidative stress playing an important role in genesis of hypoxia across kidney eventually leading to diabetic kidney disease. In oxidative stress defensive factors like SOD, CAT and GSH offer protection by scavenging generated free radicals and hence get used up rapidly by issue. [17] Present study supports this hypothesis as levels of SOD, CAT and GSH were found to be declined in the diabetic control group which affirms induction of oxidative damage to kidneys in diabetic kidney disease. Treatment with M+E+HP illustrated restoration of the levels of this antioxidant enzymes inhibiting peroxidation of lipids thereby exhibiting antioxidant effect. Literature reports progressively increased expression of TGF-ß1 is accompanied with extracellular matrix (ECM) accumulation and glomerular basement membrane (GBM) thickening. Mesangial expansion and glomerular basement membrane thickening are the pathological hallmark in diabetic kidney disease. [18] TGF-ß1 being an inflammatory mediator in diabetic nephropathy it was analyzed as a parameter. It was found to be elevated in STZ induced diabetic rats wherein treatment with M+E+HP is found to be capable of restoring it back to normalcy. Exact pharmacological mechanism behind it is to be ascertained but aforesaid antioxidant potential of herbal medicine could be one of the contributing factors to debilitate release of TGF-ß1.

CONCLUSION

DKD has emerged as a major aftermath of the worldwide diabetes pandemic. Although the existing therapies hold promise for improving outcomes among patients with DKD yet control of multiple risk factors and definite outcome often remain suboptimal. The result of the present study showed that concomitant administration of *Brihatyadi Kashaya Ghana,* a poly-herbal formulation in combination with standard allopathic medicine (M + E) not only exerted superior glycemic control but also produced desired effects in various parameters like kidney weight, urinary volume along with prevention of perturbation in levels of urinary albumin, total protein, BUN and Serum creatinine levels. It also exhibited improvement in body weight and creatinine clearance indicating protection from diabetic nephropathy. This study revealed that adjuvant administration of standard care and study drug ameliorated oxidative stress by attenuating lipid peroxidation with significant rise in levels of endogenous antioxidants such as SOD, CAT and GSH which in turn prevented release of TGFß, thereby protecting against inflammatory cascade in Diabetic Nephropathy. Thus, present study provides scientific evidence for utilizing antioxidant potential of *Bruhatyadi Kashaya Ghana* to potentiate better clinical outcome in terms of nephroprotection in Diabetic Kidney disease.

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