

EVALUATION OF HYDROALCOHOLIC EXTRACT OF STEM BARK OF BAUHINIA VARIEGATA (LINN.) AGAINST STREPTOZOTOCIN-INDUCED DIABETIC NEPHROPATHY IN RATS

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Abstract—the nephroprotective activity of hydroalcoholic extract of stem bark of *B. Variegata* against streptozotocin (stz) induced nephropathy was investigated in rats. Nephrotoxicity was induced by i.p. Injection of stz (90 mg/kg). After eight weeks of stz administration (diabetes confirmed), extract of *B. Variegata* at dose levels of 100 and 200 mg/kg, glimepiride used as positive control, was administered daily and 200 mg/kg, glimepiride used as positive control, was administered daily for further eight weeks in experimental animals. The nephroprotective activity was assessed using various biochemical parameters such as hba1c, bun, creatinine, $\text{tnf-}\alpha$, urine creatinine and albumin evaluation. The lower dose of standard drug glimepiride. The statistically processed results suggested the protective action of *B. Variegata* stem bark against stz induced diabetic nephropathy.

Keywords: streptozotocin, diabetic nephropathy, kanchnar, *bauhinia variegata*

I. INTRODUCTION

THIS Diabetes mellitus is known to trigger retinopathy, neuropathy and nephropathy. Diabetic nephropathy is one of the most serious complication of diabetes and the leading cause of end stage renal disease (ESRD) worldwide, and it affect about 15-25% of type-I diabetes, and 30-40% of type-II diabetes patients [Nand et al 2000 and Ramachandran et al 2001]. Metabolic factors (glucose dependent factors) are also activated within the diabetic kidney and result in enhanced oxidative stress, renal polyol formation and accumulation of advanced glycation end products (AGEs), these pathways ultimately results in progressive albuminuria, reduction in glomerular filtration rate, elevation of arterial blood pressure and fluid retention [Arora et al.2013 and Cooper et al.2008]. The use of herbal supplements for diabetic nephropathy has become increasingly popular, The plant *Bauhinia variegata* Linn. locally known as Kanchnar belonging to the family Caesalpinaceae, is a widely distributed plant throughout the

subtropical and tropical regions of the world [Mali et al.2007]. It is a widely used medicinal plant by the tribals throughout India and popular in many herbal traditions such as Ayurveda, Unani and Homeopathy. Different parts of this plant are used traditionally and have high value as antitumor, antimicrobial, anti-inflammatory, antioestrogenic, hepatoprotective, antioxidant and antihyperlipidemic [Mali et al.2007, Rajani et al.2009 and Bodakhe et al.2007]. The leaves exhibited significant anti-hyperglycemic effects [Azevedo et al.2008] The stem bark of this plant is reported to contain many active phytochemicals including stigmaterol, flavone glucoside, lupeol, kaempferol-3-glucoside, β -setosterol [Prakash et al.1978 and Kumar et al.2012]. Recent work done on this plant many active phytochemicals including stigmaterol, flavone glucoside, lupeol, kaempferol-3-glucoside, β -setosterol [Prakash et al.1978 and Kumar et al.2012]. Recent work done on this plant was using the stem for seeing its effect on alloxan-induced diabetes [Pani et al. 2011] and cisplatin-induced nephropathy in rats .

II. MATERIALS AND METHODS

Hydroalcoholic extract of stem bark of *Bauhinia variegata* was collected from Council for Research in Ayurveda and Siddha (CCRAS), New Delhi, as gift sample with certificate of analysis. Calculated amount of dried hydroalcoholic extract was suspended in 0.5% w/v of sodium CMC in normal saline solution to prepare the test doses (100 and 200 mg/kg/ml). The dose limits were selected on the basis of previous available literature (Pani et al., 2011).

III. EXPERIMENTAL ANIMALS

Wistar rats of either sex, weighing 150-200 g (procured from animal house facility, Delhi Institute of Pharmaceutical Sciences and Research, New Delhi) were employed in the present study. The animals were kept under maintained laboratory conditions with adequate supply of drinking water *ad libitum* and pallet diet. The experimental protocol was approved by the Institutional Animal Ethical Committee (Protocol No: DIPSAR/ IAEC/ 2009/24).

Induction of diabetic nephropathy (Streptozotocin induced neonatal rat model)

Non insulin dependent diabetes mellitus (NIDDM) was induced in rats by administration of STZ as per the method of Bonner-Weir *et al.* (1981) Three female rats were caged with one male for mating. After 21-23 (gestation period) days the animals delivered pups (neonatal rats) were used for further studies. Briefly, STZ (freshly prepared in 0.1M citrate buffer, P^H 4.5) at a dose of 90 mg/kg was injected intraperitoneally to two day old neonatal rats. After eight weeks of STZ administration, blood glucose was estimated. The animals showed blood glucose level more than 140 mg/dl were considered as NIDDM positive and they were randomly allotted to different experimental groups as per experimental protocol for further eight weeks treatment study.

IV. EXPERIMENTAL PROTOCOL

Five groups, each comprising six Wistar albino rats, were employed in the present study. Group I (Normal control): Rats were not subjected for STZ administration and were kept for 16 weeks. These animals were received 0.5% CMC solution for last eight weeks. Group II (Diabetic control, vehicle in STZ): Rats were subjected for STZ administration. After confirmation of diabetes, these animals were received 0.5% CMC solution for eight weeks. Group III & IV (BV 100 and 200 mg/kg, respectively, p.o. in STZ): Rats were subjected for STZ administration. After confirmation of diabetes, these animals were received hydroalcoholic extract of *Bauhinia variegata* for eight weeks. Group V (GL 0.35 mg/kg, p.o. in STZ): Rats were subjected for STZ administration. After confirmation of diabetes, these animals were received standard drug glibenclamide for eight weeks.

2.7 Biochemical examination

After eight weeks of drug treatment, urine was collected over 24 h by keeping the test animals in individual metabolic cages and employed for estimation of biochemical parameters, namely urine creatinine and urine albumin. Morning fasting blood sample was withdrawn by retro orbital puncture under mild ether anesthesia from overnight fasted rats before sacrifice and employed for HbA1c estimation. Further, blood samples were centrifuged at 4°C (3000 rpm, 15 min). The plasma separated as straw colored supernatant was employed to estimate the glucose, total protein, urea, creatinine and TNF α level. The biochemical estimations were done in biochemical-semi-auto analyzer by standard procedure using commercial kits.

After collecting the blood samples, animals were sacrificed by an overdose of anesthetic ether. The kidneys were immediately excised and transferred into formalin (10% formaldehyde v/v) for histological observation.

Statistical analysis

All the results were expressed as mean \pm SEM. The data obtained were statistically analyzed by one-way ANOVA followed by student Newman-Keuls test by using the Graph pad prism Version-5.0 software. The $p < 0.05$ was considered statistically significant. The diabetes control group was compared with the normal control group and all other treatment groups were compared with the diabetic control group.

V. RESULTS AND DISCUSSION

Effect on biochemical parameters

The results as cited in Table 1 and Figure. 2, includes change in blood, plasma and urine biochemical parameters. In the present study, STZ given in the dose range of 90 mg/kg body weight, produced as significant rise in blood HbA1c, plasma glucose, total protein, urea, creatinine & TNF- α and urine creatinine & albumin levels, indicating considerable evidenced for diabetic nephropathy. Hydroalcoholic extract of stem bark of *B. variegata* (HABV) at the dose levels of 100 and 200 mg/kg treated rats showed significant fall in the blood HbA1c, plasma glucose, total protein, urea, creatinine & TNF- α and urine creatinine & albumin levels in a dose-dependent manner when compared with the diabetic control group, except plasma urea in case of dose level of 200 mg/kg body weight. Treatment of standard drug glibenclamide at dose level 0.35 mg/kg body weight also produced similar effects as seen in higher dose of HABV (200 mg/kg).

Table 1.

Effect of hydroalcoholic extract of the bark of *B. variegata* on biochemical parameters against STZ induced diabetic nephropathy in rats.

Groups	Blood % HbA1c	Plasma total protein (g/dl)	Plasma BUN (mg/dl)	Plasma creatinine (mg/dl)	Plasma TNF- α (pg/ml)	Urine creatinine (mg/dl)	Urine albumin (mg/dl)
NC	2.98 \pm 1.13	5.82 \pm 0.05	14.29 \pm 1.11	0.52 \pm 0.1	201.33 \pm 4.0	3.45 \pm 0.82	0.28 \pm 0.02
DC	6.47 \pm 1.2 ^a	8.1 \pm 0.54 ^a	18.99 \pm 2.22 ^a	0.94 \pm 0.17 ^a	255.26 \pm 2.08 ^a	6.95 \pm 0.75 ^a	0.49 \pm 0.05 ^a
GL	3.15 \pm 0.93 ^c	6.45 \pm 0.48 ^b	16.28 \pm 3.18	0.47 \pm 0.08 ^b	206.81 \pm 6.93 ^b	3.87 \pm 0.58 ^b	0.29 \pm 0.03 ^b
BV100	3.97 \pm 1.1 ^b	6.42 \pm 0.55 ^b	14.1 \pm 0.89 ^b	0.57 \pm 0.05 ^b	210.14 \pm 4.77 ^b	3.6 \pm 0.72 ^b	0.32 \pm 0.03 ^b
BV200	3.98 \pm 0.97 ^b	6.33 \pm 0.51 ^b	17.09 \pm 1.74	0.49 \pm 0.08 ^b	211.26 \pm 2.01 ^b	3.48 \pm 0.68 ^b	0.27 \pm 0.04 ^b

NC: Normal control, DC: Diabetic Control, GL: Glibenclamide 0.35 mg/kg, BV100: *Bauhinia variegata* 100 mg/kg, BV200: *Bauhinia variegata* 200 mg/kg. Data expressed as mean \pm SEM, n = 6. ^aP < 0.05, ^bP < 0.01, ^cP < 0.001 DC Vs NC & ^aP < 0.05, ^bP < 0.01, ^cP < 0.001, GL, BV100, BV200 Vs DC (One-way ANOVA followed by Student-Newman-Keuls test).

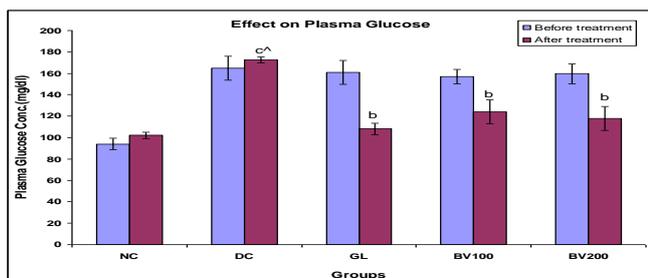


Figure 2: Effect of *Bauhinia variegata* on blood glucose level (mg/dl) of different groups before and after drug treatment. NC: Normal control, DC: Diabetic Control, GL: Glimepiride 0.35 mg/kg, BV100: *Bauhinia variegata* 100 mg/kg, BV200: *Bauhinia variegata* 200 mg/kg. Data expressed as mean \pm SEM, n = 6. ^{c^A}P < 0.001 DC Vs NC & ^bP < 0.01 GL, BV100, BV200 Vs DC (One-way ANOVA followed by Student-Newman-Keuls test).

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Conclusions:

The statistically processed results suggested the protective action of *B. variegata* stem bark against STZ induced diabetic nephropathy.

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