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Effect of a Polyherbal Formulation (*Diarun plus*) on Streptozotocin Induced Experimental Diabetes

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Abstract: *Diarun plus* was evaluated for its therapeutic antihyperglycemic activity in streptozotocin-induced diabetes in animal models. It was effective in controlling hyperglycemic status in rats and mice. The drug (500 mg/kg) showed significant antidiabetic property as judged by serum glucose levels. However, it did not significantly influence the levels of serum insulin in both diabetic and normoglycemic rats. This is a promising drug to develop standardized phytomedicine for diabetes mellitus. The findings suggest that it can be considered as a therapeutic agent, wherever elevated glycemic status is observed.

Key words: *Diarun plus*, polyherbal, hyperglycemia, diabetes mellitus, streptozotocin

INTRODUCTION

Diabetes mellitus is a metabolic disorder, characterised by lack/deficient secretion of insulin from β cells of islets cells of pancreas[1]. Available therapeutic agents include administration of insulin as in Insulin Dependent Diabetes Mellitus (IDDM) or along with oral hypoglycemic agents in Non-Insulin Dependent Diabetes Mellitus (NIDDM)[1]. Oral hypoglycemic agents can produce serious side effects and in addition, they are not suitable for use during pregnancy[2]. Though effective glycemic control is achieved by these, certain undesirable effects such as hypoglycemia, resistance etc. occurs[3]. Since the theraphy is life long, therapeutic agents devoid of side effects will be appreciated, one such approach is the use of alternate system of medicine comprising of herbal products[4].

Diarun plus, a poly herbal formula containing *Emblica officinalis* Gaertn. (Family: Euphorbiaceae), *Curcuma longa* Linn. (Family: Zingiberaceae), *Momordica charantia* Linn. (Family: Cucurbitaceae), *Eugenia jambolana* Lam. (Family: Myrtaceae), *Trigonella foenum-graecum* (Retz.) R. Br. ex Schult. (Family: Fabaceae), *Gymnema sylvestre* R. Br. (Family: Asclepiadaceae) and *Salacia reticulata* Wight (Family: Celastraceae), of proven antidiabetic effect with folkloric claim[4]. Previous studies carried out on the glycemic status modified by physiological means in non-diabetic animals[5] and physiologically modified glycemic and insulinemic status revealed this formulation can maintain near euglycemic state irrespective of the altered glycemic status[6].

In the present study, as a supportive measure, an attempt has been further made to evaluate the glucose-lowering effect of *Diarun plus* in conventional experimentally induced diabetic model using Streptozotocin (STZ)[7,8].

MATERIALS AND METHODS

Animals: Male Swiss albino mice (25-30 g) and Male Wistar albino rats (180-220 g) were housed under normal laboratory conditions at least one week prior to experimentation under 12:12 light:dark cycle. They had free access to food (Standard pellet from Lipton India, Ltd) and water *ad libitum* and maintained at 24-28°C temperature, 60-70% relative humidity. Experiments were conducted during light hours. The study proposal approved by the Institute Animal Ethics Committee.

Drugs used: *Diarun plus* was gifted by Rumi Herbals, Chennai, Streptozotocin was procured from Sigma chemicals, USA and Human insulin was purchased from Smithkline Beecham Pharmaceuticals, Mumbai.

Streptozotocin-induced diabetes: Hyperglycemia in animals was achieved by tail vein injection of STZ[7] (75 mg kg^{-1} i.v.) dissolved in normal saline. After 72 hrs, 1 mL blood sample was drawn by venipuncture and glucose levels were determined using glucometer[9]. The rats exhibiting plasma glucose level above 200 mg% were considered hyperglycemia and was selected for this

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study. Blood glucose level was measured prior to STZ administration and 72 hrs after and every week thereafter for a period of 4 weeks. Animals were maintained with exogenous insulin supplement (2 IU/week). Another group of animals received *Diarun plus* orally as suspension 500 mg kg^{-1} twice a day along with insulin 1 IU/wk. Changes in glycemic status, body weight and percentage of mortality were noted. Appropriate vehicle treated animals served as control. Results are expressed as actual blood glucose value and percentage change, considering the initial value of each animal as 100%.

STZ received hyperglycemic animals were divided into the following sub groups of six animals each. The first group received insulin 2 IU/wk. The second group received *Diarun* plus (500 mg kg^{-1} b.d.; orally) along with either insulin 1 IU/wk or vehicle. The last group received vehicle only. The parameters measured as described above besides monitoring mortality.

Measurement of blood glucose: The blood glucose was measured using Ames glucometer (Bayers Diagnostics with appropriate glucostix) from a drop of blood collected by venipuncture of the tail, which has been validated in an earlier study[9] by comparing with blood glucose level estimated by autoanalyser.

Statistical evaluation: Statistical significance between treated and control groups were analysed using Student's 't' test[10]. $p<0.05$ was considered as statistically significant.

RESULTS

Administration of STZ produced marked elevation of glycemic status ranging from 53 to 71%. This hyperglycemia was effectively controlled to near euglycemic state with weekly administration of insulin. The enhanced glycemic status in STZ animals was attenuated to 28% in animals which received *Diarun plus* alone. However, in these animals still a significant hyperglycemic status persisted (Fig. 1 and 2). However, when *Diarun plus* was co-administered with half the maintenance dose of insulin (1 IU/wk), near euglycemic status (comparable with insulin received groups) was achieved. The results of the extended period revealed that administration of *Diarun plus* alone for three weeks attenuated the hyperglycemic effect of STZ more than 50% (Fig. 3 and 4).

The present mortality in STZ hyperglycemic animals 83% was significantly reduced to 66.67% by 1 IU of insulin, to 16.67% by 2 IU of insulin and *Diarun plus* and

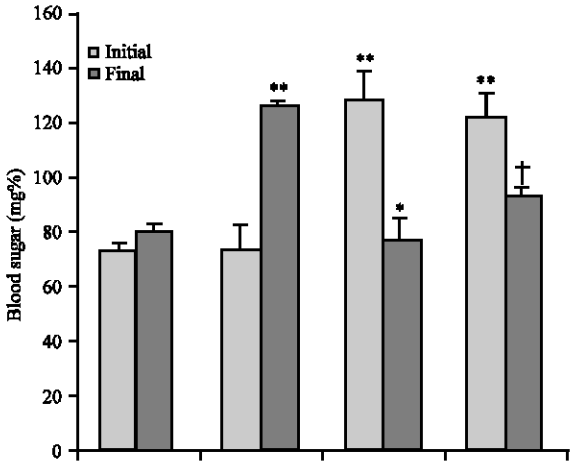

Fig. 1: Effect of *Diarun plus* on experimental hyperglycemia in mice

Values represent Mean ± SEM of six observations. A represents control; B for STZ induced without drug; C for treatment with Diarun plus with Insulin 2 IU/week and D for treatment with Diarun plus with Insulin 1 IU/week. *$p<0.05$ and **$p<0.001$ as compared with Control (n = 6) †$p<0.05$ as compared with initial response

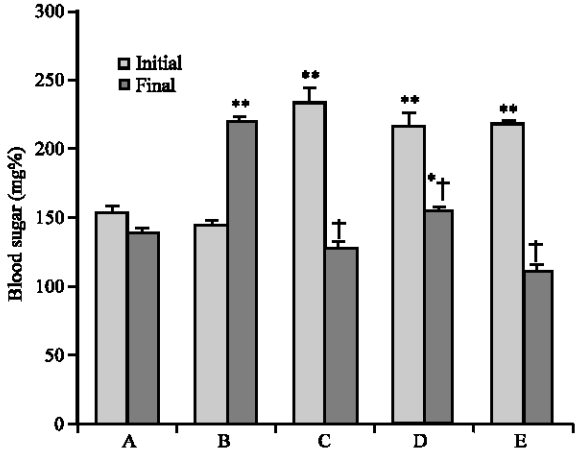

Fig. 2: Effect of *Diarun plus* on experimental hyperglycemia in rats

Values represent Mean ± SEM of six observations. A-Control; B - STZ induced without drug; C- treated with 2 units of insulin; D - treated with 1 unit of insulin and Diarun plus; E - treated with Diarun plus alone* $p<0.05$ and **$p<0.001$ as compared with Control (n = 6) †$p<0.05$ as compared with initial response

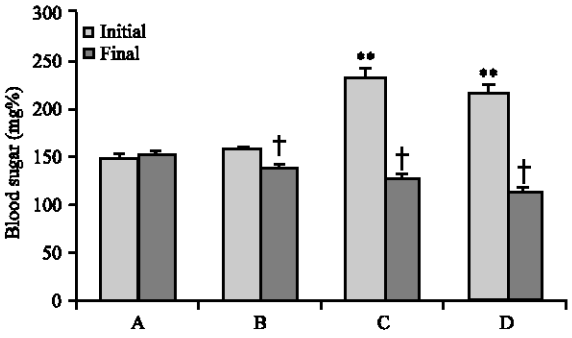

Fig. 3: Effect of *Diarun plus*, insulin alone and in hyperglycemia included by STZ in rats for 3 weeks

Values represent Mean ± SEM of six observations. A-Control; B-STZ induced treated with Diarun plus; C-STZ induced treated with 2 units of insulin; D-STZ induced treated with 1 unit of insulin and Diarun plus. * * $p<0.001$ as compared with Control † $p<0.05$ as compared with respective final glucose

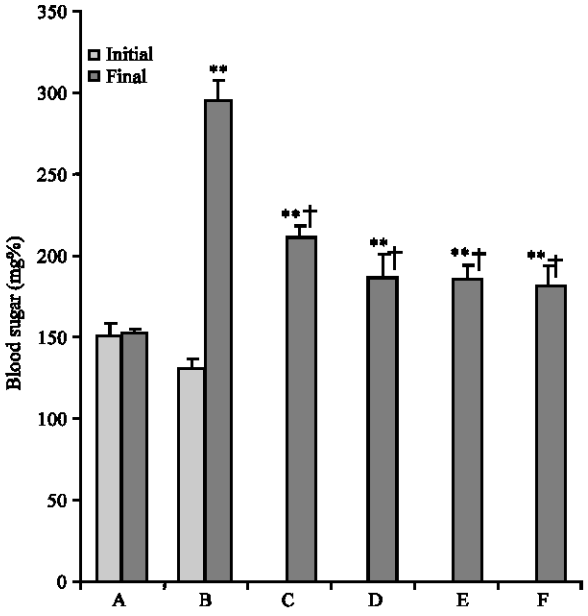

Fig. 4: Effect of *Diarun plus*, alone on the STZ included hyperglycemia in rats

Values represent Mean ± SEM of six observations. A-Control; B-STZ induced without drug; C-STZ induced treated with Diarun Plus alone for 1 week; D-STZ induced treated with Diarun plus alone for 2 weeks; E-STZ induced treated with Diarun Plus alone for 3 weeks; F - STZ induced treated with Diarun Plus alone for 4 weeks.* $p<0.05$ and **$p<0.001$ as compared with Control ($n = 6$) † $p< 0.05$ as compared with initial response

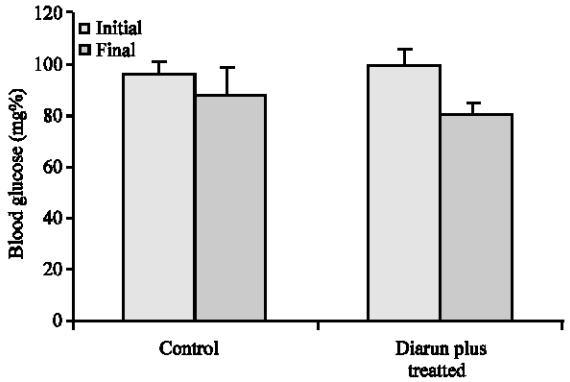

Fig. 5: Effect of *Diarun plus*, on the blood glucose levels in normal rats

Values represent Mean ± SEM of six observations. Drug was administered 60 min prior to final glucose measurement

to 0% by *Diarun plus* alone. Additionally the loss of body weight induced by STZ was also antagonized by *Diarun plus* and in fact weight gain was noticed (Data not shown).

The study exhibited antidiabetic property in streptozotocin-induced diabetic mice (Fig. 1) and rats (Fig. 2) as evident by serum glucose. STZ elevated the blood glucose from 142.8 to 217 mg% indicating diabetic state. Reduction in serum glucose (124.7 mg%) by insulin administration (Fig. 2) was observed. Supplement of *Diarun plus* 500 mg kg^{-1} with insulin 1 U/wk almost produced a similar response by bringing down the blood sugar to 153.1 mg%. *Diarun plus* (500 mg kg^{-1}) supplementation brought down the blood glucose in diabetic animal to euglycemic state. Furthermore in insulin (2U) supplemented animals mortality (16.67%) was noticed at the end of 4 weeks (Fig. 3). In *Diarun plus* supplemented animals there was no mortality (Fig. 4) upto 4 weeks.

DISCUSSION

Previous study[5,6] and the findings of the present one on *Diarun plus* clearly indicate that this formulation is not a hypoglycemic agent since if *per* se failed to produce any significant changes in the glycemic status (Fig. 5). This can be considered as an important advantage since the available agents like insulin and oral hypoglycemic agents are hypoglycemic, when in euglycemic individuals[11]. Additionally, longer duration of *Diarun plus* theraphy of in combination with insulin significantly attenuated the STZ induced hyperglycemia. This suggests that *Diarun plus* administration possibly

might have reduced the daily insulin requirements and thereby can avoid the development of insulin resistance. This anti-hyperglycemic activity could be attributed to the folkloric claims of the ingredients contained in it[12].

The herbal ingredients present in the formulation had been proved to exhibit hypoglycemic effect in many experimental diabetic models and beneficial effect in diabetic patients. *C. longa* present in this formulation, lowers blood sugar in alloxan-induced diabetic rats by vitalization of pancreatic cells and stimulation of insulin production[13]. *M. charantia* seeds lowers fasting blood glucose and improved glucose tolerance[14]. *E. officinalis* is a potent antioxidant, free radical scavenger and lipid peroxide inhibitor[15]. *E. jambolana* lowers blood glucose in diabetic rabbits[16] and produce symptomatic relief in diabetic patients[17]. *T. foenum-graceum* exhibits potent hypoglycemic effect in alloxan induced diabetic rats[18]. The seed extract also induced hyperinsulinemia and hypercholesterolemia[19]. *G. sylvestre* lowers blood glucose in diabetic animals and stimulates insulin secretion[20]. Salacino isolated from *S. reticulatta* is a potent inhibitor against several alpha-glucosidases, such as maltase, sucrase and isomaltase and the inhibitory effects on serum glucose levels in maltose- and sucrose-loaded rats (*in vivo*) were found to be more potent than that of acarbose, a commercial alpha-glucosidase inhibitor[21].

Based on the above observations, it can be concluded that the folkloric claim of the anti-diabetic activity of the ingredients of *Diarun plus* is scientifically substantiated. Besides, the reduction in mortality, absence of hypoglycemic effect and reversal effect of hyperglycemia induced weight loss are potential added advantages of this formulation. It is suggested that *Diarun plus* is an effective formulation for diabetic patients with a potential of decreasing the prognosis of the disease, which deserves for the experimentation.

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