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Influence of Aqueous Extract of *Emblica officinalis* Fruits on Tolbutamide Induced Hypoglycemia/Antihyperglycemia in Normal and Diabetic Rats

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This work reports the evaluation of the influence of aqueous extract of *Emblica officinalis* fruits on tolbutamide activity in normal and diabetic rats. Tolbutamide produced hypoglycemic activity in a dose dependent manner in normal and diabetic condition. In the presence of *Emblica officinalis* fruits, tolbutamide produced early on set of action and maintained for longer period compared to tolbutamide group. An aqueous extract of *Emblica officinalis* fruits was found to improve tolbutamide response in normal and diabetic rats.

Key Words: *Emblica officinalis*, Tolbutamide, Hypoglycemia, Antihyperglycemia.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism. It may be due to decrease in the secretion of insulin (Type-1 diabetes) or decrease in the secretion of insulin ((Type-2 diabetes) from β-cells of the islets of Langerhan's in the pancreas. Type-2 diabetes is a heterogenous disease with both genetic and environmental contributory factors, involved multiple defects in insulin action and insulin secretion leads to hyperglycemia and effecting nearly 10 % of the population[1].

Diabetes is one of the stress related disorder. Diabetic patients are shown to have increased oxidative stress and decreased antioxidant levels[2-4]. There is an evidence that tight control of blood glucose is possible with decrease in oxidative stress[5]. The initial treatment of type-2 diabetes has always been optimization of diet and physical activity. However, the benefits of nonpharmacological therapy are not dependable due to non compliance of patients to the prescribed diet and exercise.

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Insulin is the drug of choice in type-1 diabetes and sulfonylureas are the drug of choice in type-2 diabetes. Among sulfonylureas, tolbutamide is the drug of choice for geriatrics because of its short duration of action and lower incidence of hypoglycemia in early hours of night.

The limitations of currently available pharmacological agents for the control of blood glucose have promoted physicians to prescribe antioxidants as supplemental agents with antidiabetic drugs[6]. In many antioxidant formulations *Emblica officinalis* is the active ingredient. There is an evidence that *Emblica officinalis* has antidiabetic activity[7,8].

Present study was conducted to find the influence of aqueous extract of *Emblica officinalis* fruits on the hypoglycemic and antihyperglycemic activities of tolbutamide in normal and diabetic rats.

EXPERIMENTAL

Albino rats of either sex weighing between 175-200 g were procured from Mahaveer Enterprises, Hyderabad, India, were used in the study. They were maintained under standard laboratory conditions at ambient temperature of 25 ± 2 ºC and 50 ± 15 % relative humidity with a 12 h light/12 h dark cycle. Rats were fed with a commercial pellet diet (Rayans Biotechnologies Pvt. Ltd., Hyderabad) and water *ad libitum*. All experiments were performed in accordance with the institutional animal ethics committee. The animals were divided into 3 groups of 6 each. They were fasted for 18 h prior to the experiment (allowing access to water) and during the experiment, food and water were withdrawn.

An aqueous extract of *Emblica officinalis* fruits (AEE) was obtained from M/s Laila Impex, Vijayawada as a gift sample and it was used throughout the study. Alloxan monohydrate was purchased from LOBA Chemie, Mumbai, India. Tolbutamide was supplied by Dr Reddy's Laboratories, Hyderabad. Glucose kits (Span diagnostics) were purchased from the local pharmacy.

Study in normal rats: Group I/II/III were treated with AEE (50 mg/kg body weight)/tolbutamide (20 mg/kg body weight)/ AEE (50 mg/kg body weight) prior to the administration of tolbutamide (20 mg/kg body weight), respectively.

Study in diabetic rats: Albino rats of either sex (175-200 g) were treated with alloxan monohydrate (100 mg/kg body weight i.p.). Alloxan monohydrate was dissolved in saline solution and was administered. Animals were treated with 10 % dextrose orally to combat the early phase of hypoglycemia. Rats showing fasting blood glucose levels above 150 mg/dL were selected for the study. These rats were divided in to 3 groups. Group I/II/III were treated with AEE (40 mg/kg body weight)/tolbutamide (20 mg/kg body weight)/ AEE (40 mg/kg body weight) prior to the administration of tolbutamide (20 mg/kg body weight), respectively. AEE dose was fixed based on its response, which produced above 50 %.

Collection of blood samples: Blood samples were collected from the retro orbital plexus of each rat at 0, 0.5, 1, 1.5, 2, 4 and 6 h after drug administration. Blood glucose levels were determined by using GOD-POD method[9].

Statistical analysis: Data were expressed as mean ± standard deviation (SD). The significance of blood glucose reduction produced by AEE with tolbutamide compared to tolbutamide control was determined by applying student's unpaired t-test.

RESULTS AND DISCUSSION

Effect in normal rats: In normal rats AEE at the dose of 50 mg/kg body weight administered orally produced 50.86 % blood glucose reduction at 0.5 h and 20 mg/kg body weight of tolbutamide produced 35.20 % at 4 h as peak effects. In the presence AEE 50 mg/kg body weight, the action of tolbutamide was early in onset and maintained for 6 h. The data was presented in Table-1.

TABLE-1
PER CENT BLOOD GLUCOSE REDUCTION WITH AEE / TOLBUTAMIDE / AEE + TOLBUTAMIDE IN NORMAL RATS (n = 6)

Treatment	Dose	Time (h)						
		0	0.5	1	1.5	2	4	6
AEE	50 mg/kg bd.wt	-	50.86 ± 1.07	21.25 ± 1.46	10.44 ± 1.19	2.10 ± 0.28	-2.34 ± 0.32	-
Tolbutamide	20 mg/kg bd.wt	-	12.52 ± 0.39	17.41 ± 0.47	20.49 ± 0.48	25.25 ± 0.52	35.20 ± 0.43	11.32 ± 0.72
AEE + Tolbutamide	50 mg/kg bd.wt + 20 mg/kg bd.wt	-	58.21 ± 0.55*	32.27 ± 0.28*	29.27 ± 0.32*	27.26 ± 0.36*	47.49 ± 0.85*	15.93 ± 0.78*

All values are expressed as mean ± SD; *Statistically significant $p < 0.001$.

Effect in diabetic rats: In diabetic rats, oral administration of AEE at the dose of 40 mg/kg body weight produced 41.87 % blood glucose reduction at 1.5 h and tolbutamide 20 mg/kg body weight produced 45.27 % at 4 h. In the presence of AEE 40 mg/kg body weight, the tolbutamide produced antidiabetic activity at 0.5 h and was maintained for 6 h. The data was presented in Table-2.

TABLE-2
PER CENT BLOOD GLUCOSE REDUCTION WITH AEE / TOLBUTAMIDE / AEE + TOLBUTAMIDE IN DIABETIC RATS (n = 6)

Treatment	Dose	Time (h)						
		0	0.5	1	1.5	2	4	6
AEE	40 mg/kg bd.wt	-	18.29 ± 0.56	35.13 ± 0.49	41.87 ± 0.76	34.35 ± 0.31	20.77 ± 0.39	-
Tolbutamide	20 mg/kg bd.wt	-	3.50 ± 0.38	7.40 ± 0.47	18.41 ± 0.41	38.04 ± 0.56	45.27 ± 0.51	23.51 ± 0.15
AEE + Tolbutamide	40 mg/kg bd.wt + 20 mg/kg bd.wt	-	25.43 ±0.63 *	38.44 ± 0.32 *	59.32 ± 0.37*	62.45 ± 0.27*	65.39 ± 0.34*	40.77 ± 0.36*

All values are expressed as mean ± SD; *Statistically significant $p < 0.001$.

Drug-interaction studies are usually conducted in animal models to assess the safety of the combination, before they are conducted in humans. The normal rat model served to quickly identify the interaction and the diabetic rat model served to validate the interaction in an actual-use condition of the drugs. The rat model was used for the pharmacodynamic-interaction study, since it is the most widely used species in drug metabolism and drug interaction studies.

Oxidative stress has been implicated in the pathogenesis of various diseases[10]. Both poorly controlled type-1 and type-2 diabetes mellitus are characterized by reduced capacity of peripheral tissues (*i.e.* skeletal muscle and adipose tissue) to respond to the metabolic effects of insulin. Several lines of evidences suggest that increased oxidative stress occurs in diabetes mellitus and could have a role in the development of deterioration of peripheral insulin resistance. Alloxan has been observed to cause a massive reduction of the β-cells of the islets of Langerhans and induce hyperglycemia[11]. Thus, it is logical to think that antioxidants can prevent precipitation of diabetes mellitus and also control hyperglycemia. Use of typical antioxidants alone/in combination may retard or even prevent the normal progression of diabetic complications. Emblica officinalis has a potent antioxidative capacity in wide variety of experimental systems[12,13]. *Emblica officinalis* fruits, being rich in both vitamin-C and polyphenols, may be a rich source of antioxidants and function as a potent integral component of antioxidant protection systems to relieve oxidative stress during diabetes[14,15]. As per literature Emblica officinalis has been shown to improve glucose metabolism in diabetic condition[7,8].

The present study was conducted in rats with AEE as an antioxidant. AEE was selected because it is used as an ingredient in antioxidant formulations that are used in therapy.

The AEE and tolbutamide showed hypoglycemia/antihyperglycemia in normal/diabetic rats in dose dependent manner. AEE when administered alone produced an early onset of action at 0.5 and 1.5 h in normal and diabetic rats respectively. Tolbutamide when administered in therapeutic dose produced the maximum effect at 4 h and was maintained up to 6 h in both normal and diabetic rats. In presence of AEE the onset of action of tolbutamide was early and maintained for longer duration compared to tolbutamide control. Tolbutamide acts by stimulating insulin secretion[16] and also by increasing tissue up take of glucose[17]. The early on set of action was noticed to be due to vitamin-C of AEE, which was maintained later due to tolbutamide activity since both are reported to have influence on insulin secretion[16].

Conclusion

The results indicate that additive action of AEE on pharmacodynamic response of tolbutamide may be useful to improve the tolbutamide activity in insulin resistant cases and to postpone the occurrence of diabetic complications. However further work on human patients is required to confirm the observation in diabetic condition and usefulness of AEE as supplemental antioxidative agent for improved control of blood glucose levels when administered orally along with sulfonylureas.

REFERENCES

1. A. Kar, B.K. Choudary and N.G. Bandhopadhyay, *J. Ethnopharmacol.*, **64**, 179 (1999).
2. S.K. Jain, R. Mcvie, J.J. Jaramillo, M. Palmer and T. Smith, *J. Am. Coll. Nutr.*, **15**, 458 (1996).
3. J. Nourooz-Zadeh, A. Rahimi, J. Tajaddini-Sarmadi, H. Tritschelr, P. Rosen, B. Halliwell and D.T. Betteridge, *Diabetologia*, **40**, 647 (1997).
4. S. Sardas, M. Yimazz, U. Oztak, N. Cakir and A.E. Karakaya, *Mutation Res.*, **490**, 123 (2001).
5. L.B. Owens, J. Wright and E. Brown, *New Eng. J. Med.*, **224**, 319 (1941).
6. S. Satyanarayana, K.E. Kumar and J.R. Sekhar, *Therapy*, **3**, 613 (2006).
7. M.C. Sabu and R. Kuttan, *J. Ethnopharmacol.*, **81**, 155 (2002).
8. P.S. Babu and M.P.P. Stanely, *J. Pharm. Pharmacol.*, **56**, 1435 (2004).
9. P. Trinder, *Ann. Clin. Biochem.*, **6**, 24 (1969).
10. A. Rudich, A. Tirosh, R. Potashnik, M. Khamaisi and N. Bashan, *Diabetologia*, **42**, 949 (1999).
11. M. Goldner and G. Gomori, *Endocrinology*, **33**, 297 (1943).
12. A. Bhattacharya and S. Ghosal, *Indian J. Exp. Biol.*, **38**, 877 (2000).
13. S.K. Bandyopadhyaya, S.C. Pakrashi and A. Pakrashi, *J. Ethnopharmacol.*, **70**, 171 (2000).
14. J.K. Jose and R. Kuttan, *J. Clin. Biochem. Nutr.*, **19**, 63 (1995).
15. T.P. Rao, N. Sakaguchi, L.R. Juneja, E. Wada and T. Yokozawa, *J. Med. Food*, **8**, 362 (2005).
16. R. Vigneri, V. Pezzino and K.Y. Wang, *J. Clin. Endocrinal Metab.*, **54**, 95 (1982).
17. M.A. Peifer, J.B. Halter, J.C. Beacd and D.J. Partel, *J. Clin. Endocrinal Metab.*, **53**, 1256 (1981).

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