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EVALUATION OF HYPOGLYCEMIC ACTIVITY OF HOLARRHENA ANTIDYSENTERICA LEAVES EXTRACT IN STREPTOZOTOCIN INDUCE DIABETIC TARS

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ABSTRACTS

The current study is an effort to examine the effect of *Holarrhena antidysenterica* leaves extract on streptozotocin induced diabetes in Wistar rats. Two groups of streptozotocin induce diabetic rats were orally treated through *Holarrhena antidysenterica* leaves extract (250 and 500 mg/kg) respectively. The blood glucose level, body mass, Glycosylated hemoglobin, liver glycogen, lipid profile, Antioxidant status were measured at the end of the study that is after 28 days of treatment. *Holarrhena antidysenterica* leaves extract were originate to be significant ($p < 0.001$) in dropping the blood glucose level, liver and renal function, hemoglobin, triglyceride, cholesterol, blood urea, lipid profile, while both the treatments improved body weight, liver glycogen satisfied status once compared to the diabetic control. It have been concluded that *Holarrhena antidysenterica* leaves extract, in adding to the antidiabetic activity in the streptozotocin induced diabetic model.

KEYWORDS:

INTRODUCTION

Diabetes is a chronic disorder. It may be characterized by hyperglycaemia. These may help in insulin secretion defects and both insulin action. Due to development of insulin resistance the inadequate insulin secretion and tissues dimension may lead to abnormalities of fats, carbohydrate and metabolism of protein. These may lead to change or may increases the concentration of blood glucose level. These may damage many systems of the body like blood vessels, nerves. Diabetes is unique of the most important causes of morbidity and death in all over the world. According to the survey it was concluded that 0.5 to 3% of person was surfer from diabetes. Now a days its reaches to more than 7%. Around 200 to 300 million people are affected and it should be double or triple in next few years.

It is a heterogeneous disorder. These may arise from interactions of genetic and environment and a lifestyle factor. Insufficient insulin production and a genetic factor are causing the type 2 diabetes. It may be resistance to the insulin target tissues. Erectile dysfunction, blindness, poor healing wound, failure of kidney and heart diseases may occur during long term diabetes. Type 2 diabetes is more common than type 1. Scientific data of India shows that around 57 to 60 million of patients is been affected in year 2025. This will make the India in world largest diabetic population.

MATERIALS AND METHODS

The design of the system consists of a series of steps that are taken systematically to achieve specific objectives according to specific guidelines and recommendations. It covers all stages as of field visits to remark, selection and assortment of therapeutic plants, quantitative assortment, compliance regulation, equipment use, material preparation, and selection of specific extraction solvents, etiquette formulation. Final implementation of the standard procedure. Completely of this requires a decent sense of humor and a decent technical and professional hand.

PREPARATION OF PLANT EXTRACTS

The leaves of the plant are cleaned, dried in the air in the shade at room temperature and dried by logs in a mill. The dry object was kept or taken out through the extraction process.

The dry matter was collected with distilled water using petroleum ether, methanol, hydro alcohol (1: 1) and sox extractor respectively. The item was placed in a large container and placed in a bottle containing an extracting ingredient. The socket is fitted with a power supply. The bottle is heated; the solvent evaporates and flows addicted to the condenser, where it is converted into a liquid that enters the discharge chamber holding the sample.

Solids are slightly filed with hot solvent. When the

socket chamber is almost full, the chamber is automatically removed and the solvent returns to the distillation flask. This process is repeated several times, in hours or days, till the solvent colour fades from the socket of the socket. Each discharge is filtered at the end of the heat dissipation process. The filtrate was concentrated and the solvent was extracted using a rotating evaporator. This extract was placed in desiccators to take away residual moistness and was eventually kept in airtight container at 40^0 C for further use.

INITIAL PHYTOCHEMICAL EXAMINATION OF ABSTRACTS

New hydrochloric abstracts are analyzed by phytochemical elements as described by Trace and Evans (1989) to identify different plant components.

Tannins

2ml of sample extract mixed with 2ml 5% FeCl3; the yellowish-brown colour precipitate was appeared.

Test for Alkaloid

The 2ml of methanolic extract mixed with 1.5ml of 1% hydrochloric acid and heated on the water bath. After heating the solution added six drops of wagner regents/ mayers reagent/ dragendroff regents. Orange colour precipitate were formed, alkaloid was present.

Saponins

Approximately 1 ml of exam mixture is diluted distinctly by 20 ml of distilled water and stirred for 15 min in a graduated cylinder. I cm foam layer was produce, saponins was present.

Cardiac glycoside

In 2 ml of alcohol filtrate, 1 ml of glacial acetic acid and 1-2 drops of $FeCl_3$ were added and 1 ml of concentrated H_2SO_4 was added. Indicated by a brown ring on the visible connector the presence of a deoxygenase element of cardinals. Infringement ring may appear under the brown ring, but the green ring may be on top of the brown ring in the acetic acid layer and spread slowly throughout the layer. (Trease & Evans, 1989).

Terpenes

2ml of exam sample, 5ml of chloroform, 2ml of acetic acid and concentrated sulfuric acid was mixed in each other, a reddish brown is appeared.

Flavonoids

Taken 2gm of plant material in 10ml of alcohol or water. Same drops of concentrated hydrochloric acid mixed with 0.5gm of zinc or magnesium transfer to 2ml filtrate. After three minutes the pink or red magenta colour was appeared.

Phenolics

2ml of exam sample was mixed with 1ml 1% ferric chloride mixture, blue or green colour was appeared.

Anthraquinones

0.5ml of extract was heated with 10ml of sulfuric acid and filter. The filtrate was shaking with 5ml of chloroform. The layer of chloroform is piped out into other test tube, mixed with 1ml of diluted ammonia. Resulting mixture was observed colour change.

PHYSICOCHEMICAL PARAMETERS

The standard drug is made according to WHO guidelines. Various physicochemical parameters have been determined.

Description

Leaves can be examined by nerves (skin, eyes, tongue, nose and ears) or by extensive examination and testing of the plant by certain factors such as color, aroma, taste, size, shape and touch, texture.

Loss on Drying

About 10 g of coarse flour (without basic suspension) is placed in a wire bowl after weighing (weighing 0.1 g). Suspended for 5 hours at 105^oC and measured. Drying and weeding was performed over a period of 1 hour until the difference between the two consecutive weights was more than 0.25%. After 30 minutes of suspension two consecutive weights have reached a permanent weight. Also put in the fridge for 30 minutes. In one desiccator, no difference of more than 0.1 g was observed.

Extractive Values

A known quantity of shrub substance is booked and completely the sugar is washed with cold water, dried in a desiccator until the weight is stabilized and a saxtile containing petroleum ether, methanol, hydro alcohol and water is extracted in a complete extractor respectively. Special release. Ingredients have a moderate weight and are calculated as a percentage related to the weight of the extracted substance.

Total Ash Value

The flowers, which weigh about two ounces [2-3 g], are burned in stellar silica crucible at temperatures above 45^0 C until there is absolutely no carbon. Then cooled, If carbon free ash cannot be found in this technique, the burnt mass can be removed by warm water, residues, residues and filter paper collected from non-ash paper will burn, the filtrate will evaporate to dry, and the temperature will not exceed 45^0 C.

Acid-Insoluble Ash Value

25ml of distilled hydrochloric acid was added to the crucible holding all the ashes. The unsolvable material is poised in a non-abrasive filter paper (Whatman) and wash away by warm water until the filter is no longer working. A paper filter containing non-soluble material is transferred to the original crucible and dried on a hot plate and then burned with a permanent mass. The residue was permitted to cool for 30 minutes at the appropriate desiccator and was measured without delay.

The unresolved ash content is calculated in relation to air-dried plant material.

Water Soluble Ash Value

The ash was heated in 25ml of water for 5 minutes; Collect non-melting material in a non-abrasive filter paper, rinse by warm water and bake intended for 15 minutes at a temp not above 45^0 C. The mass of an unsolvable substance is removed by the weight of the ash; weight difference indicates ash dissolved in water. Ratio of water solvable ash is calculated in relation to airborne plant matter.

Residue on Ignition/ Sulfated Ash test

The silica crucible was burned red for ten minutes and permitted to cool and weigh on the desiccator. 1-2g of shrub material is poured into the crucible, which weighs exactly; do not take it lightly until something is completely burned. The residue is cooled, moistened with 1 ml of H2SO4, heated gently until white smoke evaporates and set at 800°C±25 C until all the dark atoms are gone. The burning took place in a place endangered from radio broadcasts. The crucible is permitted to cool; little droplets of sulphuric acid are mixed and the crucible is burned. It was then heated as previously, permitted to cool again. Performance is repeated until two consecutive weights do not exceed 0.5mg.

pH Value at 10 % and 1 % Dilution

pH of 10% solution: Specify ten grams of the drug accurately measured in 100ml of water and test the pH of the filtrate with a standard glass electrode.

pH of 1 % solution: Straight dilute one mg of the drug in 100ml of water and check the pH of the filter with a standard glass electrode.

EXPERIMENTAL ANIMALS

Healthy albino wistar mice weighing 200–220 g were selected for study. The animals were found in an animal shelter at the Transmal Institute of Pharmaceutical Education and Research. Animals are kept in standard cages and are kept under normal conditions. They are given regular food and water. Animal studies are first approved by the IAEC, TIEPE, and Meerut, established by the Committee on Animal Control and Experimental Control and Examination (CPCSEA).

RESULTS AND DISCUSSION

Table 1: Effect of *Holarrhena antidysenterica* leaves extracts on food eating, liquid drinking and urine defecated by STZ cured diabetic mice.

Parameter→ Groups↓	Liquid Drinking (ml/day)	Diet Eating (g/day)	Urine (Wetting of Bedding)
Normal Control	39.33±2.01	28.71±1.23	+
Diabetic Control	64.66±3.05#	40.63±2.86#	++++
Gliclazide Std.(10mg/kg)	47.36±1.40**	26.24±1.56**	++
Methanol Extract (500mg/kg)	57.59±2.02*	38.40±1.87	++
Hydro alcohol Extract (500mg/kg)	50.12±1.98**	26.23±1.98**	++
Aqueous Extract (500mg/kg)	52.66±2.36*	33.32±1.78*	++

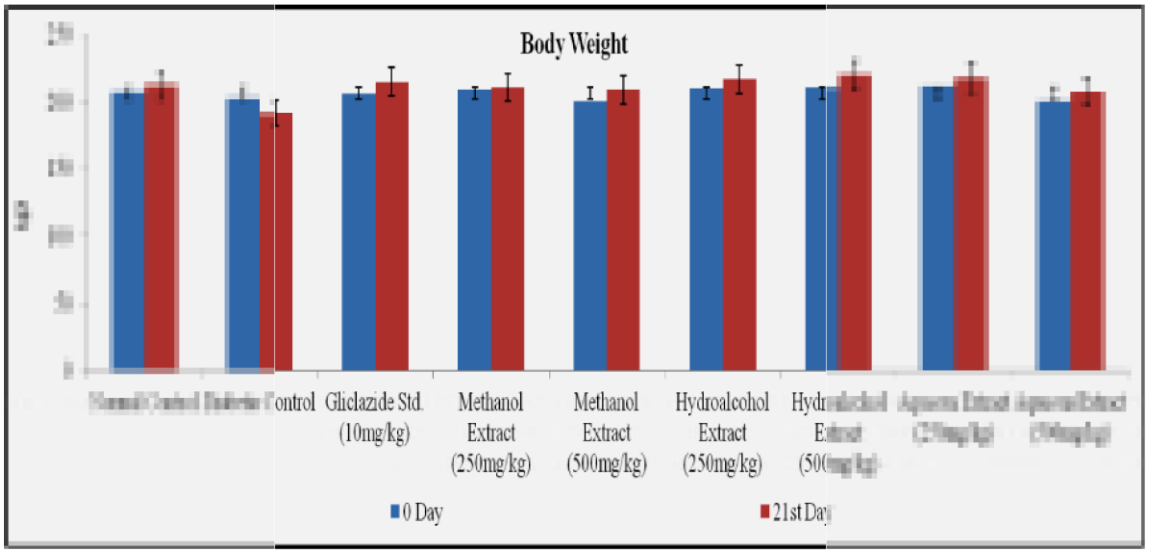

Fig 1: Result of leaves extract of on body weight of STZ persuaded diabetic mice.

Table 2: Result of leaves extract of *Holarrhena antidysenterica* on body mass of STZ persuaded diabetic mice.

Parameter→ Groups↓	BodyWeight (gm)		
	0Day	21st Day	%Variation
Normal Control	207±4.19	212±3.77	2.36
Diabetic Control	202±4.75	191±2.48*	-5.76
Gliclazide Std. (10mg/kg)	205±5.08	214±3.62*	4.21
Methanol Extract (250mg/kg)	208±4.34	210±4.18	0.95
Methanol Extract (500mg/kg)	200±4.10	208±3.77*	3.85
Hydroalcohol Extract (250mg/kg)	209±5.03	216±3.76	3.24
Hydroalcohol Extract (500mg/kg)	210±5.09	218±3.44*	4.11
Aqueous Extract (250mg/kg)	211±4.78	217±3.12	2.76
Aqueous Extract (500mg/kg)	200±3.85	207±3.26*	3.38

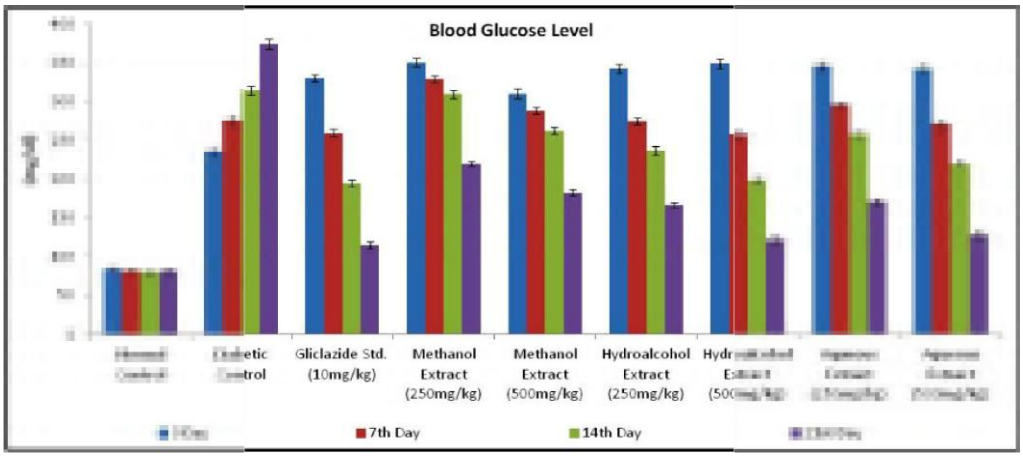

Figure 2: Result of extract on fasting plasma glucose levels of diabetic mice.

Table.3: Result of dissimilar extract on fasting plasma glucose levels of STZ induced diabetic mice.

Parameter→ Groups↓	Plasma glucose level (mg/dl)			
	0 Day	7th Days	14th Days	21st Days
Normal Control	85.02±2.65	83.32±2.60	81.30±3.14	82.34±2.42
Diabetic Control	234.78±4.52*	276.21±6.54*	314.00±5.19*	374.01±6.49**
Gliclazide Std. (10mg/kg)	329.91±4.23	258.10±5.07*	194.48±4.89**	114.61±4.13**
Methanol Extract (250mg/kg)	350±6.52	330.80±4.60*	310.61±5.10*	219.45±3.12**
Methanol Extract (500 mg / kg)	310.11±6.24	289.01±4.55*	263.41±4.98*	182.61±4.18**
Hydro alcohol Extract (250 mg / kg)	343.31±5.52	275.71±4.62*	237.70±5.44*	166.59±3.44**
Hydro alcohol Extract (500 mg / kg)	348.81±6.15	258.329±5.16*	198.51±5.12*	121.54±4.05**
Aqueous Extract (250 mg / kg)	346.50±5.91	297.11±3.89*	258.69±4.60*	171.32±3.33**
Aqueous Extract (500 mg / kg)	343.72±4.89	271.79±4.86*	219.89±3.98*	128.22±4.02**

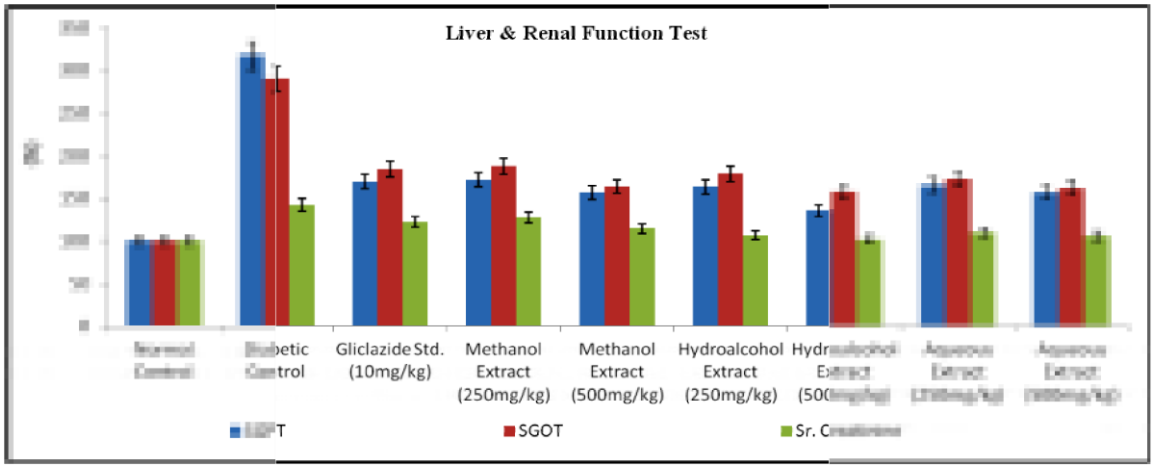

Fig 3: Result of dissimilar extract *of Holarrhena antidysenterica leaves* on SGOT, SGPT and serum creatinine levels of diabetic mice.

Table 4: Result of dissimilar extract *of Holarrhena antidysenterica leaves* on SGOT, SGPT and serum creatinine levels of STZ induced diabetic mice.

Parameter→ Groups↓	SGPT(IU/L)	SGOT(IU/L)	Sr. Creatinine (mg/dl)
Normal Control	31.2±1.4	32.8±1.7	0.86±0.12
Diabetic Control	98.7±2.39	95.0±2.66	1.22±0.19
Gliclazide Std. (10mg/kg)	52.4±1.4*	60.2±1.61*	1.046±0.13
Methanol Extract (250mg/kg)	53.2±1.72**	61.3±2.19**	1.09±0.15*
Methanol Extract (500mg/kg)	48.5±1.47**	53.5±2.34**	0.98±0.14*
Hydroalcohol Extract (250mg/kg)	50.6±2.06**	58.5±2.42**	0.91±0.12**
Hydroalcohol Extract (500mg/kg)	42.02±1.38**	51.54±1.40**	0.87±0.12**
Aqueous Extract (250mg/kg)	51.24±2.16**	56.42±1.58**	0.94±0.13**
Aqueous Extract (500mg/kg)	48.68±1.15**	53.02±2.16**	0.90±0.15**

Table 5: Effect of extracts of *Holarrhena antidysenterica leaves* on serum lipid profile of STZ induced diabetic mice.

Parameter→ Groups↓	TG(mg/dl)	TC(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDLmg/dl)
Normal Control	150.67±3.65	119.22±2.9	37.40±1.03	51.69±1.47	30.13±1.53
Diabetic Control	263.55±3.59**	219.62±3.02**	19.93±0.67**	146.98±3.26**	52.71±1.32*
Gliclazide Std. (10mg/kg)	183.79±3.21**	143.92±3.15**	29.33±1.20 **	77.83±1.40**	36.76±1.44**
Methanol Extract (250mg/kg)	239.49±3.82	180.98±4.01*	22.77± 1.04*	110.31±2.77*	47.90±1.76*
Methanol Extract (500mg/kg)	206.34±3.10*	166.53±3.01*	27.71±1.15*	97.55±2.10*	41.27±1.42*
Hydroalcohol Extract(250mg/kg)	184.36±3.89*	158.51±3.92*	26.66±1.06*	94.97±2.90*	36.87±1.38*
Hydroalcohol Extract(500mg/kg)	163.0±4.01**	138.04±3.21**	35 ±1.18**	70.22±2.3**	32.72±1.85**
Aqueous Extract (250mg/kg)	193.67±4.11*	155.6±4.12*	24.9±1.02*	91.91±2.10*	38.77±1.11*
Aqueous Extract (500mg/kg)	170.8±4.32**	143.84±3.61**	29.9±1.2**	79.78±2.11**	34.16±1.20**

Result of Dissimilar Abstracts of Holarrhena Antidysenterica Leaves On Normal Mice

Oral-Glucose Acceptance Examination

Management of the release of Holarrhena antidysenterica before loading glucose leads to a substantial decrease in plasma glucose levels inside 120 minutes of glucoseload (Table 3.8). Uploading of glucose 60 minutes before administration does not allow blood glucose levels to rise above normal. The same was observed with hydro alcohol and liquid extracts.

Table 6: effect of different extracts of holarrhena antidysenterica leaves on oralglucose acceptance in normoglycaemic mice.

Time→ Groups↓	0Min.	30Min.	60Min.	90Min.	120Min.
Normal Control	80.5±4.5	170.3±12.6	160.4±10.6	140.6±8.8	115.3±8.6**
Methanol Extract (250mg/kg)	84.66±4.4	162.40±10.1	150.72±9.6	127.21±7.7	110.44±6.9**
Methanol Extract (500mg/kg)	78.6±3.8	169.7±11.8	140.9±8.10	120.1±9.8*	105.7±6.10**
Hydroalcohol Extract (250mg/kg)	80.5±4.10	168.5±9.5	130.7±4.8	110.1±6.9**	90.2±7.8**
Hydroalcohol Extract (500mg/kg)	86.2±5.9	165.3±8.9	120.4±11.9**	105.2±7.3**	80.3±9.8**
Aqueous Extract (250mg/kg)	85.3±6.2	160.5±11.3	125.5±9.0	115.2±10.1**	94.7±6.7**
Aqueous Extract (500mg/kg)	75.8±5.1	162.5±9.3	130.7±10.6	98.2±11.2**	85.1±9.9**

CONCLUSION

This study was conducted to evaluate the beneficial effects of various flowering species of Holarrhena antidysenterica on normoglycemic mice and diabetic mice produced by streptozotocin and the results indicate that this plant may have normal blood sugar levels and diabetic rats induced by streptozotocin. It has a positive effect. The results suggest that extracts from plants can be particularly protective against other diarrheal diseases caused by diabetes, allergies, and chemical disorders.

The results have shown that hydrochloric release is very effective in lowering blood sugar levels in diabetic rats, followed by fluid excretion and methanol; the outcome depends on the volume. In addition, the ingredients show improvements in parameters such as weight, diet, liquid intake and excretion. This release lowers serum SGPT, SGOT and serum creatinine levels, indicating the effect of excretion on reversing loss due to diabetes as seen in high levels of SGOT and SGPT in diabetes management. It's moving. Withdrawal also lowers serum lipid levels such as triglycerides and cholesterol. Examination of the pancreas has historically shown the restoration of damaged tissue, in which parts of the cured groups were related by the controls of diabetes. Toxicity studies have shown that the release is safe and does not show toxic reactions up to a body weight of 5000 mg / kg. Extraction also showed an increase in glucose tolerance for normal effects, while phytochemical analysis of Holarrhena antidysenterica leaves extracts revealed active formulas of alkaloids, tannins, saponins,

terpenoids, flavonoids and phenolic. From other plants it has also been found to have anti-diabetic activity.

In short Holarrrhena antidysenterica leaves extract has a significant hypoglycemic effect action.

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