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Comparative Studies on Antidiabetic effect with phytochemical screening of *Azadirachta indicia* and *Andrographis paniculata*

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Abstract: *The present study was undertaken to evaluate the antidiabetic properties with phytochemical screening of the ethanol leaves extracts of two common indigenous medicinal plants of Bangladesh -Azadirachta indica (Neem) and Andrographis peniculata (Kalomegh) on experimental animal model. Hypoglycemic activity of the concentrated ethanolic (90%) extract of Azadirachta indica and Andrographis peniculata were studied compared with that of a reference antidiabetic drug glimeperide in both glucose loaded and alloxan induced diabetic rats. The acute toxicity of these two plants extract was also studied. Experimental results showed that ethanol leaves extract (1 gm/kg) of Azadirachta indica and Andrographis peniculata significantly (P<0.01) reduced the elevated blood glucose level by 36.91 % and 40.65 % respectively in glucose loaded rats and 30.20 % and 32.18 % respectively in alloxan induced diabetic rats compared to the respective diabetic control group. This study strongly suggested that ethanol leaves extracts of Azadirachta indica and Andrographis peniculata possess significant antidiabetic activity and could be a potential source for treatment of diabetes mellitus.*

Key words: *Azadirachta indica, Andrographis peniculata, Diabetes mellitus, Alloxan induced diabetes.*

I. Introduction

Diabetes mellitus is a syndrome characterized by an increase in blood glucose level and disturbances in protein, carbohydrate and fat metabolism associated with absolute or relative deficiency in insulin secretion or insulin action. Chronic diabetes leads to secondary complications affecting the eyes, kidneys, nerves and arteries [1]. Diabetes is a major threat to global public health that is rapidly escalating [2].The increasing prevalence of diabetes is now reaching epidemic proportion worldwide. It is estimated that more than 170 million people are suffering from diabetes globally and this number is expected to double by 2030. The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable [3].

Diabetes and its multiple complications are extremely burdensome on the health and economics of countries worldwide. If not successfully managed, diabetes along with other chronic diseases will become the most expensive problem faced by the health care systems. Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed [4], because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries.

Herbal remedies from medicinal plants have been used traditionally in many parts of the world where access to formal healthcare is limited. Recent experiences have shown that the plant drugs are relatively non-toxic, safe and free from serious side effects [5]. Therefore, plants used in traditional medicine to treat diabetes mellitus may be a valuable alternative for the control of this disease.

Currently available oral agents for the treatment of type 2 diabetes mellitus include a variety of compounds from 5 different pharmacologic classes with differing mechanisms of action, adverse effect profiles, and toxicities. The oral antidiabetic drugs can be classified as either hypoglycemic agents (sulfonylureas and benzoic acid derivatives) or antihyperglycemic agents (biguanides, α-glucosidase inhibitors, and thiazolidinediones). In this review, a brief discussion of the pharmacology of these agents is followed by an examination of the adverse effects, drug-drug interactions, and toxicities. Finally, treatment of sulfonylurea-induced hypoglycemia is described, including general supportive care and the management of pediatric sulfonylurea ingestions. The adjunctive roles of glucagon, diazoxide, and octreotide for refractory hypoglycemia are also discussed [6].

Azadirachta indicia (Neem) and *Andrographis paniculata* (Kalomegh) are two widely reputed medicinal plants in Bangladesh, which have been used traditionally for a long time to treat various diseases including diabetes [7]. In this study, we have evaluated the hypoglycemic activity of ethanol leaves extract of *Azadirachta indica* and *Andrographis paniculata* to establish scientific evidence in support of the folklore claim.

II. Materials and methods

2.1 Animals and Diets

Albino Wistar rats weighing about 200 ± 20 g of the either sex obtained from animal house of BCSIR laboratories, Chittagong were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet and water ad libitum.

2.2 Collection of Plant materials

The leaves of *Azadirachta indicia* and *Andrographis paniculata* were collected from the plantation area of BCSIR laboratories, Chittagong. The plants were taxonomically identified by the Botanical Research Division of the BCSIR laboratories, Chittagong.

2.3 Preparation of ethanol leaves extract

The collected leaves of two plants were cut into small pieces, air dried at room temperature for about 10 days and ground into powder form and stored in an airtight container. 500 gm of powder of leaves of each plant was then macerated in 90% ethanol at room temperature with occasional stirring. The ethanol extract of each plant was collected in a separate container and concentrated under reduced pressure below 50 ºC through rotatory vaccum evaporator. The individual extracts were filtered through fresh cotton bed and finally with Whatman no.1 filter paper. The filtrates were concentrated with a rotary evaporator at low temperature (40o-50oC) and reduced pressure to provide *Azadirachta indicia, Andrographis paniculata* extracts.

2.4 Assay for anti-diabetic properties

2.4.1 Experimental protocol

For Alloxan induced experiments: 50 Wistar albino rats of either sex were randomly divided into five experimental groups (Marked as Group I to V), where ten rats were taken in each group. Diabetes was induced in three groups (Group II to V) of rats by intraperitoneal injection of alloxan tetrahydrate (40mg/kg body weight). After 18 hrs of fasting alloxan induced diabetic rats were treated orally by following manner-

Group I (Normal Control) : Normal rats received only distilled water.
Group II (Diabetic Control) : Alloxan induced diabetic rats received only distilled water.
Group III (Positive Control) : Diabetic rats received reference antidiabetic drug Glimepiride (4 mg/kg).
Group IV (Sample Treated) : Diabetic rats treated with ethanol leaves extract of *Azadirachta indicia* at the rate of 1 gm/kg body weight.
Group V (Sample Treated) : Diabetic rats treated with ethanol leaves extract of *Andrographis paniculata* at the rate of 1 gm/kg body weight.

Two hours after drug treatment all the animals were anesthetized with diethyl ether and blood was collected from cardiac vessel using disposable syringe.

2.4.2 For glucose loaded experiments

Rats were grouped in an identical manner to alloxan induced classification. Group I rats received only distilled water and termed as normal control. Rats of Group II, III, IV and V were administered orally with 1.5 g/kg body weight glucose solution. Group II rats were considered as diabetic control (only glucose), Group III rats received 4-mg/kg body weight of reference antidiabetic drug Glimepiride and served as positive control. Sample treated groups- Group IV and V were treated with the ethanol leaves extract of *Azadirachta indicia* and *Andrographis paniculata* respectively at the rate of 1 gm/kg body weight.

Time schedule: All the animals were primarily fasted for 18 hrs and then glucose solution were given through feeding needle. After two hours, distilled water, reference drug solution, *Azadirachta indicia* and *Andrographis paniculata* leaves ethanol extract were given orally according to classified. Two hours later all the animals were anesthetized with diethyl ether and blood was collected from cardiac vessel using disposable syringe.

2.4.3 Serum Preparation

The blood collected from cardiac vessel was kept undisturbed in room temperature for 20 minutes. Serum from blood after clotting separated out and collected in a clean tube by centrifugation at 2000 rpm for 15 minutes.

2.4.4 Estimation of blood glucose level (BGL)

The level of glucose in blood samples from each of the experimental and control rat was determined spectrophtometrically by using glucose kit essentially followed by glucose oxidase- per-oxidase (GOD-POD) method.

2.4.5 Acute toxicity studies

For acute toxicity studies groups of 10 Wistar albino rats weighing 140-150gm were administered orally as well as intraperitoneally with two test compounds at high dose level (4g/kg body weight). The rats were closely observed for 24 hours for any mortality and next 10 days for any delayed toxic effects. Their food consumption and growth rate were also examined once daily up to ten days.

2.5 Phytochemical Screening

The ethanol extract of Saurauia roxburghii was screened for phytochemical constituents using standard procedures of analysis [8, 9, 10].

2.6 Statistical Analysis

All the values in the test were expressed as Mean± SEM (Standard Error of Mean). Statistical differences between the means of the various groups were analyzed by using Student's t test. P values < 0.05 were considered as significant. Percent increase or decrease was calculated by using the following formula:

$$\frac{(\text{Mean Control- Mean Treatment})}{\text{Mean control}} \times 100$$

III. Results

3.1 Effect of ethanol extracts of *Azadirachta indicia* and *Andrographis paniculata* on BGL of alloxan induced diabetic rats

Table-1 shows the effect of ethanol extract of the leaves of *Azadirachta indicia* and *Andrographis paniculata* on BGL of alloxan induced diabetic rats. Alloxan increased the BGL by 100.73 % when compared with the normal control rats. Treatment of rats with ethanol extract of *Azadirachta indicia* and *Andrographis paniculata* significantly ($p<0.01$) lowered the BGL by 36.91 % and 40.65 % respectively as compared to diabetic control group. In this condition percent of BGL decreasing effect of the standard drug of glimepiride was 51.05 %. Results show that ethanol extract of *Andrographis paniculata* possess almost the similar effect as that of reference antidiabetic drug glimepiride.

3.2 Effect of ethanol extracts of *Azadirachta indicia* and *Andrographis paniculata* on BGL of glucose loaded rats

The effect of ethanol extract of the leaves of *Azadirachta indicia* and *Andrographis paniculata* on BGL of glucose loaded rats is presented in Table-2. Administration of glucose increased the rats BGL by 70.55 % as compared to normal control rats. The ethanol extract of *Azadirachta indicia* and *Andrographis paniculata* significantly ($p<0.05$) lowered the elevated BGL by 30.20% and 32.18% respectively as compared to diabetic control (glucose loaded) rats. In this case of glimepiride treatment, the percent of BGL was 37.23.

3.3 Acute toxicity test

In the acute toxicity assay of ethanol leaves extract of *Azadirachta indicia* and *Andrographis paniculata*, no deaths were observed at high (4g /kg) dose level. Animals also did not show any stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhoea or increased diuresis. Behavior of all treated animals appeared normal and no significant change in the body weight as well as their daily food consumption were observed. Hence, both the extracts were considered to be without gross or acute toxic effects on rats.

3.4 Phytochemical screening

The result of the phytochemical screening of Azadirachta indica and Andrographis peniculata is presented in Table-3. It gives the valuable information for the present or absent of various compound.

IV. Discussion

In the present study, *Azadirachta indicia* and *Andrographis paniculata* were found to have significant ($P< 0.01$) antidiabetic effect on glucose loaded and alloxan induced diabetic rats. In accordance with the present finding other scientists also reported similar results on *Azadirachta indicia* [11, 12] and *Andrographis paniculata* [13].

In alloxan induced diabetic model, we have observed that the intraperitoneal injection of alloxan tetrahydrate (40 mg/kg) caused sharp elevation of blood glucose level (100% compared with normal BGL) in Wistar albino rats. The blood glucose levels of rats were raised due to permanent destruction of their pancreatic β cells. Alloxan is a beta cytotoxin, mediated their action by generating reactive oxygen species that cause rapid destruction of β cells and resulting in a diabetic state [14].

However, in alloxan induced model, where there is complete loss of pancreatic β cells, *Azadirachta indicia* and *Andrographis paniculata* leaves extract causes a significant reduction (36.91 % and 40.65 % respectively) of blood sugar, which indicates that both plants possess bioactive principals, which probably acts by increasing the uptake or utilization of glucose peripherally. This speculation is also supported by our observation on glucose loaded diabetic rats where *Azadirachta indicia* and *Andrographis paniculata* were found to reduces the elevated blood glucose level by 30.20 % and 32.18 % respectively. The mechanism of blood glucose reduction of *Azadirachta indicia* and *Andrographis paniculata* ethanol leaves extract may be as a result of the ability of the bioactive compounds present in the above two extracts to bind to receptor sites especially the peroxisome proliferator – activated receptors which are the chief regulators of glucose metabolism. The compounds after binding to the receptors may activate the receptors, which act on glucose metabolizing pathways and thus reduce the glucose circulation in alloxan induced diabetes [15].

Previous research on *Azadirachta indicia* and *Andrographis paniculata* showed that their leaves contain two principle bioactive compounds namely nimbidin and andrographolide respectively which have been reported to possess hypoglycemic activity [16, 17]. Kar et al. [16] reported that nimbidin significantly delayed the peak rise in blood sugar after glucose administration. Andrographolide an active principle in the leaves of A. paniculata has been shown to have significant antidiabetic activity and suggested that andrographolide can increase glucose utilization to lower plasma glucose in diabetic rats lacking insulin [17]. However, possibility of other mechanisms may be involved here in improving glucose homeostasis like inhibition of intestinal glucose absorption, reduction of glycaemic index of carbohydrates, and/ or reduction of the effect of glutathione cannot be ruled out.

In Alloxan diabetic rabbits, the blood glucose levels are raised due to permanent destruction of pancreatic Cells [18]. Moreover, the serum insulin levels are decreased in Alloxan diabetic rabbits due to destruction of pancreatic cells. The increase in serum insulin levels of diabetic rabbits as observed in the present work shows that some regeneration of pancreatic cells has occurred with the use of Compound recipe. This regeneration of pancreatic cells has occurred slowly and was maximum after a period of 4 months. This finding is in accordance with the observations of Dhaliwal.

It is claimed that Pancreas tonic, which is composed of several medicinal plants, causes a significant reduction in blood glucose levels due to the regeneration of pancreatic islet cells. The regeneration is mainly due to presence of Pterocarpus marsupium that contains significant quantity of
(-) Epicatechin. It has been reported that aqueous extracts of the plant produced a significant reduction in the blood glucose levels in rabbits [19].

Based on the above observations it could be concluded that ethanolic leaves extract of *Azadirachta indicia* and *Andrographis paniculata* have the potentiality in reducing blood sugar level in glucose loaded and alloxan induced diabetic rats and could be an alternative source for treatment of diabetes mellitus. However, further research on the extract and/or their chemical constituents are needed to elucidate the mechanism behind this effect. This report may serve as a footstep on this aspect. The result of the phytochemical screening is presented in Table-3. This reveals moderate concentration of alkaloids, carbohydrate, cardiacglycosides, flavonoids, saponins, terpenes and steroids some of which chemical compounds have been associated to antibacterial activities and thus have curative properties against pathogens [20].

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Table-1: Effect of ethanol extracts of *A. indica* and *A. paniculata* on BGL of alloxan induced diabetic rats.

Group	Treatment	Dose	Blood Glucose Level (mg/dl)	Percent increase or decrease
Gr-I	Normal control (Distilled water)	-	60.21 ± 2.25	
Gr-II	Diabetic control (Alloxan + Distilled water)	-	120.86 ± 4.83 [b]	100.73 % (□)
Gr-III	Positive control (Alloxan + Glimepiride)	4 mg/kg	63.38 ± 3.17 [a]	47.56 % (□)
Gr-IV	Sample Treated (Alloxan + *A. indica*)	1g/kg	76.24 ± 3.63 [a]	36.91 % (□)
Gr-V	Sample Treated (Alloxan + *A. paniculata*)	1g/kg	71.72 ± 5.02 [a]	40.65 % (□)

Values are mean ± SEM (n = 10)
Here, [a] P<0.01 compared with diabetic control, [b] P<0.01 compared with normal control

Table-2: Effect of ethanol extracts of *A. indica* and *A. paniculata* on BGL of glucose loaded rats.

Group	Treatment	Dose	Blood Glucose Level (mg/dl)	Percent increase or decrease
Gr-I	Normal control (Distilled water)	-	61.87 ± 2.56	
Gr-II	Diabetic control (Glucose + Distilled water)	-	105.52 ± 3.5 [b]	70.55 % (□)
Gr-III	Positive control (Glucose + Glimepiride)	4 mg/kg	66.23 ± 6.3 [a]	37.23 % (□)
Gr-IV	Sample Treated (Glucose + *A. indica*)	1g/kg	73.65 ± 5.5 [c]	30.20 % (□)
Gr-V	Sample Treated (Alloxan + *A. paniculata*)	1g/kg	71.56 ± 4.6 [c]	32.18% (□)

Values are mean ± SEM (n = 10)
Here, [a] P<0.01 compared with diabetic control, [c] P<0.05 compared with diabetic control and [b] P<0.05 compared with normal control

Table-3: Phytochemical screening of ethanol extract of *Azadirachta indica* and *Andrographis peniculata*

S/No.	Constituents	*Azadirachta indica*	*Andrographis peniculata*
1	Alkaloids		
	i.Dragendorff's test ii.Meyer's test	- -	- -
2	Cardiac glycosides		
	Killer-killanis test	+ +	-+
3	Flavonoids		
	i.Shinoda's test ii.FeCl3 test	+ +	++ +
4	Saponins		
	Frothing test	-	++
5	Terpenes	+	-
6	Quinone test	+	+
7	steroids	+	++

Key: + = Positive (slightly present), ++ = Positive (moderately present), - = Negative (absent)