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Antihyperglycemic Activity Evaluation of Antidiabetic Herbal Formulations on Alloxan Induced Rats

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Abstract: Three marketed oral antidiabetic herbal tablet formulations have been comparatively studied for their antidiabetic potentialities in alloxan induced diabetic rats (AIDRs). This sort of study is a good indicator for the evaluation of the idealness of an antidiabetic preparation. Marketed antidiabetic herbal formulations from different manufacturers were randomly chosen for this study. The blood glucose levels were investigated in AIDRs after 4 and 8 hours of single dose (5ml/kg body weight). The treatment of these test products, significantly (p<0.01) reduced blood glucose by 31.59%, 50.58% and 16.0% respectively; after 8 hours of oral administration of sample solutions in distilled water which were fitted with antihyperglycemic effects of standard metformin-HCl. All the products were found effective in lowering blood glucose level. It may be inferred that the antidiabetic herbal tablets of Bangladeshi manufacturers complies with the standard specifications for antihyperglycemic potentialities.

Key words: Antihyperglycemic · Herbal Formulation · Alloxan · Diabetes

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycaemia, hypertriglyceridaemia and hypercholesterolaemia, resulting from defects in insulin secretion or action or both [1]. Two groups of oral hypoglycaemic drugs, sulphonylureas and biguanides, have been used in the treatment of DM. They act by lowering blood glucose levels thereby delaying or preventing the onset of diabetic complications [2]. In traditional practice medicinal plants are used in many countries to control diabetes mellitus. The hypoglycemic action of these medicinal plants has been studied [3]. In recent times plant drugs are frequently considered to be less toxic since they are believed to produce lesser side effects than synthetic ones [4]. Three commonly used polyherbal formulations were procured from local market of Dhaka, Bangladesh to evaluate and compare their antidiabetic activity. The effects produced by these formulations on blood glucose were evaluated and compared with standard oral hypoglycemic agent, Metformin.

MATERIALS AND METHODS

Selection and Procurement of Antidiabetic Formulations: Three formulations were procured from the local market. The formulations selected on the basis of matching the contents maximally and are consumed by most of the diabetic patients here in Bangladesh. The formulation and chemical profiling data of sample 1 & 2 ware as per BNFUM, while the sample 3 was as per BP.

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Sample No.	Botanical Source	Manufacturer
Sample 1*	*Stevia rebaudiana*	*Green Laboratories, BD.*
		Batch no: 970
		MFG date: Jun 2010
Sample 2*	*Bambusa arundhacea*	*Sadek Pharmaceuticals limited, BD.*
	Rumex vesicarius	*Batch no: 9060*
	Gymnema sylvester	*MFG date: Sep 2009*
Sample 3**	*Panax ginseng*	*Square Herbal and Neutraceuticls Limited, BD.*
		Batch no: 011036
		MFG date: Nov 2010

*Bangladesh National Formulary of Unani Medicine, 1993. ** British Pharmacopoeia, 2011.

Experimental Animals: A total number of 20 Wistar albino rats of either sex (Weight 105-160 g, age 2 months) were purchased from animal house of International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). Prior to the commencement of the experiment, all the rats were acclimatized to the new environmental condition for a period of one week. During the experimental period, the rats were kept in a well-ventilated animal house at room temperature of 25°C and were supplied with standard pellets collected from ICDDR, B and fresh drinking water *ad libitum*. All the rats were kept in cages with wide square mesh at the bottom to avoid coprophagy and maintained with natural 12 h light and dark cycle. Rats were divided randomly into 5 groups.

Collection of Diabetic Inducer: Alloxan monohydrate was used as the Diabetes inducer in animals and was procured from the Loba Chemicals, Mumbai, INDIA. The blood samples were analyzed by Bioland-423, glucometer from Germany.

Experimental Induction of Diabetes: Animals were allowed to fast for 12 hrs and were administered freshly prepared alloxan (110 mg/kg body weight i.p.) in freshly prepared saline water. The alloxan treated animals were allowed to food over night to overcome drug-induced hypoglycemia. After 48-72 hrs to allow for the development and aggravation of diabetes, rats with moderate diabetes having persistent glycosuria and hyperglycemia [5] were considered diabetic for further experimentation.

Experimental Design: In the experiment, a total of 30 rats (25 diabetic surviving rats, 5 normal rats) were used. Group I (Normal group) receives only vehicle. Group II was selected for diabetic control, which does not receive either metformin, or plant formulations. Group III stands for metformin control group in which metformin was administered intraperitoneally at a dose of 150 mg/ kg body weight. Group IV, Group V and Group VI received sample formulations 1, 2 and 3 respectively. The dosage was prepared in solution form in such a concentration that, each 0.5 ml of solution contains those drugs corresponding to the volume of 5 ml/kg body weight. The blood samples collected from tail vain were analyzed for blood glucose content at 0, 4 and 8 hours respectively by using glucometer.

Acute Toxicity Test: Groups of albino rat comprising 5 animals each, fasted for 12 hrs prior to the experiment and were given the formulations 1, 2 and 3 respectively up to 2 g/kg p.o. The control group received distilled water 2 ml/kg p.o. Mice were closely observed for 2 hrs post-treatment for behavioral changes and signs of toxicity. Mortality in each group within 24 hrs was recorded and surviving animals were observed for a further 14 days for any signs of delayed toxicity.

Statistical Analysis: Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by one-way ANOVA followed by Dunnett's Multiple Comparison Test (DMCT) and the values were considered statistically significant when $p<0.01$. Statistical calculations and the graphs were prepared using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

RESULTS

Acute Toxicity Studies: In acute toxicity study no toxic symptoms were observed for all formulations up to dose 2g/kg body weight. All animals behaved normally. No neurological or behavioral effects could be noted. No mortality was found up to 14 days study.

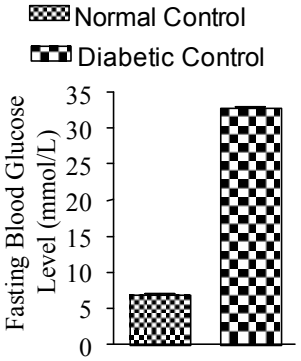

Fig. 1: Blood glucose levels of normal and diabetic rats. Values are mean ± SEM, (n = 5), p<0.01, one-way ANOVA followed by Dunnett's Multiple Comparison Test

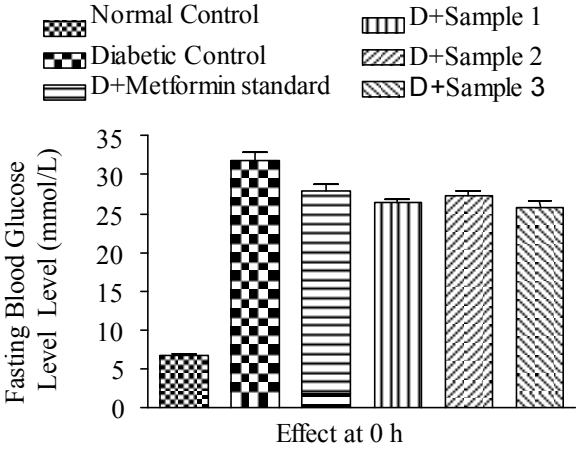

Fig. 2: Effects of the three products at 0 hour after oral administration in alloxan induced diabetic rats. Values are mean ± SEM, (n = 5), p<0.01, one-way ANOVA followed by Dunnett's Multiple Comparison Test.

Effect of Alloxan on Blood Glucose: After administration of Alloxan (110 mg/kg) the effects on the experimental animals (Blood glucose levels of normal and diabetic rats) were measured and injection of Alloxan resulted in significant elevation of blood glucose level (Figure 1) during the period of experiment.

Effects of Herbal Formulations on Serum Glucose Level in Alloxan-Induced Diabetic Rats at Zero Hour: The effects of sample formulations during the period of experiment indicated as 1, 2 & 3 at zero hour (at the time of administration) on alloxan induced diabetic rats ware evaluated and among three samples, sample 2 found highest fasting blood glucose level (mmol/L) which has been illustrated in Figure 2.

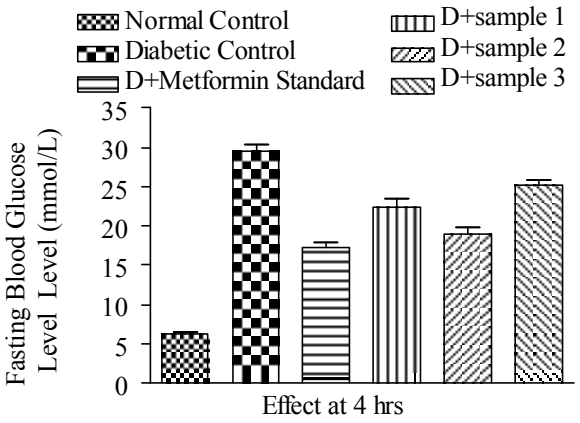

Fig. 3: Effects of the three products after 4 hours on oral administration in alloxan induced diabetic rats. Values are mean ± SEM, (n = 5), p<0.01, one-way ANOVA followed by Dunnett's Multiple Comparison Test

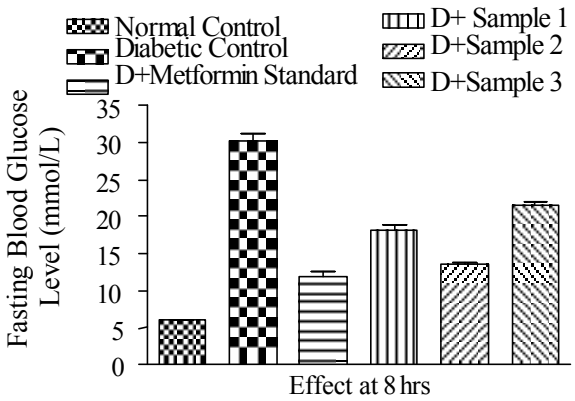

Fig. 4: Effects of the three products after 8 hours after oral administration in alloxan induced diabetic rats. Values are mean ± SEM, (n = 5), p<0.01, one-way ANOVA followed by Dunnett's Multiple Comparison Test

Effects of Herbal Formulations on Serum Glucose Level in Alloxan-Induced Diabetic Rats at Four Hours: The effects of sample formulations indicated as 1, 2 & 3 at four hours (at the time of administration) in alloxan induced diabetic rats where sample 3 showed highest and sample 2 showed lowest blood glucose level (Figure 3).

Effects of Herbal Formulations on Serum Glucose Level in Alloxan-Induced Diabetic Rats at Eight Hours: The effects of sample formulations indicated as 1, 2 & 3 at eight hours (at the time of administration) in alloxan induced diabetic rats presented in Figure 4.

DISCUSSION

The present study was conducted to evaluate the antidiabetic activity of some marketed herbal formulations available in Bangladesh. The experimental groups were treated with standard (Metformin) and test formulations. The experiment was conducted in accordance with parallel design i.e. each group received single formulation, single time. After completion of the study protocol, it was found that with test and standard treatment, the serum level of glucose improved significantly ($p<0.01$) as compared to diabetic control. The samples 1, 2 and 3 all are effective in controlling the blood glucose level of the diabetic rats. Among them sample 2, i.e. Polyherbal formulation is most effective whereas sample 1, i.e. stevia plant formulation is more effective than sample 3, i.e. ginseng plant formulation.

Sample 1 is a powder formulation obtained from *Stevia rebaudiana.* Stevia is a perennial shrub. It belongs to the Compositae family, which is indigenous to the northern regions of South America [6]. Stevia is still found growing wild in the highlands of border area between Brazil and Paraguay. It is grown commercially in many parts of Brazil, Paraguay, Uruguay, Central America, Israel, Thailand and China. Now it is cultivated in Bangladesh and used as folk medicine for diabetes control. The leaves have been known to contain 100 useful alkaloids among other pharmacologically active compounds. It has been used for the treatment of diabetes and its anti-diabetic effect has been evaluated in diabetic animals in many countries and significant hypoglycemic activities of powdered form of Stevia (*Stevia rebaudiana* Bertoni) leaves have been reported. However, its antidiabetic effects have not yet been investigated in Bangladesh [7]. Stevioside, an important glycoside of stevia green is responsible for its antidiabetic activity. *In vitro* hypoglycemic actions of stevioside and steviol are a result of their ability to stimulate insulin secretion via a direct action on beta cells. Results indicate that the compounds may have a potential role as antihyperglycemic agents in the treatment of type 2 diabetes mellitus. Other compounds of stevia responsible for antidiabetic activity in this are steviolbioside, rebausiosides A-E and dulcoside A.

Sample 2 is a polyherbal formulation obtained from three sources *Bambusa arundhacea, Rumex vesicarius* and *Gymnema Sylvester.* Gymnemic acid isolated from *G. sylvestre* and triterpene glycosides isolated from plant inhibited glucose utilization in muscles [8]. The drug influenced the disturbed carbohydrate metabolism in hyperglycaemic animals by limiting the carbohydrate turnover and thus inhibiting the vicious cycle of hyperglycaemia.

Sample 3 is capsule formulation obtained from *Panax ginseng.* The active components of ginseng are considered to be ginsenosides, a group of steroidal saponins. The identification of a significant antihyperglycemic activity in ginsenoside has provided an opportunity to develop a novel class of antidiabetic agent.

Alloxan, a beta cytotoxin, induces diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic beta cells resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues [9]. Insulin deficiency leads to various metabolic aberrations in the animals including increase in blood glucose [10]. This herbal therapy appeared to bring about blood glucose homeostasis through increased serum insulin levels provided by repair/regeneration of the endocrine pancreas [11].

The significant antidiabetic activity of formulations may be due to inhibition of free radical generation and subsequent tissue damage induced by alloxan or potentiation of plasma insulin effect by increase of either pancreatic secretion of insulin from existing beta cells or its release from bound form as indicated by significant improvement in glucose and protein level because insulin inhibits gluconeogenesis from protein. In comparative evaluation all brands found to be safe as they did not show any sign of acute toxicity. The formulation Sample 2 was found to be more efficacious as compared to others.

CONCLUSION

Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and efficacy but they lack an experimental base and therefore have second class status whereas modern medicines have a very strong experimental basis for their use but have side effects. Thus, it seems, to get a new class of drugs, the researchers are increasingly blending the traditional knowledge with modern experimental methodology for testing the efficacy and safety of herbal drugs. This inclination seems to be a result of people all over the world looking to various alternative systems of medicine, especially herbal drugs which are claimed to be safe, equally effective in comparison to allopathic drugs and which provide

some answer to chronic diseases. Developing countries like Bangladesh with traditional knowledge base have leadership potential to develop globally acceptable newer opportunities and applications for herbal industry. Our study has provided strong evidence that the antidiabetic herbal formulations available in Bangladesh confirm all the standard specifications and can be used by the patients of type 2 diabetes in Bangladesh.

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