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Antihyperglycemic and Antihyperlipidemic Effects of Methanolic Extract of *Holarrhena antidysenterica* Bark in Alloxan Induced Diabetes Mellitus in Rats

D.D. Bandawane, K.H. Bibave, A.V. Jaydeokar, U.S. Patil and M.G. Hivrale

Department of Pharmacology, Progressive Education Society's Modern College of Pharmacy, Sector No. 21, Yamunanagar, Nigdi, Pune-411044, Maharashtra, India

ABSTRACT

Background: *Holarrhena antidysenterica* L. (family Apocynaceae) is traditionally used in Ayurvedic system of Indian medicine for the treatment of diabetes mellitus. Therefore, the present work was undertaken to evaluate the antidiabetic and antihyperlipidemic effects of methanolic extract of *Holarrhena antidysenterica* Bark (MEHA) in alloxan induced diabetes mellitus and to focus on its possible mechanism. **Materials and methods:** Wistar albino rats (150-220 g) of either sex were used for the study. Diabetes was induced in rats by injecting alloxan (150 mg kg^{-1}) intraperitoneally. Group I served as normoglycemic rats. Group II served as diabetic control. Group III and IV served as diabetic rats treated with 200 and 400 mg kg^{-1} of MEHA, respectively. Group V served as diabetic rats treated with oral hypoglycaemic agent, glibenclamide (4 mg kg^{-1} p.o.). Group VI and VII served as normoglycemic rats treated with 200 and 400 mg kg^{-1} of MEHA. All the treatments were given for 28 days. At the end of study, on 28th day, overnight fasted rats were sacrificed and blood was collected to determine fasting blood glucose, triglycerides, total cholesterol, high density lipoprotein, low density lipoprotein, very low density lipoprotein, total protein, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. To study *in vivo* antioxidant activity, liver tissues of different groups were homogenized to determine maloaldehyde (MDA), catalase (CAT), Super Oxide Dismutase (SOD) and reduced glutathione (GSH). Additional parameters were estimated to focus on mechanism of action were liver glycogen content and glucose uptake from hemidiaphragms. **Results:** Diabetic rats treated with MEHA in doses of 200 and 400 mg kg^{-1} significantly ($p<0.01$) reduced fasting blood glucose and normalized the lipid profile in comparison to diabetic control group. There was dose dependent decrease observed in transaminases, BUN and MDA whereas there was significant ($p<0.01$) improvement in total proteins, liver catalase, SOD and GSH in MEHA treated groups. MEHA (200 and 400 mg kg^{-1}) treated diabetic rats showed significant improvement in liver glycogen and glucose uptake by rat diaphragm. Improvement in histopathology of pancreas of MEHA treated rats confirmed its protective role in alloxan induced diabetes. **Conclusion:** It can be concluded that MEHA possesses antihyperglycemic activity with antihyperlipidemic and antioxidant potential which may prove beneficial in cardiovascular complications associated with diabetes mellitus.

Key words: *Holarrhena antidysenterica* L., diabetes mellitus, alloxan, glibenclamide

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INTRODUCTION

Diabetes mellitus is a common endocrine disorder caused due to either deficiency in insulin production or due to ineffectiveness of the insulin produced. Such a deficiency of insulin results in impaired metabolism of glucose and other energy yielding fuels like lipids and proteins[1]. Globally diabetes has shadowed the spread of modern lifestyle and it can be linked to an increase overweight and sedentary population[2]. The metabolic disturbances contribute massively to most of the cardiovascular, neurological, renal and retinal diabetic complications[3]. The estimation that diabetes mellitus will affect more than 300 million people by the year 2025 shows the need for improvement in the treatment aspect of this chronic disorder[4].

Currently available drugs for the treatment of diabetes mellitus do not restore normal glucose homeostasis and they are associated with number of side effects[5]. Moreover due to high cost of these drugs, it is

Corresponding Author: D.D. Bandawane, Department of Pharmacology, Progressive Education Society's Modern College of Pharmacy, Sector No. 21, Yamunanagar, Nigdi, Pune-411044, Maharashtra, India
Tel: (+91)9822425748 Fax: 020-27661315

difficult to provide modern medical healthcare especially in developing countries. It therefore becomes necessary to make use of vast reserves of plant origins for medical purposes which will help to search effective as well as safer drug remedy for diabetes mellitus. Traditional medicines derived mainly from plants play measure role in the management of diabetes mellitus[6]. World Health Organization (WHO) has recommended evaluation of traditional plants as a source of antidiabetic remedies since they are having less side effects, have easy regional availability and are cost effective[7]. In Indian traditional medicinal system, over 150 plants and some of their active principles including flavonoids, tannins, alkaloids are reported for the management of diabetes[8].

Holarrhena antidysenterica (Apocynaceae) popularly known as kurchi bark is a traditional Indian medicinal plant. Traditionally decoction of *Holarrhena antidysenterica* bark is used as astringent, anthelmintic, stomachic, diuretic, antipyretic, tonic and is used as a principle remedy in cases of various types of diarrhoea and dysentery[9,10]. The bark and seeds of *Holarrhena antidysenterica* are used to treat asthma, bronchopneumonia and malaria[11]. In Asian countries seeds of *Holarrhena antidysenterica* are used as antidiabetic[12,13]. The phytochemical constituents reported in *Holarrhena antidysenterica* bark are alkaloids, flavonoids, tannins, saponins and phenolic compounds[11]. In Maharastra state of India, decoction of *kurchi* bark is used for treating diabetes[14]. Hence the present study was undertaken to provide a scientific basis for traditional use of *Holarrhena antidysenterica* bark in the treatment of diabetes mellitus.

MATERIALS AND METHODS

Collection of plant material and extraction: Dried bark of *Holarrhena antidysenterica* was procured from Yucca Enterprises, Dombivli, Maharashtra, India. The specimen was identified and authenticated by Dr. Diwakar, Director, Botanical Survey of India (BSI), Pune and a voucher specimen (V. No. KHB-1) was deposited at BSI for future reference. The dried bark was powdered coarsely and exhaustively extracted with methanol by maceration method[15]. The extract was concentrated to dryness under vaccum to obtain a residue.

Preliminary phytochemical screening: Methanolic extract of *H. antidysenterica* bark was screened for presence of various phytoconstituents like steroids, flavonoids, tannins, alkaloids, glycosides, carbohydrates, amino acids, phenolic compounds and saponins[16].

Animals: Healthy albino Wistar rats of either sex weighing 180-220 g were procured from the National Institute of Biosciences (NIB), Pune, Maharashtra. The animals were housed in polypropylene cages in groups of six at an ambient temperature of 25±1°C, under a 12:12 h light-dark cycle, with free access to standard diet and water throughout the study. The study protocol was approved by Institutional Animal Ethical Committee of Modern College of Pharmacy in accordance with the regulations of CPCSEA (884/PO/ac/05/CPCSEA).

Chemicals: Alloxan was procured from Spectrochem, Mumbai. Glibenclamide was procured from Aventis Pharma limited, Mumbai. All other chemicals and reagents used were of analytical grade procured from SRL Mumbai, E. Merck India.

Acute toxicity study: Acute toxicity study was performed as per OECD guideline 423[17]. Female wistar rats selected by random sampling technique were employed in this study. The animals were fasted overnight with free access to water. Methanolic extract of *Holarrhena antidysenterica* was administered orally to different groups at the dose levels of 300, 500, 2000 and 5000 mg kg^{-1}. The animals were observed 24 h for mortality with special attention during first 2 h and then intermittently for 14 days. At the end of the study, the animals were observed for general toxic signs, morphological behaviour and mortality.

Experimental design

Induction of diabetes: Rats were fasted for 12 h prior to the induction of diabetes. Alloxan freshly prepared in ice cold citrophosphate buffer (pH 4.3) and was administered intraperitoneally in single dose of 150 mg kg^{-1}. Development of diabetes was confirmed by measuring blood glucose level 3 days after the administration of alloxan. Rats with blood glucose level more than 250 mg dL^{-1} were considered to be diabetic and used for the studies[18].

Short term antihyperglycemic activity: The antihyperglycemic activity of MEHA was first evaluated on a short term basis in normal and alloxan diabetic rats. Overnight fasted rats were divided into seven groups (six animals per group):

Group I : Normal control; 10 mL kg^{-1}distilled water
Group II : Diabetic control; 10 mL kg^{-1} distilled water
Group III : Diabetic rats treated with single dose of MEHA; 200 mg kg^{-1} p.o.
Group IV : Diabetic rats treated with single dose of MEHA; 400 mg kg^{-1} p.o.
Group V : Diabetic rats treated with single dose of glibenclamide; 4 mg kg^{-1} p.o.

Group VII : Normoglycemic rats treated with single dose of MEHA; 200 mg kg^{-1} p.o.
Group VIII : Normoglycemic rats treated with single dose of MEHA; 400 mg kg^{-1} p.o.

Blood was collected from tail tip at 0, 2, 4, 6 and 8 h after administration of extracts. Blood glucose levels were estimated using glucometer (Ultra Touch Two, Johnson and Johnson).

Sub-acute antihyperglycemic activity (Study of 28 days): Wistar albino rats were randomized into five groups comprising of six animals in each group as given below:

Group I : Normal control; 10 mg kg^{-1} distilled water
Group II : Diabetic control; 10 mg kg^{-1} distilled water
Group III : Diabetic rats treated with single dose of MEHA; 200 mg kg^{-1} p.o.
Group IV : Diabetic rats treated with single dose of MEHA; 400 mg kg^{-1} p.o.
Group V : Diabetic rats treated with single dose of glibenclamide; 4 mg kg^{-1} p.o.

All the drugs or extracts were administered orally once daily for 28 days.

Oral glucose tolerance test (OGTT): Before the termination of the experiment, Oral Glucose Tolerance Test (OGTT) was performed to assess the glucose tolerance. For this purpose, overnight fasted rats were fed glucose (2 g kg^{-1}) orally and blood was collected at 0, 30, 60, 90 and 120 min interval from tail tip for glucose estimation[19].

Biochemical analysis: At the end of the study (28th day) blood samples were collected by cardiac puncture method for biochemical estimations. Serum total cholesterol, HDL-cholesterol and triglycerides were estimated using standard kits (Span Diagnostics, Gujrat). LDL-cholesterol and VLDL-cholesterol were calculated using Friedewald's equation[20]. Serum levels of AST and ALT were assayed by method of Reitman[21] and ALP was assayed by the method of King[22]. Total protein and albumin were estimated by the method of Kingsley[23]. Blood urea nitrogen was estimated by the method of Doumas[24]. Lipid peroxidation was measured as malondialdehyde (MDA) by method of Kumar[25]. Liver SOD was determined according to the method of Marklund[26]. Liver catalase (CAT) was determined according to the method of Sahreen[27] and liver glutathione (GSH) was determined according to the method of Kaur[28]. Liver glycogen content was determined according to the method of Carroll[29]. *In vitro* glucose uptake by rat diaphragm was determined according to the method of Walaas[30] and Chattopadhyay[31].

Histopathology of pancreas: Rats were sacrificed under light ether anaesthesia. Pancreas was removed and placed in formalin for histopathological studies. Sections of pancreas were stained with haematoxylin eosin stain and the exposed sections were examined under resolution power of 40X using digital microscope[32].

Statistical analysis: Experimental results were presented as Mean±SEM of six animals. Analysis of variance was performed by ANOVA followed by Dunnet's multiple comparison test. The p-values less than 0.05 were considered to be statistically significant.

RESULTS

Extraction: The yield of methanolic extract of *Holarrhena antidysenterica* was 10.7% w/w.

Preliminary phytochemical screening: Preliminary phytochemical analysis of MEHA showed presence of alkaloids, flavonoids, tannins, saponins, phenolic compounds and steroids.

Antihyperglycemic effect of MEHA: The effect of short term antihyperglycemic study is shown in Table 1. Alloxan induced diabetic rats treated with MEHA (200, 400 mg kg^{-1}) showed significant ($p<0.01$) reduction in fasting blood glucose levels by 50.15% and 40.27%, respectively at 8 h as compared to standard drug glibenclamide which showed reduction up to 44.90% at 8 h. However there was no significant effect of extract on blood glucose level in normal rats. The effect of 28 days antihyperglycemic activity was depicted in Fig. 1. There was significant ($p<0.01$) dose dependent reduction in fasting blood glucose levels with the doses of 200 and 400 mg kg^{-1} of MEHA as compared to diabetic control group.

Effect of MEHA on oral glucose tolerance: The effect of MEHA on Oral Glucose Tolerance Test (OGTT) was shown in Figure 2. Blood glucose levels of normal and diabetic rats were increased at 30 min after glucose administration. MEHA (200, 400 mg kg^{-1}) significantly ($p<0.01$) reduced the increase in blood glucose levels at 60, 90 and 120 min in glucose loaded rats when compared with diabetic control rats (Fig. 3).

Effect of MEHA on lipid profile: The protective effect of MEHA on lipid profile has been shown in Table 2. Diabetic control rats showed significant ($p<0.01$) elevation in the levels of total cholesterol, triglycerides,

Table 1: Effect of acute administration of aqueous and methanolic extract of *Holarrhena antidysenterica* bark in fasting blood glucose level in alloxan induced diabetic rats

		Fasting blood glucose level (mg dL^{-1})				
Experimental groups	Dose (mg kg^{-1})	0 h	2 h	4 h	6 h	8 h
Normal control (NC)		77.33±2.29	75.66±2.98	77.16±2.54	77.66±2.15	75.5±2.48
Diabetic control (DC)		490.83±14.49##	492.00±10.25##	493.00±17.06##	506.84±20.96##	504.84±18.30##
DC+MEHA	200	487.16±27.59	399.33±18.21	307.00±16.49*	254.50±28.07**	242.66±11.51**
DC+MEHA	400	404.33±18.68	445.66±23.24	387.66±16.36	325.16±19.92*	241.50±21.03**
DC+GL	4	481.16±21.23	470.33±25.37	265.00±21.15**	283.16±12.96*	313.34±11.66*
DC+MEHA	200	79.50±2.29	77.16±2.31	75.66±1.97	72.83±1.93	70.33±1.80
DC+MEHA	400	80.50±2.47	78.66±2.47	74.33±2.5	72.66±2.21*	71.00±1.89

n = 6, Values are Mean±SEM, ##p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to diabetic control group, MEHA: Methanolic extract of Holarrhena antidysenterica, GL: Glibenclamide, Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple test for comparison

Table 2: Effect of subacute administration of MEHA on lipid profile in alloxan induced diabetic rats

		Lipid profile (mg dL^{-1})				
Experimental groups	Dose (mg kg^{-1})	T-CH	TG	HDL	VLDL	LDL
Normal control (NC)		87.95±8.89	121.08±11.07	35.83±4.56	23.57±0.55	28.55±1.26
Diabetic control (DC)		172.75±9.22##	196.08±10.88##	22.79±1.84##	38.77±1.03##	111.49±3.65##
DC+MEHA	200	109.33±1.35*	141.21±6.07*	34.66±1.72*	33.23±0.75**	41.44±3.46**
DC+MEHA	400	105.51±2.26*	136.10±2.442*	33.83±1.30*	27.95±0.70**	43.73±1.25**
DC+GL	4	91.25±3.24**	136.16±3.34*	33.40±5.30**	27.95±0.70**	29.89±2.60**

n = 6, Values are Mean±SEM, ##p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to diabetic control group, MEHA: Methanolic extract of Holarrhena antidysenterica, GL: Glibenclamide, Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple test for comparison

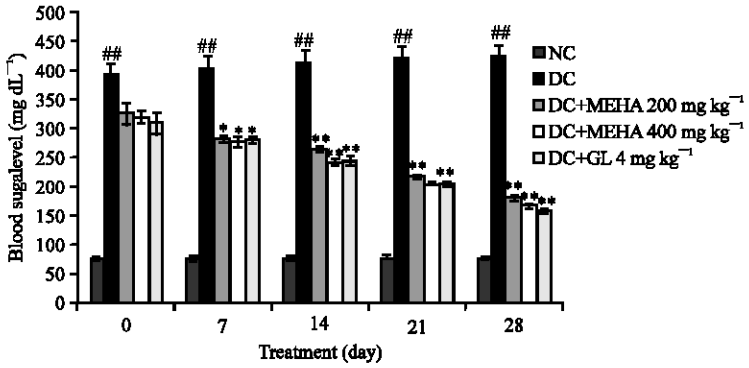

Fig. 1: Effect of subacute administration of MEHA (200 and 400 mg kg^{-1}) on fasting blood glucose level in alloxan induced diabetic rats, ##p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to diabetic control group

LDL and VLDL while level of HDL was significantly decreased when compared to normal rats. Diabetic rats treated with MEHA (200, 400 mg kg^{-1}) and glibenclamide (4 mg kg^{-1}) showed a significant (p<0.05) reduction in elevated total cholesterol level in diabetic rats. MEHA (400 mg kg^{-1}) treated diabetic rats decreased T-CH by 38.66%, TG by 32.05%, LDL by 62.21% and VLDL by 27.87%. Whereas, 400 mg kg^{-1}MEHA treated groups showed a significant (p<0.05) increase in HDL levels in diabetic rats.

Effect of MEHA on biochemical parameters: As shown in Table 3, Diabetic control rats showed significant (p<0.01) reduction in total proteins and albumin levels and a significant (p<0.01) increase in blood urea nitrogen levels as compared to normal rats. MEHA (200, 400 mg kg^{-1}) showed significant (p<0.01) increase in total protein level by 56.14 and 59.77% in comparision to 50.69 and 64.42% increase by glibenclamide (4 mg kg^{-1}) treated groups, respectively. MEHA at test dose of 200 mg kg^{-1}showed significant (p<0.05) increase in albumin level by 56.71%. while 400 mg kg^{-1}dose showed significant (p<0.01) increase in albumin level by 63.36%. Standard drug glibenclamide (4 mg kg^{-1}) increased the albumin levels by 66.37 and 68.80%, respectively. MEHA at 200 and 400 mg kg^{-1} dose levels showed decrease in blood urea nitrogen by 31.59 and 32.53%, respectively.

Table 3: Effect of subacute administration MEHA on biochemical parameters in alloxan induced diabetic rats

Experimental groups	Dose (mg kg^{-1})	Total proteins (g mL^{-1})	Albumin (g dL^{-1})	Blood urea nitrogen (mg dL^{-1})
Normal Control (NC)		11.47±0.34	9.46±0.24	19.63±0.61
Diabetic Control (DC)		3.89±0.76##	2.48±0.12##	47.03±1.83##
DC+MEHA	200	8.87±0.47**	5.73±0.20*	32.17±0.81*
DC+MEHA	400	9.67±0.48**	6.82±0.33**	31.73±1.40**
DC+GL	4	9.25±0.58**	7.37±0.38**	29.99±0.93**
Normal Control		10.87±2.33**	7.95±0.28**	30.17±7.33**

n = 6, Values are Mean±SEM, ##p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to diabetic control group, MEHA: Methanolic extract of Holarrhena antidysenterica, GL: Glibenclamide, Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple test for comparison

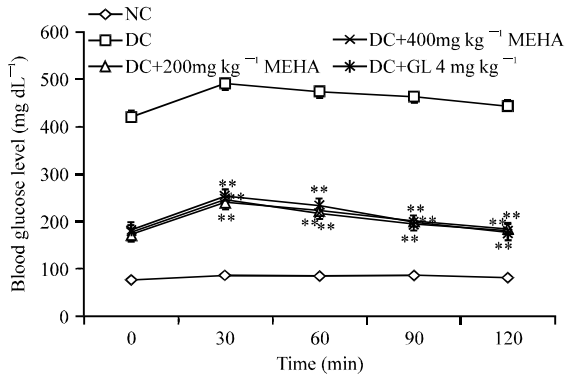

Fig. 2: Effect of MEHA on oral glucose tolerance test in alloxan induced diabetic mellitus rats, ##p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to diabetic control group

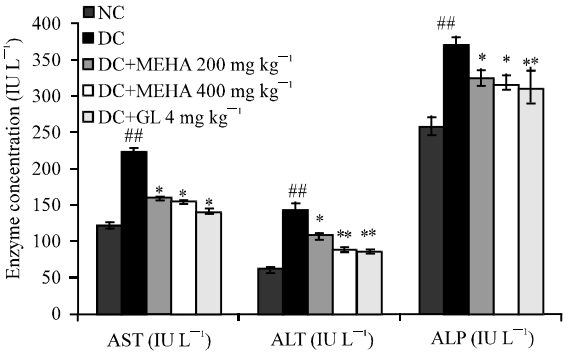

Fig. 3: Effect of subacute administration of MEHA on serum AST, ALT, ALP in alloxan induced diabetic rats, ##p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to diabetic control group

Effect of MEHA on transaminases: As shown in Fig. 3, diabetic control rats showed significant (p<0.01) increase in levels of AST, ALT and ALP as compared to normal rats. After 28 days of MEHA (200, 400 mg kg^{-1}) suplimentation, there was significant (p<0.05) reduction in ALT levels by 24.97 and 37.45%, in AST levels by 28.28 and 30.44% and in ALP levels by 12.49 and 14.51%.

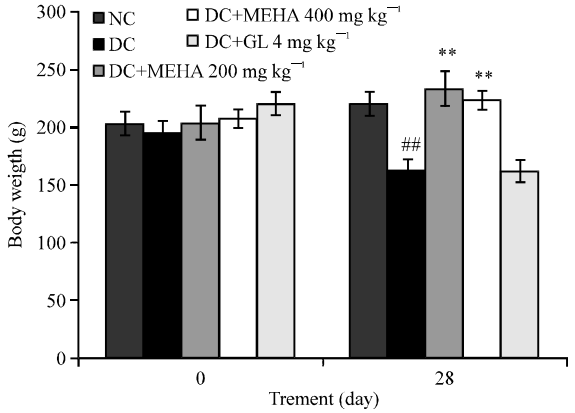

Fig. 4: Effect of subacute administration of MEHA on body weight in alloxan induced diabetic mellitus rats,**p<0.01 as compared to diabetic control group

Effect of MEHA on body weight: Effect of MEHA was shown in Fig. 4. Alloxan induced diabetic rats showed significant (p<0.01) reduction in body weight as compared to normal rats. Treatment of diabetic rats with 200 and 400 mg kg^{-1} of MEHA for 28 days showed significant (p<0.01) increase in body weight by 12.29 and 13.79% as compared to glibenclamide which improved the body weight by 14.39%.

Effect of MEHA on water and feed intake: As shown in Fig. 5 and 6, diabetic control rats showed significant (p<0.01) increase in water and feed intake as compared to normal rats. Diabetic rats treated with MEHA (200 and 400 mg kg^{-1}) showed significant (p<0.01) reduction in water intake from 14th day of treatment as compared to diabetic control rats. The effect of MEHA (200, 400 mg kg^{-1}) on water intake of the animals was comparable to that of the standard drugs glibenclamide (4 mg kg^{-1}). Diabetic rats treated with MEHA (200, 400 mg kg^{-1}) showed significant (p<0.01) reduction in feed intake from 7th day of treatment as compared to diabetic control rats. As shown in Fig. 6, the effect of MEHA (200, 400 mg kg^{-1}) on feed intake of the animals was comparable to that of the standard drugs glibenclamide (4 mg kg^{-1}).

Table 4: Effect of subacute administration of MEHA on liver antioxidants

Experimental groups	Dose (mg kg^{-1})	Catalase (IU mg^{-1} protein)	SOD (U mg^{-1} protein)	MDA (n mol mg^{-1} protein)	GSH (nmoles mg^{-1} of protein)
Normal control (NC)		74.9±1.29	13.67±1.67	197.7±2.1	47.21±2.05
Diabetic control (DC)		45.8±1.27$^{##}$	5.63±0.32$^{##}$	460.2±4.5$^{##}$	15.25±1.98$^{##}$
DC+MEHA	200	49.2±2.30*	10.11±0.37*	294.4±3.6*	24.28±1.26*
DC+MEHA	400	51.8±2.40*	11.21±0.41**	284.5±4.2**	32.47±2.18**
DC+GL	4	68.5±1.03**	11.47±0.42*	235.7±1.3**	35.85±1.74**

n=6, Values are Mean±SEM, $^{##}$p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to diabetic control group, MEHA: Methanolic extract of Holarrhena antidysenterica, GL: Glibenclamide, Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple test for comparison

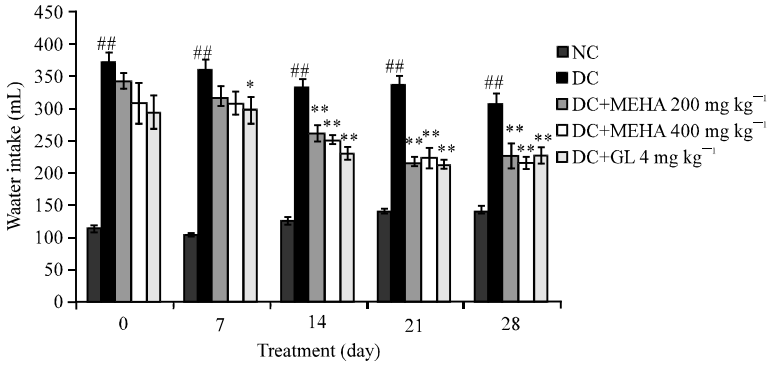

Fig. 5: Effect of subacute administration of MEHA on water intake in alloxan induced diabetic rats, $^{##}$p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to diabetic control group

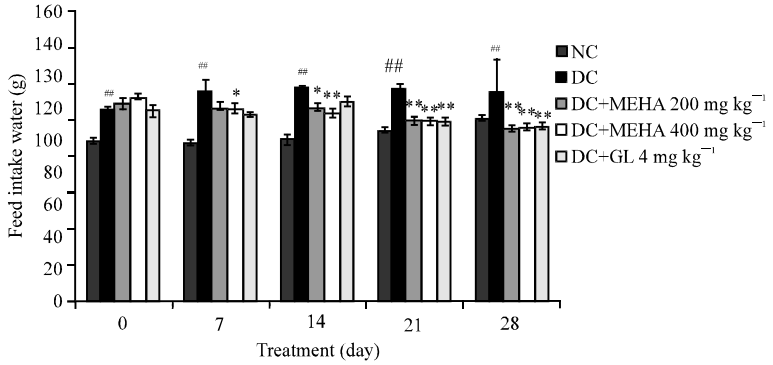

Fig. 6: Effect of subacute administration of MEHA on feed intake in alloxan induced diabetic rats, $^{##}$p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to diabetic control group

Effect of MEHA on liver antioxidant enzymes: Effect of MEHA on liver SOD, catalase and GSH was shown in Table 4. Diabetic control rats showed significant (p<0.01) decrease in liver CAT, SOD and GSH levels while significant (p<0.01) increase in level of liver MDA as compared to normal rats. Treatment with MEHA for 28 days resulted in significant (p<0.01) reduction in liver tissue MDA. MEHA (200, 400 mg kg^{-1}) treated diabetic rats also showed significant (p<0.05) elevation in CAT and SOD levels (Fig. 7).

Effect of MEHA on liver glycogen: As shown in Table 5, liver glycogen was significantly (p<0.01) decreased in diabetic control rats as compared to normal rats. There was significant (p<0.01) increase in glycogen content of liver with MEHA (200, 400 mg kg^{-1}) and glibenclamide (4 mg kg^{-1}) treated groups as compared to diabetic control rats.

Effect of MEHA on *in vitro* glucose uptake: As shown in Table 5, diabetic control group shows significant decrease in uptake of glucose compared to normal rats. Administration of MEHA significantly (p<0.01) enhances the uptake of glucose by isolated rat hemi-diaphragm as compared to diabetic control group. It was found to be more effective than insulin.

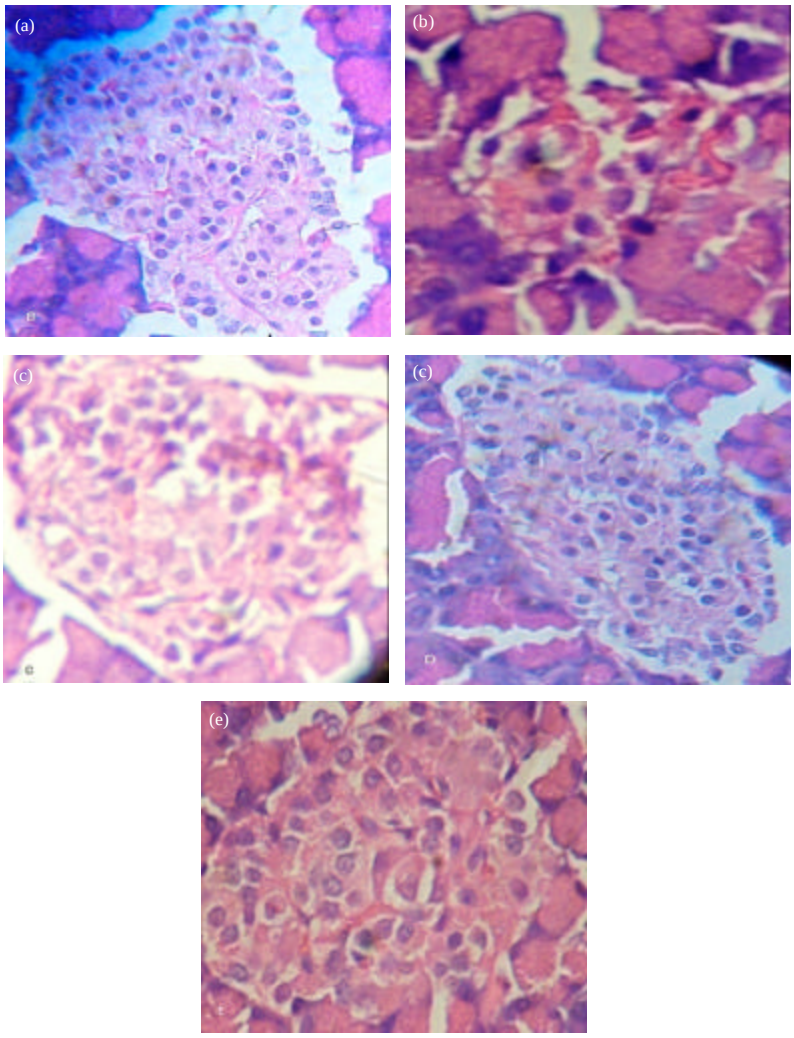

Fig. 7(a-e): Hitopathology of pancreas in different experimental groups in alloxan induced diabetic rats

Table 5: Effect of subacute administration of MEHA on liver glycogen and glucose uptake by rat hemi-diaphragms in alloxan induced diabetic rats

Experimental groups	Dose (mg kg^{-1})	Liver glycogen (mg 100g^{-1})	Uptake of glucose from rat hemi-diaphragm (mg g^{-1} 30 min^{-1})
Normal control (NC)		20.15±1.28	38.61±2.17
Diabetic control (DC)		5.69±0.72**	10.23±2.35**
DC+MEHA	200	9.54±0.48*	19.09±2.37*
DC+MEHA	400	11.15±0.63**	24.66±3.08*
DC+GL	4	15.56±1.70**	29.73±3.98*

n=6, Values are Mean±SEM, **p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to diabetic control group, MEHA: Methanolic extract of Holarrhena antidysenterica, GL: Glibenclamide, Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple test for comparison

Administration of *H. antidysenterica* and insulin together was found to be less effective than MEHA alone but significantly (p<0.01) higher than insulin treated group.

Histopathology study: Histopathology of pancreas of different experimental groups was shown in Fig. 7. The section of pancreatic tissue of diabetic control group showed extensive beta cell degranulation and decreased cellular density. Diabetic rats treated with MEHA (200, 400 mg kg^{-1}) showed dose dependent remarkable improvement in beta cell degranulation and cellularity changes. Histopathological

improvement in pancreatic tissue was comparable to that caused by glibenclamide treatment.

DISCUSSION

Medicinal plants being the potential sources of bioactive agents are gaining acceptability worldwide. A number of studies on ethnomedicinal plants and herbal medicines have been conducted in the past and plants have been reported for being used medicinal purpose by tribals in several countries. The ethnobotanical survey can bring out many different clues for the development of drugs to treat human diseases like diabetes. Safe, effective and inexpensive indigenous remedies are gaining popularity equally among the people of both the urban and rural areas, especially in developing countries like India[33].

Alloxan induced diabetes has been commonly utilized as an animal model to study diabetes in experimental animals. Alloxan exerts its diabetogenic actions when administered intravenously, intraperitonealy or subcutaneously. The action of alloxan in the pancreas is preceded by its rapid uptake by the insulin-secreting beta cells[34]. The cytotxic action of alloxan is mediated by reactive oxygen species which leads to rapid destruction of β cells, thereby reducing levels of insulin and increasing the blood glucose[35,36]. However there is possibility for the survival of a few beta-cells and this has been proved by several workers who observed antihyperglycemic activity with oral hypoglycemic agents like glibenclamide in alloxan induced diabetic animals[37,38]. Alloxan treated rats in our study, in accordance with earlier reports thus represents experimental model for studying non-insulin dependent diabetes mellitus[39].

In the present study, acute antihyperglycemic effect of methanolic extract of *Holarrhena antidysenterica* bark have been evaluated on alloxan induced diabetic rats. Maximum antihyperglycemic effect shown by MEHA at 8 h after administration indicates that it takes about 8 h or more time for the active ingredients of the extract or its metabolites to enter into circulation and their target tissues to bring antihyperglycemic activity. The present study showed that MEHA when given for 28 days significantly reduced elevated blood glucose level in alloxan induced diabetic rats. Results of OGTT in present study indicated that the control of post-prandial glucose level shown by MEHA may be mediated by regulation of glucose uptake from the intestinal lumen, through the inhibition of carbohydrate digestion or absorption[40]. Thus increased utilization of glucose by the body may be responsible for antihyperglycemic activity of the extract.

In the present study, the decrease in body weight of alloxan induced diabetic rats has been attributed to gluconeogenesis, which is associated with increased muscle wasting and loss of tissue proteins[41,42]. Diabetic rats treated with MEHA showed an increase in body weight as compared to the diabetic control rats, which may be its protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis. In the present work, diabetic rats showed polyphagia and polydypsia which may be due to metabolic changes caused by lack or deficiency of insulin. MEHA showed dose dependent decrease in feed and water intake in diabetic rats. This could be due to increase in insulin release from remnant beta cells or increase in utilization of glucose by the body.

In the present study dyslipidemia has been shown by alloxan induced diabetic rats. High levels of T-CH and TG in diabetic rats is associated with insulin deficiency resulting in failure to activate lipoprotein lipase[43]. Improvement in serum total cholesterol and triglycerides in alloxan diabetic rats after administration of MEHA could be due to insulin mediated inhibition of lipase and consequently decreasing rate of lipolysis and decrease in conversion of free fatty acids to phospholipids and cholesterol in the liver. Effect of MEHA on lipid profile may be due to the presence of flavonoids, which significantly increased LDL receptors, that in turn increase hepatic uptake and degradation of LDL causing decrease in serum LDL levels.

High LDL and VLDL levels in diabetic condition are usually associated with atherosclerosis. HDL level is inversely associated with coronary heart disease and its elevation is considered as an anti-atherosclerotic factor. Thus, MEHA showed antihyperlipidemic effect which could play a protective role against the development of atherosclerosis and cardiovascular complications in diabetes mellitus[44].

In the present study, decreased level of total proteins in diabetic rats may be due to increased rate of amino acids conversion to glucose, decreased amino acid uptake, increased conversion rate of glucogenic amino acids to carbon dioxide and water and reduction of ribosomal protein synthesis[45]. Improvement in serum total proteins and albumin levels in MEHA treated diabetic rats may be because of increase in insulin secretion from remnant beta cells. This leads to decreased utilization of total proteins or increased protein synthesis or decreased proteolysis by activating the enzyme that catalyzes amino acids. In the present investigation, elevated levels of blood urea nitrogen in the diabetic control group has considered as significant marker for renal disorder[46]. This elevation in serum urea nitrogen was controlled significantly by treatment with MEHA possibly due to controlled gluconeogenesis.

Alkaline phosphatase (ALP) levels were increased significantly in alloxan induced diabetic rats. It has been

reported that the increased transaminases under insulin deficiency were responsible for the increased gluconeogenesis and ketogenesis during diabetes[47]. AST is an enzyme found mainly in the cell of the liver, heart, skeletal muscle, kidney and pancreas and to lesser amount in red blood cells. Its serum concentration is proportional to the amount of cellular leakage or damage and it is released into serum in larger quantities when any one of these tissue is damaged. AST increase is usually associated with heart attack or liver disease. ALT is an enzyme found mainly in the liver and elevated levels in serum indicate liver damage. Significant reduction in the activities of these enzymes in MEHA treated diabetic rats may be because of revival of insulin secretion into circulation or decrease cellular damage[48].

Free radicals are formed disproportionately in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins and the subsequent oxidative degradation. Increased oxidative stress is involved in diabetes. Hypoinsulinemia in diabetes also increases the activity of the enzyme fatty acyl coenzyme-A-oxidase which initiates beta oxidation of fatty acids resulting in lipid peroxidation. The determinations of MDA level provides a good measure of peroxidation which is one of the chief mechanisms of cell damage leading to necrosis or apoptosis[49]. Our present study showed a significant elevation in liver MDA content of diabetic rats suggesting involvement of peroxidative injury in development of diabetic complications. In our study MEHA significantly reduced the MDA level in liver tissue of diabetic rats indicating its protective role during oxidative damage.

Associated with the changes in lipid peroxidation, diabetic animals showed decreased activity of the key antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) which play an important role in scavenging the toxic intermediates of incomplete oxidation. SOD is one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen[50]. SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical, which damages the membrane and biological structures[51]. Catalse is a hemeprotein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals[52]. GSH protect the cellular system against the toxic effects of lipid peroxidation. MEHA treatment showed a significant restoration in SOD, CAT and GSH contents in liver of diabetic rats. It may be because of antioxidant effect of MEHA or could be due decrease in hyperglycemia induced oxidative stress.

The conversion of glucose to glycogen in the liver cells is dependent on the extracellular glucose concentration and the availability of insulin. The reduced liver glycogen store in the diabetic rats has been attributed to the reduced activity of glycogen synthase and increased activity of glycogen phosphorylase. This is probably due to lack of insulin in the diabetic state, which results in the inactivation of the glycogen synthetase systems[53]. Treatment with MEHA significantly increased the liver glycogen levels of the diabetic animals and this may be because of insulin mediated increase in conversion of glucose to glycogen in liver.

In the present study, we estimated glucose uptake from rat diaphragms which is commonly used and reliable method for *in vitro* study of peripheral uptake of glucose. Increased glucose uptake by rat diaphragm of diabetic rats after treatment of MEHA confirms that MEHA increases peripheral utilization of blood glucose and thereby reduce blood glucose level.

The possible mechanisms by which MEHA brings about its antihyperglycemic action may be through potentiation of pancreatic secretion of insulin from the remnant beta-cells of islets. It could also be due to extra-pancreatic mechanisms like enhanced glycogenesis or decreased glycogenolysis by liver. It may be due to enhanced transport of blood glucose to peripheral tissues as seen by stimulatory effect on glucose uptake in rat diaphragm or due to inhibition of α-glucosidase[40]. The restoration of architecture of islets of Langerhans by MEHA in histopathological studies further showed its insulinomimetic activity. The antihyperglycemic potential of MEHA could be due to presence of tannins[54], alkaloids and flavanoids[55] present in the extract.

CONCLUSION

Thus our study proves the beneficial effects of MEHA in management of diabetes and its associated complications. MEHA is not only acting by enhancing peripheral utilisation of glucose but also on beta cells and hepatocytes to increase insulin and glycogen synthesis respectively. Thus *Holarrhena antidysenterica* bark can be beneficial in the management of type II diabetes mellitus. Furthermore studies are required to isolate the active constituent responsible for antihyperglycemic activity and also to study the effect of extract on insulin resistance.

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