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PHARMACOLOGICAL SCREENING OF ANTIDIABETIC HERBAL PREPARATIONS IN STREPTOZOTOCIN INDUCED TYPE-2 DIABETIC MODEL RATS

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Abstract

Antidiabetic potency of locally produced antidiabetic herbal preparations (ADHPs) are unknown. In this study, a pharmacological screening of antihyperglycemic potency of locally available six ADHPs were evaluated on streptozotocin (STZ) induced type 2 diabetic rat models. ADHPs were administered to the diabetic rat and blood glucose levels were monitored before (0 day) and after administration of ADHPs (at 14th day and 28th day) by measuring serum glucose concentration using oxidase method. Serum glucose levels in different groups of ADHP treated rats were compared against baseline glucose levels and water control and reference drug by t test. Serum glucose levels were found to be similar at baseline in all groups against water control and reference drug. The administration of herbal preparations except ADHP-3 and ADHP-5 resulted in a non-significant reduction of blood glucose level of the diabetic rats when compared with diabetic rats that received no treatment at 14th day or compared to the diabetic rats received standard or baseline. ADHP-3 was found to be most effective hypoglycemic herbal preparation at 14th day. At 28th day, all ADHPs were found to have hypoglycemic potential compared to water control and only ADHP-3 showed similar hypoglycemic effect compared to reference drug and baseline glucose levels at 28th day. This study revealed that a minor fraction of the locally available antihyperglycemic herbal preparations are truly effective and among the six ADHPs studied, only ADHP-3 possesses hypoglycemic effect.

Keywords: *Anti diabetic herbal preparation, diabetic model rat, antihyperglycemic potency, Serum glucose*

Introduction

In vertebrates, the pancreas is a glandularorgan in their digestive and endocrine system. It is located in the abdominal cavity behind the stomach in human body and produces several important hormones, including insulin, glucagon, somatostatin, and pancreatic polypeptide. These hormones circulate through the blood stream. Among these hormones, insulin, produced by beta cell in Pancreas compensate resistance by releasing more amount of insulin to maintain glucose homeostasis, thereby reduces blood glucose concentration. Mechanism of beta cell incorporates quick response to spikes in blood glucose concentrations by means of secreting some of their stored insulin and the shortage insulin thereby is made up simultaneously. Dysfunction to this pancreatic beta cell led to its inability to compensate for insulin resistance and is characterized by a progressive decline in insulin action, lead to type-2 diabetes mellitus. Type-2 diabetes develops when over time span, function of beta cell gets impaired which leads to deterioration in glucose homeostasis. This condition ultimately turned into impaired glucose tolerance and frank diabetes.

As most of the individuals diagnosed with this disorder is found to be obese **(1,2)** it is inked as key cause of diabetes of this kind. However, genetic predisposition and ageing also culpritized in developing this disorder **(3)** Glutathioneperoxidase, catalase, and superoxide dismutase (SOD) metabolize oxidative toxic intermediates are antioxidant enzymes. These enzymes scavenge reactive oxygen species, often found associated with patients with type- 2 diabetes, inked as oxidative stress. Diabetes also induces changes in the tissue content and activity of the antioxidant enzymes **(4-7).** The number of people suffering from complications related to diabetes will surge to 552 million by 2030 with ninety percent cases will be attributed to the type-2 one **(8),** amassing 7% of the population of the world by then. In another estimation, the recent one, concludes by 2035, 10% population of the world will be suffering from diabetes which amounts 592 million people (**9**)**.** It will not be extravagant to lebel the prevalence of diabetes is at pandemic level. The report of people suffering from diabetes increasing worldwide with faster growth in developing countries **(10,11)**.

Medications in lowering blood sugar level acts as insulin sensitizers, insulin secretagogues, DPP4 inhibitors and alpha glucosidase inhibitors. Along with modern therapeutics, anti- diabetic herbal preparations (ADHPs) are being used in fighting with this chimera. Severe hypoglycemia, idiosyncratic liver cell deficit, digestive discomfort, lactic acidocis, permanent neurological deficit has been reported with people being treated with modern therapeutics. Adverse effect of modern drugs escalating death in some cases **(12).** Moreover, modern drugs due to their high price and unavailability don't make them to the end user patients **(13).** Moreover, having been of natural origin, the herbal medicines are considered harmless. Cumulating these all, including their ease of access, therapeutic efficacy, relative low cost in comparison with other medications and low side effects **(14,15)** gained attention towards using anti diabetic herbal preparations as ailment of diabetes type-2. With this surge, in the utilization of medicinal herbs the vast majority of the medicinal herbal products made their way to the end user without licensing. In most of the developed countries, herbal preparations no compliance is maintained regarding their safety and efficacy of herbal preparations. **(16)** And, thus, a lack in demnstratrating potency and safety is a big question after overlooked by the government healthcare authorities **(17).**

Now there is a raising question whether pharmacological procedures are in place after it has found like several countries the herbal preparations are approved dosage system without guidelines and regulations in Bangladesh. So without authenticated and categorized scientific procedure ensuring reproductively of these preparations is a challenge. Story is in the making, in the form of gossip, without guidelines and scientific proof end users are being left in where-definitely in blur and they are puzzled whether the medications are on action in treating their ailment. To give an answer to this public plea, a scientific procedure has been undertaken to evaluate the efficacy of some anti- diabetic herbal preparations available in Bangladesh.

Methods

This experimental study has been conducted at Department of Pharmacy, Daffodil International University during the period of 2015-16. In this study, Streptozotocin was purchased from the supplier Supertech co. Ltd (sigma chemical company, Sigma No. S0130, German) and antidiabetic herbal medicines/preparations in different dosage form (Table 1) were collected from herbal medicine shops in Dhaka city. Standard drug, Glimepiride, was obtained from the Beximco pharmaceuticals, Bangladesh. Plant composition of each antidiabetic herbal preparations with their demonstrated adverse effects have been shown in Table 2.

Animal collection and conditioning

Male Long Evans rats weighting 200-300gm were collected from the Bangladesh Institute of Research, & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM). After collection they were kept in animal research lab, Dept. of Pharmacy, Daffodil Int. University where they let to adopt standard environmental conditions for a period of 7 days (temperature 22±5°c relative humidity 45-65% and 12h dark/light cycle). The rats were fed the standard laboratory pellet diet with water maintaining hygienic protocol which involved 15gm of food as pellet per day for each rat for twice a day, one half given in the morning, another half in afternoon. bavit GS (Vitamin mixture) 250gm was added per 100kg of rat food. All rats were feed HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) ad libitum, respectively, for the initial period of two weeks.

Sample preparation

BUFFER: To prepare 0.1 M 50 ml buffer solution of pH 4.4, disodium phosphate 0.1 M 30.5 ml and monosodium phosphate.1M 19.5ml are added together in a beaker. Then pH meter were used for measure pH. If *pH* variations were observed from the target *pH*, phosphoric acid or sodium hydroxide was added to adjust optimum *pH*.

STREPTOZOTOCIN (STZ): Streptozotocin powder was taken in a 25ml beaker then dissolved it with phosphate buffer (0.01 M, pH 4.4) for the concentration of dose 60mg/kg body weight.

SUSPENSION: Syrup and suspension was made to fed the type 2 diabetes model rats from tablet and other dosage form. First crush the tablet by mortar and pestle to makes it powder.1.25gm of herbal powder medicine was taken in a conical flask then dissolve it with distil water and added distil water up to 10ml. Thus the concentration of dose was 1.25gm/10ml.

STANDARD: Glimepiride was prepared at a dose of 5mg per 10 ml of solvent (9.9 ml distill water + 0.1ml tween 20)/ kg body weight of T2DM rat.

Development of Type 2 diabetes model rats

After the 2 weeks of dietary manipulation, the rats were injected intraperitoneally (i.p.) with dose of STZ (60 mg kg−1; biochemical estimations (plasma glucose (PGL), were carried out just before and 7 days after STZ injection, i.e. The rats with the non-fasting PGL of ≥300 mg dl−1 were considered diabetic and selected for further pharmacological studies. The feed and water intake of the animals were also

measured. The rats were allowed to continue to feed on their respective diets until the end of the study.

Investigation of pharmaceutical screening

Grouping

Diabetic model rats were divided into three groups and treated once a day for 28 days as follows: received fresh water 10 ml/kg body weight for groups I, received Glimepiride 5mg/kg body weight for groups II, Received herbal preparation1.25g/kg body weight for group III. Grouping was done according to administration.

Group	Sample number	Type of group
Group-I	n = 6	Water control
Group-II	n = 6	Positive control (Ref. drug control)
Group-III	n = 6*6 =36	Herbal preparation (six for each preparation)

Results

Baseline glucose levels

Baseline serum glucose (SG) levels of type 2 diabetic model rats of different experimental groups were measured on 0 day (baseline) and presented in Fig 1. The mean values of baseline (on 0th day) glucose level were 8.6 ± 0.15 mmol/L for water control and 8.5 ± 0.36 mmol/L for reference drug control. The mean values of baseline glucose level were found to be present at 8.4 ± 0.25 mmol/L, 8.5 ± 0.30 mmol/L, 8.6 ± 0.35 mmol/L, 8.8 ± 0.25 mmol/L, 8.5 ± 0.20 mmol/L and 8.7 ± 0.35 mmol/L for ADHP-1, ADHP-2, ADHP-3, ADHP-4, ADHP-5 and ADHP-6 groups respectively.

Antihyperglycemic effect

Table 3 shows serum glucose concentrations in different groups at baseline, 14th day and 28th day. The mean value of glucose levels were 9.5 mmol/L (±0.35) for water control and

7.0 mmol/L (±0.20) for reference drug control on 14th day after administration of antibiabetic herbal preparation. The mean value of 14th day's glucose levels were observed 9.3 mmol/L (±0.65), 9.3 mmol/L (±0.40), 7.1 mmol/L (±0.25), 8.7 mmol/L (±1.0), 8.3 mmol/L (±0.36), 8.9 mmol/L (±0.46) for ADHP-1, ADHP-2, ADHP-3, ADHP-4, ADHP-5 and ADHP-6 respectively.

The mean value of 28th day's glucose levels were measured to be 13.8 mmol/L (±1.50) for water control and 6.9 mmol/L (±0.20) for reference drug control. The mean value of glucose levels were found to be 8.5 mmol/L (±0.45), 11.0 mmol/L (±0.80), 7.3 mmol/L (±0.15), 8.1 mmol/L (±0.65), 9.6 mmol/L (±0.76), and 9.9 mmol/L (±0.45) for ADHP-1, ADHP-2, ADHP-3, ADHP-4, ADHP-5 and ADHP-6 respectively on 28th day after antidiabetic herbal medicine administration.

Figure 2 represents the effect of different herbal preparations on glycemic status. ADHP-3 significantly reduced blood glucose levels compared to baseline at 14th day ($p=0.004$) and at 28th day ($p=0.004$). Compared to water control, blood glucose concentrations were significantly reduced at 14th day ($p<0.001$) and at 28th day ($p=0.002$).

Discussion

Baseline glucose levels

From Figure I the mean values of baseline (on 0th day) glucose level were 8.6 ± 0.15 mmol/L for water control and 8.5 ± 0.36 mmol/L for reference drug control. Here no significant difference was observed in baseline serum glucose levels compared to water control and reference group ($p>0.05$).

Anti hyperglycemic effect

Compared to reference drug, blood glucose levels were similar to that observed for reference drugs at 14th day ($p=0.617$) and 28th day ($p=0.050$). AHDP-3 was found

to be most potent antihyperglycemic preparation among the ADHPs studied.

Figure 2 represents the effect of different herbal preparations on glycemic status. ADHP-3 significantly reduced blood glucose levels compared to baseline at 14th day ($p=0.004$) and at 28th day ($p=0.004$). Compared to water control, blood glucose concentrations were significantly reduced at 14th day ($p<0.001$) and at 28th day ($p=0.002$).

A considerable number of antihyperglycemic herbal preparations available have claimed to be effective against hyperglycemia. In this study, albeit of ADHP-3's promising effect in reducing blood sugar level, the others have found to be ineffective in reduction of blood glucose level of the diabetic rats on 14th day. On 28th day, all ADHPs were found to have hypoglycemic potential compared to water control, indicate that it may be slow acting or play indirect role to maintain normal glucose homeostasis. Only, ADHP-3 showed similar hypoglycemic effect compared to reference drug on 28th day. Thus, among the six ADHPs studied, only one was found to have hypoglycemic effect. This is consistent with the previous study conducted in this region (Ranzu et al.) et al (Ref) studied 6 ADHPs for its antihyperglycemic potency and found only two ADHPs are effective.

Frequent use of herbal preparations is reported in Bangladesh (Mosihuzamman et al., 2010). When the antidiabetic herbal preparations that claimed to be effective in management of type-2 diabetes is being passed through pharmacological screening, efficacy of only one was found with strong anti- diabetic properties, keeping others with question. Therefore, to ensure the quality of herbal drugs in the management of type-2 diabetes proper clinical intervention is a must to set scientific evidence for effective use of the identified medicine.

With claimed efficacy herbal drug manufacturers frequently distributing herbal drugs. However, the same drugs when have been subjected to pharmacological screening, only one was found potent. Therefore, the whole covering sale of anti diabetic preparations of herbal origin is in question whether it is pharmacologically potent or not. For effective use of herbal drugs, herbal drugs must be provided with scientific evidence. To do so, proper pharmacological screening with scientifically aproved techniques through an internationally recognized pharmacopoeia to ensure the quality of herbal drugs in the management of type-2 diabetes is required. Further study should address to the herbal drugs available in the market.

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Table 1. Different Dosage form of ADHP

Serial no	Product ID	Dosage form	Indication
01	ADHP-1	Tablet	4 tablet two times Daily
02	ADHP -2	Capsule	1-2 capsule at morning.
03	ADHP -3	Tablet	1-2 tablets two times daily.
04	ADHP -4	Capsule	2 Capsule 2 times daily.
05	ADHP -5	Syrup	3-4 spoonful 3 times daily after meal.
06	ADHP -6	Capsule	2 capsule 2 times

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Table 2. Product Composition

Serial no	Product ID	Herbal Drug Composition	Adverse effect [17]- [24]
1	ADHP-1	Gymnema Sylvester	Hypoglycemia
		Mytilus margaritiferus	None
			Eye, nasal, skin and throat irritation. Head, neck, arm hand and legs burning. Lung cancer
2	ADHP-2	Bambusa arundinacea	NF
		Rumex vesicarius	Impotence, fatigue and Weakness
		Gymnema Sylvester	Hypoglycemia
		Hens egg shell	NF
		Ferrous sulphate	NF
		Mytilus margaritiferus	None
			Eye, nasal, skin and throat irritation. Head, neck, arm hand and legs burning. Lung cancer
3	ADHP-6	Bambusa bambos	Myosistis, abnormal liver
		Gymnema Sylvester	Hypoglycemia
		Acacia arabica	
		Rumex vesicarius	Impotence, fatigue and weakness
4	ADHP-3	Neem leaf	NF
5	ADHP-4	Roshiund	NF
		Lou	NF
		Bon	NF
		Ohif	NF
		Jonghodumur seed	NF
		Bilbo	NF
		Kabab	NF

		Syzygium cumini skeels	On CNS
		Syzygium cumini seed	On CNS
		CentellaAsistica Urban	Safe
		Coccinia indica	None
		Mesua	None
			Liver and kidney abnormality due to fatty changes and hyaline degenarative changes respectively

Table 3. Effect of different antidiabetic herbal preparations on glycemic status

		Baseline		P value
Sample	0 day	14 th days	28 th days	
				0 vs 14: 0.015
Water Control	8.5±0.15	9.5±0.35	13.8±1.50	0 vs 28: 0.004
				14 vs 28: 0. 008
Reference drug	8.5±0.36	7.0±0.20	6.9±0.20	0 vs 14: 0.003
		(<0.001)a	(0.001)a	0 vs 28: 0.003
				14 vs 28: 0.573
ADHP-1	8.4±0.25	9.3±0.65	8.5±0.45	0 vs 14: 0.089
		(0.663)a	(0.004)a	0 vs 28: 0.753
ADHP-2	8.5±0.30	(0.004)b 9.3±0.40	(0.005)b 11.0±0.80	14 vs 28: 0.155 0 vs 14: 0.050
		(0.550)a	(0.046)a	0 vs 28: 0.007
ADHP-3	8.6±0.35	(<0.001)b 7.1±0.25	(0.001)b 7.3±0.15	14 vs 28: 0.030 0 vs 14: 0.004
		(<0.001)a	(0.002)a	0 vs 28: 0.004
ADHP-4	8.8±0.25	(0.617)b 8.7±1.0	(0.050)b 8.1±0.65	14 vs 28: 0.301 0 vs 14: 0.875

		(0.261)a		
			(0.004)a	0 vs 28: 0.157
ADHP-5	8.5±0.20	(0.045)b 8.3±0.36	(0.038)b 9.6±0.76	14 vs 28: 0.433 0 vs 14: 0.445
		(0.014)a	(0.012)a	0 vs 28: 0.072
ADHP-6	8.7±0.35	(0.005)b 8.9±0.46	(0.004)b 9.9±0.45	14 vs 28: 0.055 0 vs 14: 0.581
		(0.147)a	(0.0123)a	0 vs 28: 0.022
		(0.003)b	(<0.001)b	14 vs 28: 0.055

a, water vs target; b, standard vs target

Figure 1. Baseline serum glucose concentrations in different groups

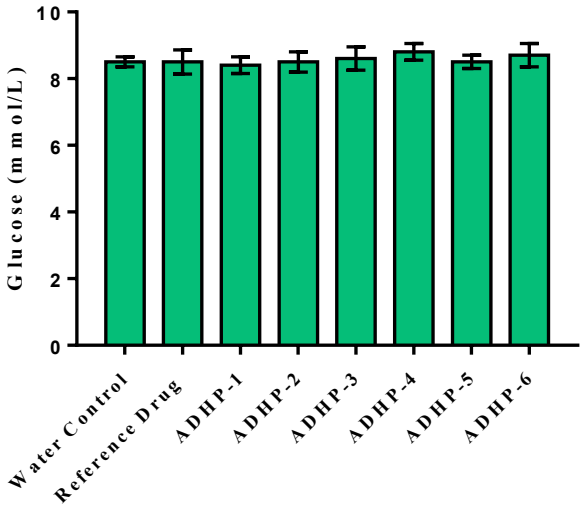

Figure 2. Comparison of antihyperglycemic effect of different ADHPs at 14th and 28th days