(Research Article)

E-ISSN: 0975-8232; P-ISSN: 2320-5148



INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH



Received on 31 December, 2017; received in revised form, 06 March, 2018; accepted, 13 May, 2018; published 01 September, 2018

ANTIHYPERGLYCEMIC AND ANTIOXIDATIVE EFFECT OF HYDRO-METHANOLIC EXTRACT OF LEAF OF ABROMA AUGUSTA IN STREPTOZOTOCIN INDUCED DIABETIC MALE ALBINO RAT

U. Saha, C. Ghosh and Chhanda Mallick *

Clinical Nutrition and Dietetics, Department of Biomedical Laboratory Science and Management, (UGC Innovative Funded Department), Vidyasagar University, Midnapore - 721102, West Bengal, India.

Keywords:

Diabetes, *Abroma augusta*, Streptozotocin, Hexokinase, Insulin

Correspondence to Author: Chhanda Mallick

Assistant Professor, Clinical Nutrition and Dietetics, Department of Biomedical Laboratory Science and Management, (UGC Innovative Funded Department), Vidya sagar University, Midnapore - 721102, West Bengal, India.

E-mail: chhanda_mallick@yahoo.com

ABSTRACT: The present study was conducted to investigate protective effect of hydro-methanolic (2:3) extract of leaf of *Abroma augusta* in streptozotocin induced diabetic male albino rat. For this purpose animals were divided into three treatment groups (n = 6): control group, STZ induced diabetic group (STZ 4 mg / 0.1 ml citrate buffer/ 100 g body weight), hydro-methanolic extract of leaf of Abroma augusta treated group (20 mg / 0.5 ml distilled water/100 g body weight). Treatment was conducted for 28 days. Blood glucose, serum insulin, glycated haemoglobin levels along with activities of hepatic carbohydrate metabolic enzymes, hexokinase and glucose-6-phosphate dehydrogenase were assed in all experimental groups. Oxidative stress markers like catalase, peroxidase enzyme activities, conjugated diene and thiobarbituric acid reactive substance levels in liver were measured. All these parameters were significantly recovered towards the control level after the treatment of hydro-methanolic extract. Pancreatic islet diameter, count of islet and number of cells in islets also decreased in diabetic animals. Treatment of these animals with extract resulted significant recovery towards the control level. Serum glutamate oxaloacetate transaminase and glutamate pyruvate transaminase were assed in all the groups and it has been revealed that the said extract has no metabolic toxicity in general. From this study it may be concluded that hydro-methanolic extract of leaf of A. augusta has protective effects on diabetes induced complications.

INTRODUCTION: Diabetes mellitus (DM) is a group of metabolic syndrome ¹ associated with both micro vascular and macro vascular diseases affecting several organs ². Many complications are occurred which results increase risk of premature death. In uncontrolled diabetes risk of fatal death is increased and it is about 43% of total world population ³. About 366 million people are suffering from DM over the world and the incidence of this disease is predicted to be more than double by the year of 2030 ⁴.



DOI: 10.13040/IJPSR.0975-8232.9(9).3788-94

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(9).3788-94

Chronic hyperglycemia produces oxidative stress that also associated with dysfunction and apoptosis of several organ and cell including pancreatic β -cells, neurons and glial cells ^{5, 6}. Many drugs are used for their anti-diabetic action ⁷ but the main disadvantage of currently available drugs is that they have to be given throughout the life and produce side effects ⁷. Insulin is not also affordable in low income country ³ and long term use of insulin formed insulin resistance, anorexia nervosa, brain atrophy and fatty liver ^{8, 9}.

Medicinal plants and their bioactive constituents can be used for the treatment of DM throughout the world especially in our country as these are less toxic. These medicinal plants interact directly with our body chemistry without any side effects. There is increasing demand by patients to use natural products having anti-diabetic activity ¹⁰.

Mannheim, Germany. GOT, GPT and glycated haemoglobin kit was purchased from Span

Diagnostic Limited, Surat, India.

E-ISSN: 0975-8232; P-ISSN: 2320-5148

Abroma augusta (A. augusta) (common name is ulatkambal in Hindi) is an evergreen shrub, found throughout the hot and humid parts of India. Different parts of this tree are used widely in ayurvedic drug preparation to cure different diseases such as uterine disorder, inflammation, rheumatic pain of joints, dysmenorrhea, gonorrhea, headaches and diabetes ¹¹. From folk medicine reputation it has been revealed that people use the leaf of this plant as antidiabetic medicine. Some preliminary work has been done for the protection of diabetic complications by methanolic extract of leaf of A. augusta ¹². By trial and error method we have selected hydro-methanolic extract for better efficacy as hydro-methanolic solution 40:60 (water / methanol) ratio is the most preferred procedure for extraction of all pharmacologically active substances present in raw plant material. All kinds of polar and non-polar molecules are extracted by this technique. This present study was conducted to search out the protective effect of hydro-methanolic extract of leaf of A. augusta in diabetes induced complications.

MATERIALS AND METHODS:

Materials:

Collection of Plant Materials: Fresh and matured leaves of *A. augusta* were collected from local area in the month of June and the material was authenticated by taxonomist of Botany Department, Vidyasagar University. Leaves are dry in shed at room temperature 25 °C.

Animal Material:

Selection of Animal and Animal Care: Eighteen matured normoglycemic male albino rats, weight of 120 ± 10 were selected for this experiment and approved by institutional Animal ethical Commetee Reg no. 2013/GO/Re/S/18/CPCSEA, DOR-1/5/18. The rats were collected from Saha Enterprise, authorized dealer of CPCSEA registered under Ministry of Environment and Forest. Rats were divided into three cages and housed for 15 days in our laboratory at 25 ± 2 °C temperature with 12 h: 12 h light, dark cycle and proper humidity prior to the experiment for acclimatization. Rats were fed with normal pellet diet and water *ad libitum*.

Reagents: Streptozotocin was purchased from Alfa Aesar, United States. Insulin (ELISA) kit was purchased from Boehringer Mannheim Diagnostic,

Methods:

A. augusta Leaves Extract Preparation: Fresh leaves of A. augusta washed in normal distilled water and cut into small pieces and leaves were dried in shed at room temperature 25 °C then dried in an incubator for 2 days and crushed. 50 g of crushed leaves were dissolved in 1000 ml of hydromethanol (40:60) respectively in glass jar in the airtight condition with occasional shaking. After 7 days filtrates are collected, dried by rotary evaporator and stored in refrigerator.

Induction of Diabetes: Rats were fasted for 12 h before induction of diabetes. After this period they were subjected to single intramuscular injection of streptozotocin (STZ) at the dose of 4 mg / 0.1 ml citrate buffer / 100 g body weight/rat.

Experimental Design: Eighteen rats were taken for this experiment and they were divided in three equal groups.

Group I (Normal Control): Rats were injected single intramuscular injection of citrate buffer at the dose of 0.1 ml / 100 g body weight/rat followed by oral administration of 0.5 ml distilled water / 100 g body weight/rat/day as vehicle for 28 days.

Group II (Diabetic Group): Rats were received single intramuscular injection of streptozotocin at the dose of 4 mg / 0.1 ml citrate buffer / 100 g body weight/rat followed by oral administration of 0.5 ml distilled water / 100 g body weight/rat for 28 days.

Group III (Hydro - Methanolic Extract Treated Group): Diabetic rats of this group were subjected to oral treatment of hydro-methanolic extract of leaf of *A. augusta* at the dose of 20 mg / 0.5 ml distilled water / 100 g body weight / rat for 28 days. On the 29th day of the experiment, final body weight was recorded and blood glucose level was measured by the single touch glucometer. After the completion of treatment schedule all the animals were sacrificed by using euthanasia. Euthanasia is defined as a painless or stress free death. The experimental animal were sacrificed by inhalation of gases *i.e.* carbon dioxide, carbon monoxide, carbon dioxide plus chloroform / halothane as a

E-ISSN: 0975-8232; P-ISSN: 2320-5148

euthenics agents. After sacrificed, blood was collected from portal vein for the assessment of blood glucose, serum insulin, glycated haemoglobin level along with serum GOT and GPT levels. Liver were dissected out and stored at -80 °C for biochemical analysis of carbohydrate metabolic enzyme like hexokinase, Glucose-6-phosphate dehydrogenase and oxidative stress measuring biosensors such as catalase, peroxidase, Conjugated Diene (CD) and Thiobarbituric acid reactive substance (TBARS). Pancreas was collected from each animal for histological study.

Testing of Fasting Blood Glucose Level: Fasting blood glucose (FBG) levels were measured in each group at the time of starting of the treatment and every 7 days interval by the single touch glucometer ¹³. Blood was collected from the tip of the tail vain of all rats.

Serum Insulin Level: Enzyme Linked Immunosorbant Assay (ELISA) kit was used for the detection of serum insulin 14 . Serum insulin level was expressed in μ IU/ml.

Glycated Haemoglobin: Glycated haemoglobin was measured using the whole blood by standard kit method ¹⁵.

Biochemical Assay of Hexokinase Activity in Liver: Liver hexokinase activity was measured on the basis of reduction of NADPH coupled with hexokinase at 340 nm by spectrophotometer ¹⁶. Liver tissue was homogenized in 0.1 M ice cold phosphate buffer saline (pH 7.4) at the tissue concentration of 50 mg/ml.

Biochemical Assay of Glucose-6-Phosphate Dehydrogenase Activity in Liver: The glucose-6-phosphate dehydrogenase activity was measured by spectrophotometrically ¹⁷ at 340 nm. Enzyme activity was determined by the reduction of NADP / min.

Catalase and Peroxidase in Liver: Catalase activity was estimated by the standard method ¹⁸. Tissue concentrations was 50 mg/ml. Enzyme activity expressed as mM H₂O₂ consumption/mg of tissue/min. Liver peroxidase activity was measured by the standard method ¹⁹.

CD and TBARS in Liver: Conjugated Diene (CD) and thiobarbituric acid reactive substance

(TBARS) ²¹ levels in hepatic tissue were measured by the standard method. Tissue concentration was 50 mg/ml in phosphate buffer saline.

SGOT and SGPT: Serum GOT and GPT activities were assessed by standard kits ²² following manufacturer protocol for assessment.

Histology of Pancreas: Pancreas was fixed in bouin's solution and paraffin sections were prepared according to standard protocol. Pancreas sections were stained by haematoxylin and eosin by the standard method ²³. Pictures were taken at 400X magnification.

Statistical Analysis: ANOVA followed by multiple comparison two tail't'-test was performed for statistical analysis of collected data by the use of SPSS16 software ²⁴.

RESULTS:

Body Weight: Final body weight of the diabetic group was significantly decreased in compare to the control group. There was significant recovery of this parameter was noted to the control level after the treatment of hydro-methanolic extract of leaf of *A. augusta* to the diabetic rat **Table 1**.

TABLE 1: CHANGES IN BODY WEIGHT ON HYDRO-METHANOLIC EXTRACT OF LEAF OF A. AUGUSTA TREATED DIABETIC RATS

	Body weight (gm)			
	Before treatment	After treatment		
Control	128.75 ± 1.89^{a}	141.13 ± 0.92^{a}		
Diabetic	126.92 ± 1.44^{a}	88.67 ± 2.43^{b}		
Hydro methanolic	127.17 ± 2.97^{a}	136.54 ± 1.37^{a}		
extract treated group				

Data are expressed as Mean \pm SE, n = 6. Different superscripts (a, b) in each vertical column significantly differ from in each other (p < 0.05).

TABLE 2: EFFECT OF HYDRO-METHANOLIC EXTRACT OF LEAF OF A. AUGUSTA ON BLOOD GLUCOSE LEVEL IN STREPTOZOTOCIN INDUCED RATS

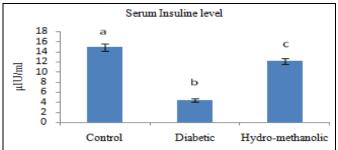
	Blood glucose (mg/dl)				
	1 st	7 th	14 th	21 th	28 th
	Day	Day	Day	Day	Day
Control	81.43	80.32	82.07	83.44	85.77
	$\pm 1.7^{a}$	$\pm 1.9^{a}$	$\pm 1.8^{a}$	$\pm 2.0^{a}$	$\pm 1.9^{a}$
Diabetic	318.78	330.71	342.66	344.46	341.04
	$\pm 2.1^{b}$	$\pm 2.5^{b}$	$\pm 2.1^{b}$	$\pm 2.5^{b}$	$\pm 2.9^{b}$
Hydro	315.23	298.29	256.24	178.43	107.71
methanolic	$\pm 2.2^{b}$	$\pm 2.1^{c}$	±1.7°	$\pm 2.0^{c}$	$\pm 1.9^{c}$
extract					
treated					
group					

Data are expressed as Mean \pm SE; n = 6. Different superscripts (a, b, c,) in each vertical column significantly differ from in each other (p < 0.05).

Fasting Blood Glucose: There was significant elevation in fasting blood glucose level was noted in diabetic group in compare to control group. When the diabetic rats were treated with hydromethanolic extract of leaf of *A. augusta*. This parameter was significantly recovered towards the control level **Table 2**.

Serum Insulin and Glycated Hemoglobin Level: Serum insulin level was decreased but simultaneously glycated hemoglobin level was elevated significantly in diabetic group in compared to control group. Both these two parametes were significantly recoverd towards the control level after the treatment of hydromethanolic extract of leaf of *A. augusta* to the diabetic rats **Fig. 1**.

Hepatic Hexokinase and Glucose-6-phosphate dehydrogenase Activity: Hepatic hexokinase and Glucose-6-phosphate dehydrogenase activities were diminished significantly in diabetic group in compared to control group. Significant recovery of these parameters was noted after the treatment of the hydro-methanolic extract of leaf of *A. augusta* in diabetic rats **Fig. 2**.



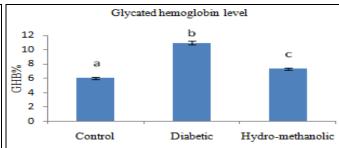
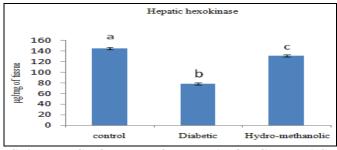


FIG. 1: RECOVERY OF SERUM INSULIN AND GLYCATED HEMOGLOBIN LEVEL AFTER THE TREATMENT OF HYDRO-METHANOLIC EXTRACT OF LEAF OF A. AUGUSTA. Data are expressed as Mean \pm SE; n = 6. Each bars with different superscripts (a, b, c) differ from in each other significantly (p < 0.05).



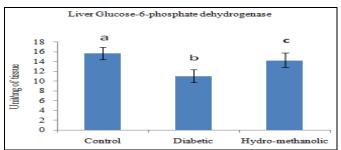
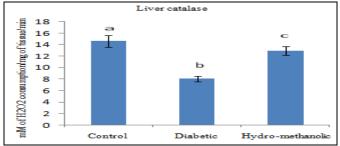


FIG. 2: EFFECT OF HYDRO-METHANOLIC EXTRACT OF LEAF OF A. AUGUSTA ON HEPATIC HEXOKINASE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITIES IN MALE RAT. Data are expressed as Mean \pm SE; n = 6. Bars with different superscripts (a, b, c) significantly differ from in each other (p < 0.05).

Catalase and Peroxidase: Hepatic catalase and peroxidase activities were significantly diminished in the diabetic group in respect to control group. After administration of hydro-methanolic extract of

leaf of *A. augusta*, both these antioxidant enzyme activities were increased towards the control level **Fig. 3**.



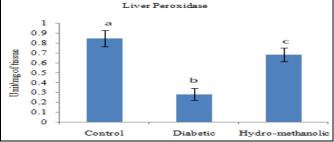
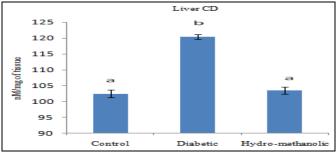


FIG. 3: CHANGES IN HEPATIC CATALASE AND PEROXIDASE ACTIVITIES IN HYDRO-METHANOLIC EXTRACT OF LEAF OF A. AUGUSTA TREATED DIABETIC RAT. Data are expressed as Mean \pm SE; n = 6. Bars with different superscripts (a, b, c) significantly differ from in each other (p < 0.05).

CD and TBARS: Oxidative stress parameters CD and TBARS levels were elevated in STZ induced diabetic rats in compared to the control rats. After the treatment of hydro-methanolic extract of leaf of

A. augusta for 28 days significant diminution in CD and TBARS levels were noted in compared to the diabetic group **Fig. 4**.



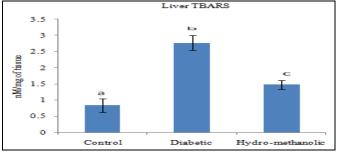
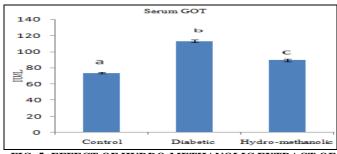


FIG. 4: EFFECT OF HYDRO-METHANOLIC EXTRACT OF LEAF OF A. AUGUSTA ON HEPATIC CD AND TBARS LEVELS IN DIABETIC MALE RAT. Data are expressed as Mean \pm SE; n = 6. Bars with different superscripts (a, b, c) significantly differ from in each other (p < 0.05).

SGOT and SGPT: SGOT and SGPT levels were elevated in STZ induced diabetic rats when compared with control rats. After the treatment of

hydro-methanolic extract of leaf of *A. augusta* the SGOT and SGPT activities were diminished towards control level **Fig. 5**.



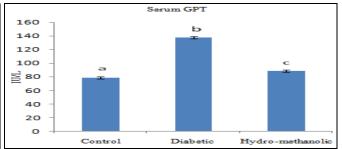


FIG. 5: EFFECT OF HYDRO-METHANOLIC EXTRACT OF LEAF OF A. AUGUSTA ON SERUM GOT AND GPT LEVELS Data are expressed as Mean ± SE; n=6. Bars with different superscripts (a, b, c) significantly differ from in each other (p<0.05).

Histology of Pancreas: Cell density of pancreatic islets, count of islets and diameter of pancreatic islets were decreased in STZ induced diabetic rat in compare to control. After the treatment of hydro-

methanolic extract of leaf of *A. augusta* to the diabetic animals resulted a marked recovery of these parameters towards the control level **Table 3**, **Fig. 6A - 6C**.

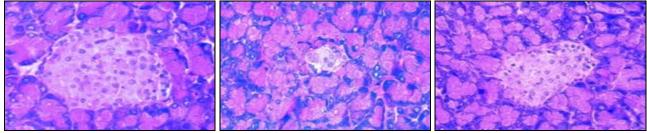


FIG. 6: HISTOLOGY OF PANCREAS, X 400 (HAEMATOXYLIN-EOSIN STAIN)

A- Control Group: Normal cell density along with normal islets size. B- Diabetic Group: Cell density along with islets size was decreased. C-Hydro-methanolic extract treated Group: Cell density and islets size both are increased towards control.

TABLE 3: EFFECT OF HYDRO-METHANOLIC EXTRACT OF LEAF OF A. AUGUSTA ON ISLET NUMBER, ISLET CELL NUMBER AND DIAMETER OF ISLETS IN STREOTOZOTOCIN INDUCED DIABETIC MALE ALBINO RAT

	Islet number count per field in 1000 X	Number of islet	Diameter of
	magnification	cells/ islet	islet
Control	22.06 ± 1.42^{a}	181.2±8.4 a	286.8±11.8 ^a
Diabetic	6.92 ± 1.02^{b}	75.9 ± 6.8^{b}	126.6 ± 9.8^{b}
Hydro-methanolic extract treated group	17.41±1.32°	162.4 ± 8.2^{c}	266.9±11.1°

Data are expressed as Mean \pm SE; n=6. Different superscripts (a, b, c,) in each vertical column significantly differ from in each other (p<0.05).

E-ISSN: 0975-8232; P-ISSN: 2320-5148

DISCUSSION: The present study was conducted the antihyperglycemic determine properties of hydro-methanolic antioxidative extract of leaf of A. augusta in STZ induced male albino rat. The specific dose and duration of experiment adopted had been selected by trial and error study. For such assessment we have studied the fasting blood glucose levels, glycated hemoglobin along with hexokinase and glucose-6phosphate dehydrogenase activities in liver as well as histoarchitecture of pancreus and serum insulin level. Hepatic catalase, peroxidase and TBARS levels of all activities. CD experimental groups were assessed as diabetes is strongly associated with oxidative stress ²⁵. Fasting blood glucose levels were recovered significantly after the treatment of hydro-methanolic extract treatment to the diabetic animals. This may be due to recovery of insulin in extract treated group. Glycated haemoglobin plays an important role in diabetic patient ²⁶. We measured the glycated hemoglobin levels in different groups and the level was elevated in diabetic group which is consistent with other report ²⁷. After the treatment of extract there was significant recovery of this parameter was noted. Hexokinase plays an important role for glucose utilization through glycolytic path way ²⁸.

In hepatic tissue this enzyme activity were decrease in diabetic rat that supported by other 13 . Extract treatment resulted significant recovery of this enzyme which results elevation in glucose utilization. Glucose-6-phosphate dehydrogenase is an important enzyme in pentose phosphate pathway for maintenance of normal blood glucose level. Activity of this enzyme decreases significantly in diabetic rats as this enzyme is also under the control of insulin 29 and STZ destroy β -cell of pancreas 30 . This result also supported by our previous work 13 .

After the administration of extract these enzyme activities were significantly increased by increased secretion of insulin 29 . Pancreatic β -cell regenerative effect of this extract was noted in this study by the rise in serum insulin as well as recovery in volume and diameter of islets found in microphotography. Diabetes is also associated with lipid peroxidation 31 as insulin secretion is closely associated with it. Elevated level of lipid peroxidation leads to islets cell damage in diabetes

³² that diminishes insulin secretion which has been supported by serum insulin levels 33 and histological study of pancreas. Diminution of catalase and peroxidase antioxidant enzyme activities and elevation of CD and TBARS levels in hepatic tissue in diabetic rats also supported by ³⁴. Administration of extract results significant restoration of antioxidant enzyme activities as well as CD and TBARS levels in hepatic tissue. These hydro-methanolic extract of leaf A. augusta has no general toxicity as body weight remain normal in extract treated diabetic group and there was no change in activities of SGOT and SGPT in extract treated group. From this discussion it may be concluded that the hydromethanolic extract of leaf of A. augusta has a significant protective effect against diabetes induced complications.

CONCLUSION: Hydro-methanolic extract of leaf of *A. augusta* have diabetic therapeutic efficacy by regeneration of pancreatic β -cell so the insulin level is increase that rectify fasting blood glucose level, glycated heamoglobin, carbohydrate metabolic enzymes and oxidative stress markers. Another mode of action may be that extract have antioxidant properties by which it may recover the diabetes induced oxidative stress. Further work is going on in our laboratory to search out the effective ingredient(s) present in the extract.

ACKNOWLEDGEMENT: Authors are great fully acknowledged the authority of Vidyasagar University for providing us infrastructural facilities for continuation of this research work.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:

Saha U, Ghosh C and Mallick C: Antihyperglycemic and antioxidative effect of hydro-methanolic extract of leaf of *Abroma agusta* in streptozotocin induced diabetic male albino rat. Int J Pharm Sci & Res 2018; 9(9): 3788-94. doi: 10.13040/JJPSR.0975-8232.9(9).3788-94.

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