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Impact of Methanolic Extract of *Chlorophytum borivilianum* Santapau & *R. R. Fern.* Leaves on dexamethasone-induced Insulin resistance in Rat

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Abstract

This study focused on antidiabetic effect methanolic extract of *Chlorophytum borivilianum* leaves in Dexamethasone-induced diabetic rats. The effect of the optimal dose of methanolic extract was measured over 21 days. During the study, diabetic rats were treated with two doses of extract (250 and 500 mg/kg). The percentage of Fasting Blood Glucose (FBG) was measured immediately after administration of the extract and at 2h, 4h and 8h after administration. The leaf extract of *Chlorophytum borivilianum* showed significant blood glucose reduction in the normoglycemic model and glucose loaded test at doses of 500 mg/kg. The results showed that the extract was rich with flavonoids, phenols and tannins. Levels of FBG and DPP-4 were significantly lower in the extract-treated group in comparison with the control group; however, the level of insulin was significantly elevated in the extract-treated group compared to the control group. This effect may be caused by two factors. First, the antioxidant effects of flavonoids that protect pancreatic beta cells from damage caused by Dexamethasone, supports regeneration of pancreatic beta cells, and therefore insulin production.

Keywords: Diabetes Mellitus, hypoglycemic, Safed Musli, Soxhlet Extraction.

Introduction

Diabetes mellitus (DM) is a metabolic syndrome characterized by hyperglycemia and other complications nephropathy, neuropathy, retinopathy, micro and macro-vascular complications [1]. It is among the highly prevalent diseases and is estimated to affect about 300 million people globally by 2025 [2]. Currently, it becomes one of the leading public health problems and the cause of morbidity and death universally [3-7]. In 2019, the International Diabetes Federation (IDF) estimates that 463 million (9.3%) adults aged 20–79 worldwide are currently living with diabetes. The total number is predicted to rise to 578.4 million (10.2%) by 2030 and 700.2 million (10.9%) by 2045 [8] with the highest increment in regions where economies are moving from low- to middle-income status without sufficient action to address the pandemic [9, 10]. The number of deaths resulting from diabetes and its complications in 2019 is estimated to be 4.2 million [8]. The existing approaches to the management of DM relied on keeping BGLs with normal limits via administration of appropriate medications together with lifestyle modifications [11]. So far, the accessible medicines for DM are various preparations of insulin and oral antihyperglycemic agents [12-16]. The older oral hypoglycemic are sulphonylureas, alpha-

glucosidase inhibitors, thiazolidinedione's, and biguanides [16,17] while the newer medicines include incretin-based therapies, sodium-glucose cotransporter 2 (SGLT-2) inhibitors, glucokinase activators, and injectable glucagon-like peptide (GLP-1) agonists [18]. These medicines are used either as mono-therapy or in combination to achieve better treatment outcomes [16].

The conventional and newer agents are still with their shortcomings, and successful treatment of diabetes is being a global challenge requiring further investigations. In fact, these medications are associated with unnecessary drug reactions or side effects [3, 16] including hepatocellular injury, exacerbate renal diseases, blood dyscrasias, gastrointestinal irregularities, hypoglycemia, hypersensitivity reactions, weight gains, and lactic acidosis [19], which decrease their effectiveness and compliance rates [4,19]. Because of the limitations of these agents, there remains a clear need for the identification of new antidiabetic drugs. Pre-clinical and clinical anti-diabetic studies on medicinal plants revealed their greater potential for drug discovery against diabetes. *Chlorophytum borivilianum* (Liliaceae) usually known as 'Safed Musli'. As per the Traditional system of medicine it is one of the most significant medicinal plants for mitigation of various ailments in human beings. The plant having therapeutic potential for diabetes, high blood pressure, arthritis, chronic leucorrhoea, delayed menopause, dysentery, diarrhea, general debility and boosts the immune system [20, 21]. To the best of the authors' knowledge, there are no previous scientific reports on the antidiabetic activity of *C. borivilianum*. Therefore, this study aimed evaluation of antidiabetic activity of leaf extract of *Chlorophytum borivilianum* in rat.

Materials and methods

Collection and authentication of plant materials

Leaves of *C. borivilianum* were collected from outskirts of Kota, Rajasthan in November 2016. The plant was identified, authenticated and certified (HPBDB/2015/1220) by Himachal Pradesh Biodiversity Board

Chemicals

All analytical grade chemicals were purchased from E. Merck Limited India and HiMedia Laboratories, Mumbai, India.

Preparation of extracts

Firstly, the plant leaves were washed with water to remove dirt and other foreign matters and were separated and shade dried. Dried leaves were then milled to coarse powder and then passed over sieve No. 14. The obtained dried powdered leaves of *C. borivilianum* (500 g) were placed in the tube of Soxhlet apparatus in the form of thimble and kept on heating mental for 6 h for extraction using methanol. The obtained extract (MECB) was filtered while hot and dried by evaporation using rotary vacuum evaporator and the final dried extracts sample was kept at low temperature in fridge for further study.

Experimental animals

Wistar rats of both sexes, weighing between 230 and 250 g and 2–3 months age, were housed in colonial cages and kept in standard laboratory environmental conditions; temperature 25 ± 2 °C, 12 h of light: 12 h of dark cycle and 50 ± 5% of relative humidity with free access to food and water ad libitum. The animals were adapted to the laboratory conditions before testing. Each group consists of six (n = 6) animals. Each of the tests was performed in the light time period (08: 00–16: 00 h). The investigations were conducted as per the standards provided by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Deshpande Laboratories Bhopal -CCP, under Reg. No. 1275/PO/Re/S/09/CPCSE.

Induction of diabetic state in rat

The Wistar albino rats (150-250 g) of either sex were allowed fasting for 24 hours prior to experimentation. Animals were divided into seven groups, each consisting of six rats. For 10 days the animals from group II-VII, dexamethasone (10 mg/kg/day, s.c.) were administered to the overnight-fasted rats and treated as follows. Drug treatments and dexamethasone treatment were started on the same day and drug treatment was continued for 21 days [22].

Experimental design

The animals were divided into seven groups of 6 rats each and treated as follows 42 Albino rats of middle-aged group were selected for the study and separated randomly in 7 groups of 6 animals in each.

Group-1: Vehicle Control Normal Control rats will treat with Normal saline; **Group-2:** Diabetic control group Rats will be treated with single dexamethasone sodium phosphate 10 mg/kg by s.c. and fed with normal pallet diet through the study period [21 days]; **Groups-3:** Diabetic- high fat diet Rats will be treated with single dexamethasone sodium phosphate 10 mg/kg by s.c. and fed with high fat diet through the study period [21 days]; **Group-4:** D-HFD + rats will be fed with methanolic extract of *C. borivilianum* (MECB) at min. dose (250 mg/kg) for [21 days]; **Group-5:** D-HFD + rats will be fed with methanolic extract of *C. borivilianum* (MECB) at max. Dose (500 mg/kg) for [21 days]; **Group-6:** D-HFD+ rats will be treated with Glibenclamide [0.5mg/kg]; **Group-7:** D-HFD + rats will be treated with Atorvastatin [10mg/kg]; **Dose: Test drug Extract:** Minimum dose; Maximum dose; **Standard drug:** Glibenclamide [0.5mg/kg]; Atorvastatin [10mg/kg].

Blood samples of each animal from respective treatment groups were collected by retro orbital on 0, 10 and 20 day of the treatment for estimation of biochemical parameters. At the end of the experimental period, i.e. on day 20, The animals were anasthesized with anesthetic diethyl ether and blood samples were collected by retro-orbital method and serum were separated for estimation of various biochemical parameters and sacrificed by cervical dislocation and viscera was exposed to remove the various tissues for estimation of physical and biochemical parameters.

Statistical analysis

The values were expressed as mean±SEM (n=6). The statistical significance was assessed using Student's t-test or one-way analysis of variance (ANNOVA) followed by Dunnett's test and $p<0.05$, $p<0.01$ and $p<0.001$ were considered as statistically significant.

Results and Discussion

Administration of dexamethasone (10 mg/kg, s.c.) showed significant ($P<0.01$) decrease in change in body weight on 10, 20 day of observational period respectively as compared to the normal group. Administration of glibenclamide (500 mcg/kg, p.o.) showed significant ($P<0.01$) increase in change in body weight as compared with diabetic control. Administration of MECB (250 and 500 mg/kg, p.o.) showed significant ($p<0.05$and $p<0.01$) increase in change in body weight as compared with diabetic control whereas RCLE (100 mg/kg, p.o.) showed insignificant effect in this regard as compared with diabetic control. Administration of atorvastatin (10 mg/kg) showed significant ($p<0.01$) increase in change in body weight as compared with diabetic control (table 1).

Table 1. Effect of MECB on Body weight in dexamethasone induced diabetes mellitus

Groups	Change in body weight	
	10 day	**20 day**
I	3.667±0.881	11.500±1.258
II	-17.333±2.333##	-25.833±3.70##
III	-12.333±1.085	2.167±3.439**
IV	-18.667±2.092	-13.333±1.406
V	-15.333±2.641	-9.333±1.646*
VI	-21.010±3.838	1.333±3.684**
VII	-12.667±2.076	7.010±3.306**

The values are expressed as mean±SEM (n=6). ##$p<0.01$, compared to normal group (Students 't' test) *$p<0.05$, **$p<0.01$, compared to diabetic control group (One way ANOVA followed by Dunnett's test).

Administration of dexamethasone (10 mg/kg, s.c.) showed significant ($P<0.01$) increase in serum glucose level on 10, 20 day of observational period respectively as compared to the normal group. Administration of glibenclamide (500 mcg/kg, p.o.) showed significant ($P<0.01$) decrease in serum glucose level as compared with diabetic control. Administration of MECB (250 and 500 mg/kg, p.o.) showed significant ($p<0.05$ and $p<0.01$) decrease respectly in serum glucose level as compared with diabetic control. Administration of Atorvastatin (10 mg/kg) showed significant ($p<0.01$) decrease in serum glucose level as compared with diabetic control (Table 2).

Table 2. Effect of MECB on Serum Glucose level in dexamethasone induced diabetes mellitus

Groups	Change in body weight		
	0 day	**10 day**	**20 day**
I	80.961±4.019	89.406±3.632	83.02±3.363
II	84.629±3.162	157.18±5.357##	187.43±8.03##
III	83.926±2.673	153.61±7.447	102.5±3.654**
IV	81.169±2.861	170.34±19.964	150.72±4.865*
V	81.786±3.120	155.61±9.933	129.25±6.65**

VI	83.531±2.434	150.27±8.825	116.32±5.597**
VII	78.296±3.010	166.66±14.929	107.23±3.569**

The values are expressed as mean±SEM (n=6). ##p<0.01, compared to normal group (Students 't' test) *p<0.05, **p<0.01, compared to diabetic control group (One way ANOVA followed by Dunnett's test).

Administration of dexamethasone (10 mg/kg, s.c.) showed significant (P<0.01) increase in SGOT as compared to the normal group. Administration of glibenclamide (500 mcg/kg, p.o.) showed significant (P<0.01) decrease in SGOT as compared with diabetic control. Administration of MECB (250 and 500 mg/kg, p.o.) showed significant (P<0.05 and P<0.01) decrease respectly in SGOT whereas MECB (250 mg/kg, p.o.) showed insignificant effect in this regard as compared with diabetic control. Administartion of atorvastatin 10 mg/kg showed significant (p<0.01) decrease in SGOT as compared with diabetic control.

Administration of dexamethasone (10 mg/kg, s.c.) showed significant (P<0.01) increase in SGPT as compared to the normal group. Administration of glibenclamide (500 mcg/kg, p.o.) showed significant (P<0.01) decrease in SGPT as compared with diabetic control. Administration of MECB (250 and 500 mg/kg, p.o.) showed significant (P<0.05 and P<0.01) decrease respectly in SGPT as compared with diabetic control. Administration of atorvastatin 10 mg.kg showed significant (p<0.01) decrease in SGPT as compared with diabetic control (Table 3).

Table 3. Effect of RCLE on SGOT and SGPT in dexamethasone induced diabetes mellitus

Groups	SGOT (U/L)	SGPT (U/L)
I	133.66±5.435	56.015±2.011
II	206.27±5.791 ##	108.24±5.898 ##
III	137.76±3.717**	56.605±2.307**
IV	187.55±4.907	82.642±2.418*
V	170.02±8.93*	69.648±3.41**
VI	139.14±3.283**	60.076±2.483**
VII	125.58±2.779**	53.249±2.379**

The values are expressed as mean±SEM (n=6). ##p<0.01, compared to normal group (Students 't' test) *p<0.05, **p<0.01, compared to diabetic control group (One way ANOVA followed by Dunnett's test).

Administration of dexamethasone (10 mg/kg, s.c.) showed significant (P<0.01) increase in cholesterol as compared to the normal group. Administration of glibenclamide (500 mcg/kg, p.o.) showed significant (P<0.01) decrease in cholesterol as compared with diabetic control. Administration of RCLE (100 and 200, 400 mg/kg, p.o.) showed significant (P<0.05 and P<0.01) decrease respectly in cholesterol as compared with diabetic control. Co- administration of RCLE 100 mg/kg and glibenclamide 250 mcg/kg showed significant (p<0.01) decrease in cholesterol as compared with diabetic control. Administration of dexamethasone (10 mg/kg, s.c.) showed significant (P<0.01) increase in triglycerides as compared to the normal group. Administration of glibenclamide (500 mcg/kg, p.o.) showed significant (P<0.01) decrease in triglycerides as compared with diabetic control. Administration of MECB (250 and 500 mg/kg, p.o.) showed significant (P<0.05 and P<0.01) decrease respectly in triglycerides as compared with diabetic

control. Administration of atorvastatin 10mg/kg showed significant (p<0.01) decrease in triglycerides as compared with diabetic control (Table 4).

Table 4. Effect of MECB on Cholesterol and Triglycerides in dexamethasone induced diabetes mellitus

Groups	Cholesterol(mg/dl)	Triglycerides(mg/dl)
I	72.658±3.879	96.302±4.389
II	147.25±3.251 ##	134.83±3.382##
III	77.044±3.171**	98.587±2.566**
IV	120.09±3.487*	107.88±3.24*
V	89.708±3.953**	103.31±2.294**
VI	81.073±3.598**	97.783±1.229**
VII	73.332±2.999**	94.081±2.603**

The values are expressed as mean±SEM (n=6). ##p<0.01, compared to normal group (Students 't' test) *p<0.05, **p<0.01, compared to diabetic control group (One way ANOVA followed by Dunnett's test).

Administration of dexamethasone (10 mg/kg, s.c.) showed significant (P<0.05) decrease in total protein as compared to the normal group. Administration of glibenclamide (500 mcg/kg, p.o.) showed significant (P<0.01) increase in total protein as compared with diabetic control. Administration of MECB (250 and 400 mg/kg, p.o.) showed significant (P<0.05) increase in total protein whereas MECB (250 mg/kg, p.o.) showed insignificant effect in this regard as compared with diabetic control. Administration of atorvastatin 10 mg/kg and glibenclamide 250 mcg/kg showed significant (p<0.05) increase in total protein as compared with diabetic control. Administration of dexamethasone (10 mg/kg, s.c.) showed significant (P<0.01) increase in blood urea nitrogen as compared to the normal group. Administration of glibenclamide (500 mcg/kg, p.o.) showed significant (P<0.01) decrease in blood urea nitrogen as compared with diabetic control. Administration of MECB (250 and 500 mg/kg, p.o.) showed significant (P<0.01) increase in blood urea nitrogen whereas MECB (250 mg/kg, p.o.) showed insignificant effect in this regard as compared with diabetic control. Administration of Atorvastatin 10 mg/kg and glibenclamide 250 mcg/kg showed significant (p<0.01) decrease in blood urea nitrogen as compared with diabetic control (Table 5).

Table 5. Effect of MECB on Total protein and Blood urea nitrogen in dexamethasone induced diabetes mellitus

Groups	Total protein (gm/dl)	Blood urea nitrogen (mg/dl)
I	6.562±0.158	16.62±0.788
II	5.345±0.199 #	23.748±1.686 ##
III	7.496±0.197**	17.723±0.895**
IV	6.268±0.173	20.869±1.205
V	7.285± 0.160*	17.859±0.748**
VI	7.452± 0.228*	17.051±0.738**
VII	7.694±0.207*	16.986±1.198**

The values are expressed as mean±SEM (n=6). #p<0.05, ##p<0.01, compared to normal group (Students 't' test) *p<0.05, **p<0.01, compared to diabetic control group (One way ANOVA followed by Dunnett's test).

The present investigation was designed to evaluate the efficacy of the MECB on dexamethasone induced diabetes mellitus. In our study, there was a significant elevation in serum glucose level in diabetic control group as compared with normal animals. The MECB-treated group exhibited significant reduction of serum glucose levels as compared to the diabetic control group. Over production of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental bases of hyperglycemia in diabetes mellitus. The decrease in body weight with diabetes mellitus has been attributed to the gluconeogenesis i.e., catabolism of proteins and fats, which is associated with the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins

In the present study, diabetic rats treated with MECB showed an increase in body weight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis. In diabetic condition, there is increase in weight of various organs such as kidney, liver, pancreas, etc. This is due to inflammation of these organs in diabetic condition. MECB reduces inflammation of such organs and reduces their weight. In dexamethasone induced diabetic rats, hyper-cholesterolemia and hyper- triglyceridemia are well documented. Insulin deficiency leads to increased serum lipids because of increased lipolysis. The elevated levels of serum total cholesterol and triglycerides were significantly decreased after treatment with MECB. The abnormally high concentration of serum lipids in diabetes mellitus is mainly due to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. It has been reported that significant reduction in total cholesterol and triglycerides is considered to be one of the desirable biochemical conditions for the prevention of diabetes mellitus.

In dexamethasone induced diabetic animals, alterations in the levels of serum enzymes are directly related to changes in the metabolic functions of SGOT, SGPT, ALP and LDH The increased levels of serum SGOT, SGPT, ALP and LDH have already been reported to be associated with liver dysfunction and leakage of these enzymes from liver cytosol into the blood stream in diabetes.

Reduction in the activity of SGOT and SGPT in MECB treated diabetic rats indicates the alleviating role of the compound against dexamethasone induced hepato-cellular necrotic changes. MECB intake produced significant decrease in plasma bilirubin of dexamethasone induced diabetic rats. Rana et al. (1996) reported that the increase in plasma bilirubin (hyper-bilirubenimia) may be resulted from the decrease of liver uptake, conjugation or increase bilirubin production from hemolysis. Also, the elevation in plasma bilirubin indicates liver damage as confirmed by the changes in the activities of plasma and liver enzymes. In diabetic group, there is decrease in serum protein level and increase in serum creatinine level. This is due to increase in catabolism of protein in diabetic condition. While MECB treated groups show increase in serum protein level and decrease in serum creatinine level by preventing the catabolism of protein. MECB in combination with Glibenclamide produced more significant effect as compared to only single MECB in diabetic animals. MECB also show protective role in diabetes mellitus in dose dependent manner.

Conclusion

The results of present study revealed that, MECB probably by its antioxidant potential prevented the oxidative stress in β-cells, induced by dexamethasone, thereby by preventing the β-cell degeneration and increasing the glucose sensitivity of β-cells leading to increase in insulin release. The effectiveness of the MECB in multiple preclinical models with desire mechanism of action might be due to the presence of flavonoids, alkaloids and tannins or its synergistic action of these phytoconstituents. However, the exact role of Phytochemical and their mechanism of action need future investigations.

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