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Effect of a polyherbal formulation (*Diarun plus*) on the glycemic status modified by physiological means in non-diabetic mice and rats

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SUMMARY

Diarun plus, a polyherbal formulation containing herbal ingredients of folkloric Antidiabetic effect, was investigated for its effect on glycemic status in rats and mice. In contrast to conventional chemical induced diabetic animal models, changes in glycemic states were induced by physiological maneuvers. Results revealed that in euglycemic animals *Diarun plus* elicited little change (-10 to +10%), which was insignificant. In food deprivation/swim exercise induced hypoglycemia, *Diarun plus* reduced the degree of hypoglycemia in both rats and mice (from 38% to 27% in rats and 45% to 32% in mice). Similarly, the marked hyperglycemia induced by dextrose (70% in rats and 95% in mice) was reduced markedly to 8% and 25% respectively. The findings of the present study suggests that the ingredients of *Diarun plus* have the unique property of maintaining near euglycemic state irrespective of the altered glycemic state, and that have no significant effect in euglycemic condition.

Key words: Hypoglycemia; Hyperglycemia; *Diarun plus*; Polyherbal

INTRODUCTION

Pre-clinical investigation on newer substances for their efficacy against diabetes mellitus is normally carried out in streptozotocin (STZ) or alloxan induced diabetic animal models, which destroys the b cells in the islets of pancreas and induce a chronic hyperglycemic status akin to diabetes mellitus (DM). However, it has been documented that these chemicals produce structural and functional abnormalities in the nervous system of these animals (Osturk *et al*., 1996). Therefore measurement of any parameter involving nerve conduction is likely to generate inconsistent results. Taking this into consideration, in the present study an attempt has been made to identify the effect of a polyherbal formulation, *Diarun plus*, on the glycemic status in physiologically altered glycemic status where the nervous system is intact.

Diarun plus is a polyherbal formulation containing seeds of *Eugenia jambolana*, *Trigonella foenum-graecum*, *Momordica charantia* leaves of *Gymnema sylvestre*, fruits of *Emblica officinalis*, rhizomes of *Curcuma longa* and roots of *Salacia reticulata*. All these ingredients have a traditional claim for their beneficial effects in the management of DM (Nadkarni, 1954; Chopra *et al*., 1956; Anonymous, 1976). Our earlier preliminary experiments (Anonymous, 2002) with

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Diarun plus in STZ-induced diabetic animal models indicated the inclusion of *Diarun plus* in the diabetic diet as an adjuvant therapeutic measure in the management of DM. In the present study, attempts were made to attain hypoglycemic status using either food deprivation or swim exercise, which is likely to utilize glucose for energy expenditure while exogenous glucose administration was used to achieve hyperglycemic state.

MATERIALS AND METHODS

Swiss male albino mice (25 - 30 g) and wistar male albino rats (180 - 220 g) were housed under normal laboratory conditions with free access to food and water except for one group in which food deprivation was included as a method to elicit hypoglycemia.

Induction of hypo- and hyperglycemia

Twenty four hour food deprivation prior to experimentation or allowing the animals to swim in water at room temperature (29 - 30°C) for 3 min in a polypropylene container (40 × 35 × 25 cm) filled with water up to 15 cm height was employed to induce hypoglycemia as described by Rajendran *et al.* (2001). In order to produce hyperglycemia, exogenous oral administration of dextrose, (2 g/kg) was carried out 15 min prior to experimentation, as validated by Reddy *et al.* (1998).

Measurement of blood glucose level

A drop of blood collected by venipuncture of the tail and the blood glucose level was measured using Ames glucometer (Bayers Diagnostics with appropriate glucostix). This method has been validated in earlier study (Rajendran *et al.,* 2001).

Drug treatment

Diarun plus was administered 60 min prior to glucose estimation as a 1% suspension in carboxyl-methyl cellulose. At the end of 60 min, the animals were allowed either to swim or received dextrose. A separate group of animals were deprived of food for 24 h. The blood glucose was measured prior to exposing the animals to food deprivation, swim exercise or dextrose administration and at 15 min after these maneuvers, previously validated by Reddy *et al.* (1998). The doses of *Diarun plus* were 100 or 500 mg/kg, i.p., which were selected based on pilot studies. Appropriate vehicle treated animals were served as control. The results were expressed as the actual blood glucose level and also as the percentage change considering the initial value as 100%. The data were subjected to statistical analysis by employing ANOVA followed by Dunnett's '*t*' test. A level of $P \leq 0.05$ was considered statistically significant.

Diarun plus was supplied by Rumi Herbals, Mugappair, Chennai, while dextrose anhydrous IP, was purchased from Glaxo, Mumbai.

RESULTS

Vehicle treatments in free fed animals, both rats and mice produced an insignificant elevation of only +2% change in blood glucose level. *Diarun plus per se* in both doses produced inconsistent and insignificant changes in the glycemic state of rats and mice (Table 1). Food deprivation for 24 h produced a significant hypoglycemia to the extent of 31.5% in rats and 33.9% in mice. In *Diarun plus* supplemented-, and food deprived-animals, this fall was attenuated both in rats and mice (Table 2). However, food deprivation-induced hypoglycemia was still significant in mice and rats which received *Diarun plus* (Table 2).

Animals, which swam for 3 min, exhibited hypoglycemia to the extent of 39% in rats and 45% in mice, which was comparable to those observed in food, deprived animals (Table 2). However, *Diarun plus* supplementation decreased significantly the hypoglycemia in mice and in rats the changes were minimum, when compared to that of the results observed in food deprived groups (Table 3). The administration of dextrose elevated the blood glucose level significantly to the extent of 70% in

Table 1. Influence of *Diarun plus* on the blood glucose levels in physiological models in free fed rats and mice

Status	Treatment mg/kg, p.o.	Blood glucose (mg/dl)		Percentage changes in blood glucose
		Initial	Final	
Rats	Vehicle	95.5 ± 5.6	97.2 ± 7.8	+ 2.1
	Diarun 100	88.6 ± 6.6	80.4 ± 7.7	- 10.0
	Diarun 500	87.6 ± 11.0	80.0 ± 4.1	- 8.1
Mice	Vehicle	71.5 ± 5.9	72.5 ± 6.9	+ 1.3
	Diarun 100	74.4 ± 7.5	82.1 ± 5.4	+ 10.3
	Diarun 500	63.4 ± 6.4	64.2 ± 4.7	+ 1.2

Each value represents the mean ± SEM of six observations; Drug was administered 60 min prior to final glucose measurement.

Table 2. Influence of *Diarun plus* on the blood glucose levels in healthy, hypoglycemic and hyperglycemic physiological models in 24 h food deprivated rats and mice

Status	Treatment mg/kg, p.o.	Blood glucose (mg/dl)		Percentage changes in blood glucose
		Initial	Final	
Rats	Vehicle	87.8 ± 6.4	60.2 ± 1.8*	- 31.5
	Diarun 100	92.4 ± 8.8	78.2 ± 6.4*	- 15.4
	Diarun 500	79.8 ± 3.4	64.2 ± 4.8*	- 19.6
Mice	Vehicle	75.4 ± 2.8	49.9 ± 4.4*	- 33.9
	Diarun 100	82.1 ± 3.5	62.0 ± 6.9*	- 24.6
	Diarun 500	77.9 ± 4.1	60.2 ± 3.4*	- 22.8

Each value represents the mean ± SEM of six observations; Drug was administered 60 min prior to final glucose measurement; $^{*}P < 0.05$ compared with respective initial value

Table 3. Influence of *Diarun plus* on the blood glucose levels in healthy, hypoglycemic and hyperglycemic physiological models by swimming in rats and mice

Status	Treatment mg/kg, p.o.	Blood glucose (mg/dl)		Percentage changes in blood glucose
		Initial	Final	
Rats	Vehicle	142.5 ± 6.9	152.8 ± 3.4	+ 7.0
	Swim alone	139.7 ± 7.2	84.6 ± 5.2**	- 39.6
	Diarun 100	128.7 ± 5.4	100.4 ± 3.2**,†	- 21.9
	Diarun 500	144.8 ± 3.9	105.3 ± 4.8**,†	- 27.1
Mice	None	122.4 ± 2.8	129.3 ± 1.9	+ 5.7
	Swim alone	142.3 ± 3.9	77.7 ± 6.4**	- 45.3
	Diarun 100	117.8 ± 4.4	80.2 ± 5.9**	- 31.9
	Diarun 500	139.4 ± 2.5	100.1 ± 7.9	- 32.0

Each value represents the mean ± SEM of six observations; Animals were allowed to swim for 3 min; Drug was administered 60 min prior to final glucose measurement; $^{**}P < 0.01$ compared with respective initial value, $^{\dagger}P < 0.05$ compared with respective vehicle treatment (final value).

rats and 95% in mice (Data not shown). In *Diarun plus* pretreated animals, dextrose was unable to produce hyperglycemia to the same extent in both rats and mice. A significant decrease was recorded in both types of animals. This effect was found to be dose-related (Table 4) among the two doses analysed.

Table 4. Influence of *Diarun plus* on the blood glucose levels in healthy, hypoglycemic and hyperglycemic physiological models in dextrose fed rats and mice

Status	Treatment mg/kg, p.o.	Blood glucose (mg/dl)		Percentage changes in blood glucose
		Initial	Final	
Rats	Swim alone	139.7 ± 7.2	84.6 ± 5.2**	- 39.4
	Vehicle	124.8 ± 4.2	212.8 ± 9.2**	+ 70.9
	Diarun 100	142.4 ± 5.2	180.2 ± 3.4**,†	+ 26.7
	Diarun 500	129.2 ± 2.9	140.2 ± 1.9*,†	+ 8.3
Mice	Vehicle	124.4 ± 4.2	242.4 ± 2.4**	+ 95.2
	Diarun 100	132.8 ± 1.9	178.2 ± 11.4**,†	+ 34.8
	Diarun 500	128.4 ± 2.9	160.0 ± 6.4**,†	+ 24.6

Each value represents the mean±SEM of six observations; Drug was administered 60 minprior to final glucose measurement; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with respective initial value, $^{\dagger}P < 0.05$ compared with respective vehicle treatment (final value)

DISCUSSION

In the present study, dose-related attenuation of the hypoglycemia and hyperglycemia elicited under various physiological circumstances was observed. This indicates that *Diarun plus* shows a tendency to maintain the euglycemic status irrespective of any alteration in the glycemic state. This restoration by *Diarun plus* to the euglycemic status suggests that *Diarun plus* supplementation will be beneficial in the situations like DM where glycemic status is altered. This effect is considered beneficial since the anti-diabetic agents like insulin or oral hypoglycemic drugs either alone or in combination have a tendency to induce hypoglycemic status. This findings suggest that *Diarun plus* would be useful in attributing the difficulty encountered in the assessment of the dose or noncompliance of the food habit advised to the DM patients. This hypoglycemic situation observed in clinical situations are considered more harmful than the disorder itself. Besides, in the present study, the polyherbal combination investigated failed to modify the glycemic status when administered alone. This is advantageous since the harmful hypoglycemic situation is not seen which is otherwise encountered with conventional hypoglycemic agents.

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 (Senthilvel *et al.,* 2004). Ethanolic extract of *Curcuma longa* rhizome lowered blood sugar in alloxan-induced diabetic rats by vitalization of pancreatic cells and stimulation of insulin production (Anonymous, 2001). Ethanolic extracts of *Momordica charantia* seeds lowered fasting blood glucose and improved glucose tolerance (Karunanayake *et al.,* 1984). Fruits of *E. officinalis* are potent antioxidant, which are free radicals scavengers and lipid peroxide inhibitors (Jose and Kuttan, 1995). Powdered seeds and aqueous extract of *E. jambolana* lowered blood glucose in diabetic rabbits (Anonymous, 1976) and produced a marked symptomatic relief in diabetic patients (Kohli and Singh, 1993). Aqueous extract of *Trigonella foenum-graceum* exhibited potent hypoglycemic effect in alloxan induced diabetic rats (Abdel Barry *et al.,* 1997). The seed extract also induced hyperinsulinemia and hypercholesterolemia (Sauvaire *et al.,* 1996, 1997). The alcoholic extract of *G. sylvestre* lowered blood glucose in diabetic animals and stimulated insulin secretion in rabbits (Chopra *et al.,* 1956). Salacino isolated from *S. reticulatta* is also been reported to have a potent inhibitory activity 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 (Yoshikawa *et al.*, 2002). A combination of these herbs can be naturally expected to lower the blood glucose level and exert beneficial effects in DM patients.

The methods employed to achieve alterations in the glycemic status are physiological and transient, thereby not causing any structural and functional damage compared to chemical models. However, a limitation in the present study is that the scope envisages only a transient stage and extrapolation to chronic situation is debatable. Despite this, the present data provide the basic information about *Diarun plus* and its possible clinical utility based on the beneficial observation recorded.

Further detailed studies on its safety and the exact mode of action are warranted.

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REFERENCES

Abdel Barry JA, Abdel Hassan IA, Al Hakiem MHH. (1997) Hypoglycemic and antihyperglycemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan induced diabetic rats. *J. Ethnopharmacol.* **58**, 149-155.

Anonymous. (1976) *Medicinal Plants of India,* In: Satyavati GV, Raina MK, Sharma M (eds.) Indian Council of Medical Research, New Delhi, Vol. 1, pp. 147.

Anonymous. (2001) *The Wealth of India,* National Institute of Science Communication, CSIR, Dr. KS Krishnan Marg, New Delhi, Vol. 2, pp. 264.

Anonymous. (2002) Proceedings of National seminar on Siddha Medicine, R & D Institutions and Pharmaceutical Industries on December 2002. In: Senthilvel G, Anoop Austin, Thirugnanasambantham P, Mayisvren E, Viswanathan S, Jegadeesan M. (2002) Effect of *Diarun plus*, a polyherbal formulation on physiologically modified glycemic and insulinemic status in mice. Tamil University, Thanjavur, India.

Chopra RN, Nayar SL, Chopra IC. (1956) *The Glossary of Indian Medicinal Plants.* Publications and Information Directorate, Council of Scientific and Industrial Research, New Delhi, pp. 127.

Jose JK, Kuttan R. (1995) Antioxidant activity of *Emblica officinalis*. *J. Clin. Biochem. Nut.* **19**, 63-70.

Karunanayake EH, Welihinda J, Sirimanne SR, Sinnadorai G. (1984) Oral hypoglycaemic activity of some medicinal plants of Sri Lanka. *J. Ethnopharmacol.* **11**, 223-231.

Kohli KR, Singh RH. (1993) A clinical trial of jambu *(Eugenia jambolana*) in non-insulin dependent diabetes mellitus, *J. Res. Ayur. Sid.* **14**, 89-97.

Nadkarni K M. *Indian Materia Medica*, Vol. 1, Popular Book Depot, Bombay, p. 1954.

Osturk Y, Alten VM, Yidizoglu Ari N. (1996) *Pharmacol. Rev.* **48**, 68-112.

Rajendran NN, Thirugnanasambantham P, Parvathavarthini S, Viswanathan S, Ramaswamy S. (2001) Modulation by insulin rather than blood glucose of the pain threshold in acute physiological and flavone induced antinociception in mice. *Indian J. Exp. Biol.* **39**, 1009-1016.

Reddy PRMK, Shankar Raman, Ramaswamy S. (1998) Effect of glucose on stress induced antinociception in noral mice. *Indian J. Physiol. Pharmacol.* 131-134.

Sauvaire Y, Broca C, Petit P, Jacob M, Baissac Y, Manteghetti M, Roye M, Ribes G. (1996, 1997) 4-hydroxy isoleucine: A novel insulinotropic amino acid isolated from fenugreek seeds. *Phytomedicine* **Suppl 1**, 272.

Senthilvel G, Jegadeesan M, Anoop Austin, Thirugnanasambantham P, Balasubramanian M, Mayisvren E. (2004) Evaluation of efficacy and tolerability of a herbal formulation (*Diarun Plus*) in the management of Type 2 Diabetes mellitus in Indian patients. *Amala Res. Bull.* **24**, 57-62.

Yoshikawa M, Morikawa T, Matsuda H, Tanabe G, Muraoka O. (2002) Absolute stereostructure of potent alpha-glucosidase inhibitor, Salacinol, with unique thiosugar sulfonium sulfate inner salt structure from *Salacia reticulata*. *Bioorg. Med. Chem.* **10**, 1547-1554.