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Anti-diabetic and antioxidant potential of *Buchanania axillaris* (Desr.) bark on Streptozotocin (STZ) induced Diabetes in rats.

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ABSTRACT

Diabetes mellitus (DM) is probably one of the oldest diseases known to man. Natural products and/or synthetic analogues of a natural lead show utmost importance in the treatment of diabetes-associated complications. The main objective is the evaluation of antidiabetic potential of *Buchanania axillaris* (Desr.) bark on Streptozotocin induced diabetes in rats. Diabetic rats were treated with *Buchanania axillaris* bark extract at a dose of 250 and 500 mg/kg orally of body weight for 28 days. Glimepiride was taken as a standard drug. After the 28 day treatment, the pancreas was assayed for antioxidant and histopathological studies. Serum glucose was examined on 15 and 28 days. Bodyweight, urine output, food and water intake, cholesterol, triglycerides, glycosylated hemoglobin, creatinine, urea, and total protein were also studied. After the 28 days study period, the maximum reduction in blood glucose level with *Buchanania axillaris* bark extract at a dose of 500 mg/kg in STZ induced diabetes in rats. There were significantly increased bodyweight, a decrease in urine output, glycosylated hemoglobin, cholesterol, triglycerides and creatinine level. However, it also influences the antioxidant parameters like reduction in TBARS and increases in GSH and CAT level. This study demonstrates that *Buchanania axillaris* (Desr.) bark extract has therapeutic value in diabetes and has antioxidant potential.

Key Words: Antidiabetic, *Buchanania axillaris*, Glimepiride, Antioxidant, Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is probably one of the oldest diseases known to man. It was first reported in the Egyptian manuscript about 3000 years ago [1]. It is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin, defective insulin action, or both [2]. World health organization has categorized DM as the 7th leading cause in the USA while it was estimated that 422 million adults present diabetes in 2014, 4 times higher than the recorded cases in 1980. The incidence of type-II DM varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factors [3].

Natural products and/or synthetic analogues of a natural lead show utmost importance in the treatment of diabetes-associated complications. Since the dawn of history, Indian traditional

practitioners have solely relied on different plant products for the treatment of diabetes and associated complications. Active components from various plants have been isolated, and some remain to be identified.

Buchanania axillaris (Desr.) is a member of family Anacardiaceae is a commercially useful tree species and well known for its medicinal and therapeutic values in Indian folk medicine. It is widely distributed in India and Srilanka. In India, it is distributed in Karnataka, Tamilnadu, Andhara Pradesh, Bihar, Chhattisgarh, Gujarat, Jharkhand, Madhya Pradesh, Maharashtra, Orissa, and Rajasthan and Varanasi and Mirzapur districts of Uttar Pradesh.

The plant is attributed with medicinal properties, bark shows antihelminthic activity in earthworms [4], leaves shows antioxidant and antibacterial [5], cardioprotective [6], aphrodisiac [7].

In combination with *Hemidesmus indicus* and *Rhus mysorensis*, they showed Dual therapy for Alzheimer's Disease (AD) and Diabetes mellitus in in-vitro experimentation [8].

In the light of the above background, the present study was designed to investigate the potential effect of *Buchanania axillaris* (Desr.) bark in the prevention of diabetes and its associated complications.

MATIERALS AND METHODS

Collection and preparation of plant extract

The bark of the plant *Buchanania axillaris* (B.A) Desr. was collected during November 2023 from local areas in Chhatarpur. The plant specimen was authenticated by Dr. Shashi Prabha Parihar, Department of Botany at Government Girls PG College, Chhatarpur, Madhya Pradesh, contained a voucher number Bot/087/2024.

The bark was shade dried and powdered coarsely with a mechanical grinder and stored in air tight container.

Preparation of extract: About 1 kg of coarsely powder drug was firstly extracted with petroleum ether (60-80 °C) for 48 hours and then the residue was extracted with 90% ethyl alcohol by using Soxhlet extraction apparatus for 48 hours. Then the solvent was completely removed under the reduced pressure and controlled temperature. The crude extract obtained was a dark red in color with the percentage age yield is 33% and stored in a refrigerator for further use. The dose of the bark was selected based on of literature [9].

Preliminary Phytochemical Screening

Preliminary phytochemical screening revealed the presence of alkaloids, glycosides, flavanoids. carbohydrates, saponins, terpenes, tannins, phenolic substances, and steroids.

Animals and Diet

Healthy adult Spargue Dawley rats (200-250g) of either sex were used for investigation and were procured from Animal house facility Daksh Institute of Pharmaceutical Science. They were housed in the polypropylene cages were kept not more than 3 in each cage; maintained under standard conditions (12:12h light: dark cycle; 25±3 ºC; 40-60 % humidity) and fed with standard rat pellet diet and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) [Protocol No.: IAEC-DIPS/2023/006 (PCL)] of the Institute under the guideline of Committee for the Control and Supervision on Experiments on Animals (CCSEA), Ministry of Environment & Forests, New Delhi.

Induction of experimental diabetes

Diabetes was induced by single intravenous injection of STZ (CDH, Delhi) at a dose of 55 mg/kg [10, 11], dissolved in fresh 0.1 M citrate buffer at pH 4.5. After STZ injections, the animals were returned to their cages and were provided with normal food and 5% glucose solution (during the first 48 h only) to minimize hypoglycemic shock. Only pre-selected diabetic animals with blood glucose levels higher than 250 mg/dL on the third day after STZ injection were used as diabetic animals for the experiments.

Experimental design

A total of 30 rats were used and divided randomly into five groups of six animals each in the present study. The treatment schedule was as follows:

Group 1: Normal untreated rats

Group 2: Diabetic control rats subjected to STZ (55 mg/kg)

Group 3: Diabetic rats treated with standard drug Glimepiride (10 mg/kg) for 28 days

Group 4: Diabetic rats treated with bark extract (250 mg/kg) for 28 days

Group 5: Diabetic rats treated with bark extract (500 mg/kg) for 28 days

Glimepiride was suspended in gum acacia and B.A in distilled water by oral route for daily administration for 28 days. Animals were kept individually in a metabolic cage for urine collection for 24 hrs to study the urine output after completion of the experiment. Blood samples were collected in plain tubes from anaesthetized animals via retro orbital plexus and serum was separated for the estimation of biochemical parameters. Finally, rats were sacrificed under deep ether anesthesia after overnight fasting, pancreas dissected out immediately. Subsequently, isolated organ was washed in ice-cold saline, patted dry, weighed and stored in formalin solution for further analysis.

Biochemical analysis

Serum parameters

Fasting glucose level, OGTT, cholesterol, triglycerides, total protein, creatinine and urea were analyzed as per the instruction of the manufacturer using commercial kits (ERBA Diagnostic Mannheim GmbH, Germany) in a semi autoanalyzer (Transasia Bio. Medical Ltd., Germany).

Tissue parameters

Pancreatic tissue was analyzed for the estimation of TBARS [8], GSH [7] and CAT activity [2].

Histopathology

The rats were sacrificed by deep anesthesia at the end of the experiment. Pancreas was removed, washed with cold saline and preserved in 10% formalin in buffered form and subsequently dehydrated in graded alcohol. After embedding in paraffin wax 4-6 µM sections were cut and mounted on aminosilane coated glass slides and stained with haematoxylin and eosin (H&E) dye. Coverslips were mounted with DPX. The tissues were examined under the microscope.

Statistical analysis

The data were expressed as mean±SEM. Statistical differences between groups were analyzed using one-way and two-way ANOVA followed by Dunnet's multiple comparison and Bonferroni test respectively by using Graph Pad Prism 5.0. A p-value of <0.05 was considered as statistically significant at a 95% confidence level.

RESULTS

Table 1: Effect of *B. axillaris* on body weight

Groups	**Initial weight (g)**	**Final weight (g)**
Normal control	252.000 ± 1.770	253.833 ± 2.400***
Diabetic control	253.000 ± 2.033	190.67 ± 4.020
Diabetic + Std. control (10 mg/kg)	239.667 ± 2.565	232.167 ± 2.93***
Diabetic + B.A (250 mg/kg)	238.833 ± 1.833	224.500 ± 1.648**
Diabetic + B.A (500 mg/kg)	239.667 ± 3.887	229.167 ± 2.613***

Values are expressed as mean±SEM, (n=6) , *** P<0.001, ** P<0.01 compared to the diabetic group, using Two way ANOVA followed by Bonferroni's test.

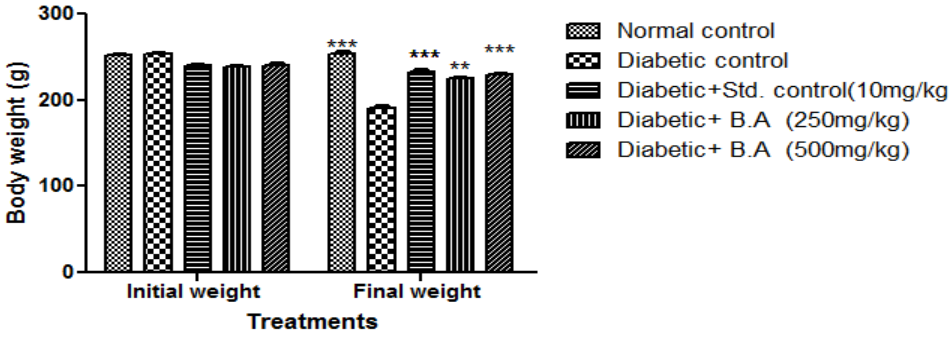

Fig 1: Effect of *B. axillaris* on body weight. On the 28th day test drug B.A shows significant result as compared to diabetic control group. "***" represents significant (P<0.001) and "**" represents (P<0.01) change as compared to diabetic control group.

Table 2: Effect of *B. axillaris* bark on urine output, food and water intake

Groups	**Urine output (ml/rat/day)**	**Water intake (ml/rat/day)**	**Food intake (g/rat/day)**
Normal control	8.417 ± 0.900***	67.833 ± 2.600***	21.333 ± 0.955***
Diabetic control	33.117 ± 1.317	133.333 ± 2.929	41.667 ± 1.085
Diabetic + Std. control (10 mg/kg)	22.967 ± 0.448***	85.500 ± 5.812***	27.833 ± 1.682***
Diabetic + B.A (250 mg/kg)	26.067 ± 0.336***	97.333 ± 5.371***	35.167 ± 0.703**
Diabetic + B.A (500 mg/kg)	24.350 ± 0.319 ***	92.167 ± 5.747 ***	32.167 ± 0.792***

Values are expressed as mean±SEM, (n=6), *** P<0.001 compared to the diabetic group, using One way ANOVA followed by Dunnet's multiple comparison test.

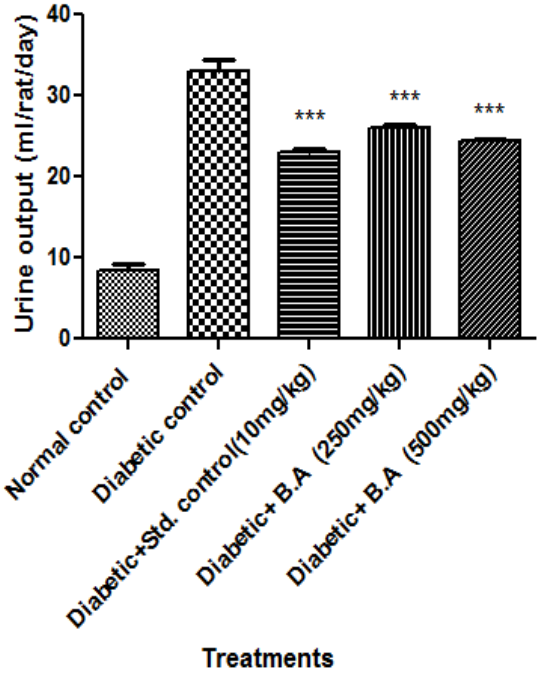

Fig 2: Effect of *B. axillaris* on urine output.

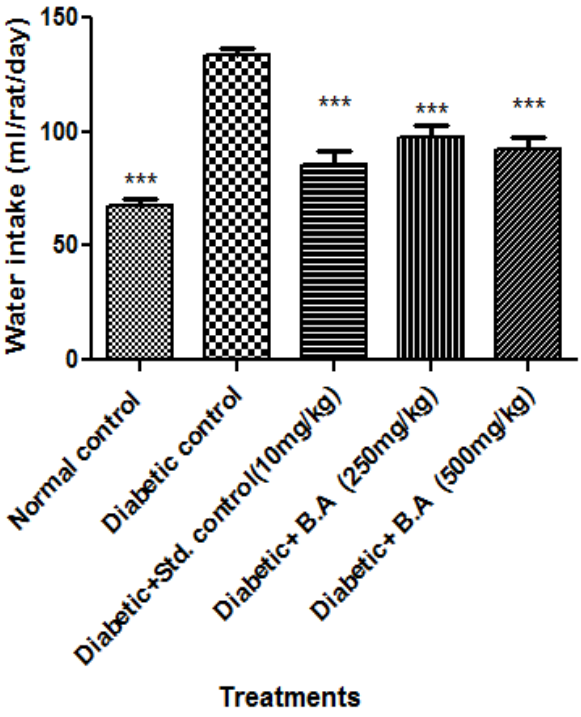

Fig 3: Effect of *B. axillaris* on water intake

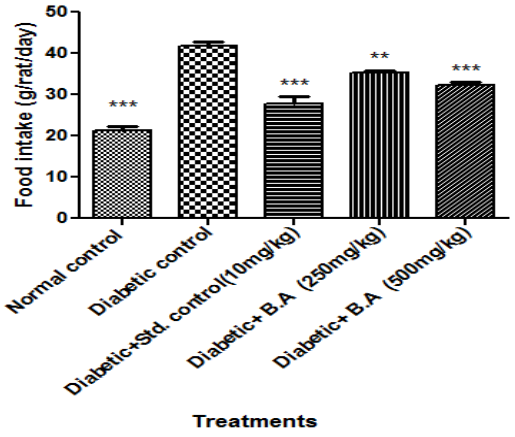

Fig 4: Effect of *B. axillaris* on food intake

Above figures shows the effect of B.A on urine output, water intake and food intake. At the 28th day test drug B.A shows significant result as compared to diabetic control group. "***" represents significant (P<0.001) and "**" represents (P<0.01) change as compared to diabetic control group.

Table 3: Effect of *B. axillaris* bark on fasting blood glucose

Groups	**0th day (mg/dl)**	**15th day (mg/dl)**	**28th day (mg/dl)**
Normal control	77.842 ± 2.67***	77.393 ± 1.905***	77.840 ± 1.620***
Diabetic control	290.400 ± 5.63	297.533 ± 4.149	297.550 ± 3.715
Diabetic + Std. control (10 mg/kg)	273.133 ± 5.673**	147.300 ± 4.197**	96.215 ± 4.812***
Diabetic + B.A (250 mg/kg)	274.067 ± 4.062***	157.917 ± 2.652***	112.213 ± 2.331***
Diabetic + B.A (500 mg/kg)	266.683 ± 5.092***	155.433 ± 2.113***	104.233 ± 2.055***

Values are expressed as mean±SEM, (n=6) , *** P<0.001, ** P<0.01 compared to the diabetic group, using Two way ANOVA followed by Bonferroni's test.

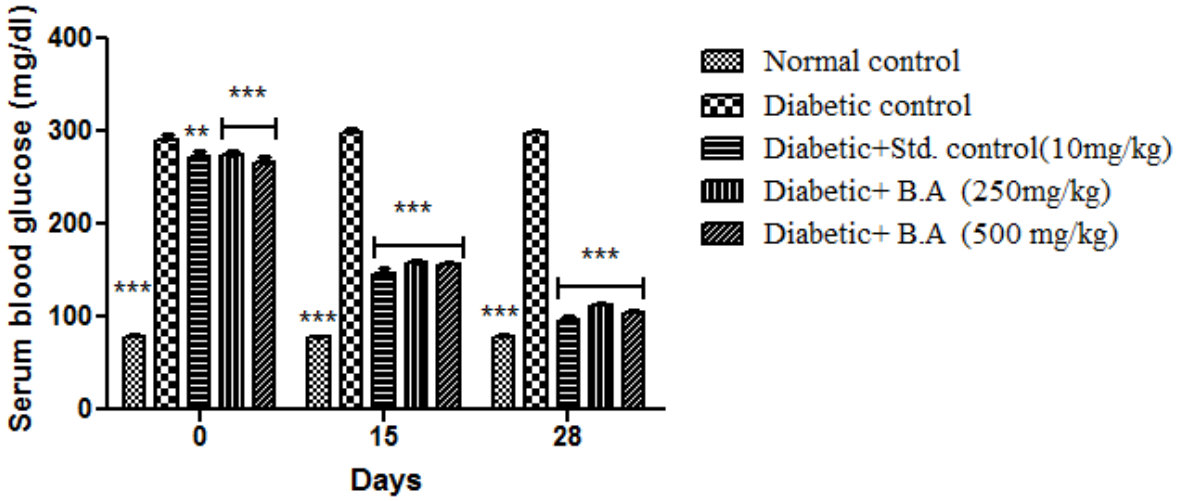

Fig 5: Effect of B.A on Fasting blood glucose. On the 28th day test drug B.A shows significant result as compared to diabetic control group. "***" represents significant (P<0.001) and "**" represents (P<0.01) change as compared to diabetic control group.

Table 4: Effect of *B.axillaris* on oral glucose tolerance test

Groups	**Blood glucose level (mg/dl)**				
	0 min	**30 min**	**60 min**	**120 min**	**180 min**
Normal control	78.50 ±2.47	139.16 ± 10.00	155.00 ± 4.66	123.66 ± 4.89***	83.00 ± 2.25***
Diabetic control	293.50 ± 2.84	315.33 ± 5.60	294.33 ± 2.45	288.53± 2.69	275.00 ± 3.01
Diabetic + Std. control (10 mg/kg)	95.66 ± 4.73	151.33 ± 3.82***	147.66 ± 3.83***	134.33 ± 5.51***	93.66 ± 4.26***
Diabetic + B.A (250 mg/kg)	108.16 ± 2.67	185.56 ± 2.97***	188.16 ± 4.37***	162.50 ± 2.17***	111.66 ± 2.66***
Diabetic + B.A (500 mg/kg)	103.00 ± 2.39	179.33 ± 3.29***	183.66 ± 3.19***	158.50 ± 3.54***	107.66 ± 2.32***

Values are expressed as mean±SEM, (n=6), *** P<0.001 compared to the diabetic group, using Two way ANOVA followed by Bonferroni's test.

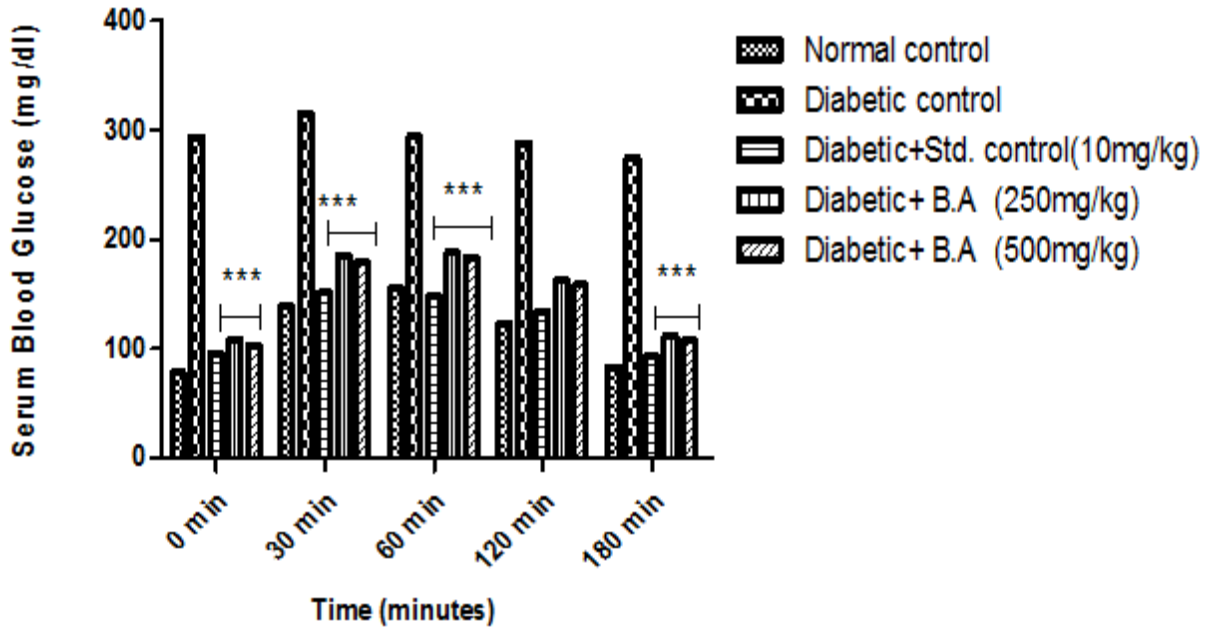

Fig 6: Effect of B.A on OGTT. On the 28th day test drug B.A shows significant result as compared to diabetic control group. "***" represents significant (P<0.001) change as compared to diabetic control group.

Table 5: Effect of B. axillaris on glycosylated Hb, cholesterol, triglycerides

Groups	**HbA1c (%age)**	**Cholesterol (mg/dl)**	**Triglycerides (mg/c**
Normal control	4.250 ± 0.05	60.323 ± 1.545	57.988 ± 2.158
Diabetic control	7.350 ± 0.05	92.110 ± 4.349	94.340 ± 1.953
Diabetic + Std. control (10mg/kg)	4.550 ± 0.05***	59.598 ± 1.915***	59.862 ± 1.130***
Diabetic + B.A (250mg/kg)	5.050 ± 0.15***	68.217 ± 2.912**	73.468 ± 1.529**
Diabetic + B.A (500mg/kg)	4.850 ± 0.05***	65.908 ± 2.466***	71.308 ± 1.905***

Values are expressed as mean±SEM, (n=6), *** P<0.001 compared to the diabetic group, using One way ANOVA followed by Dunnet's multiple comparison test.

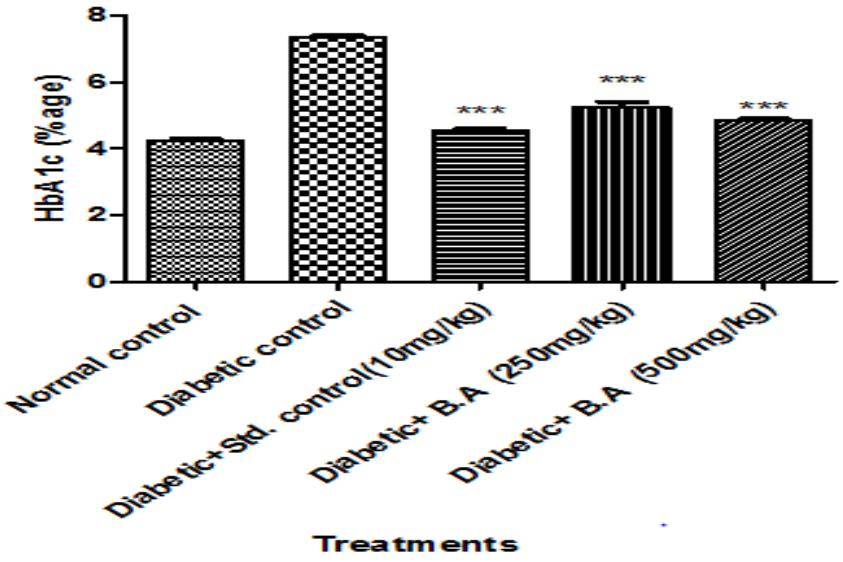

Fig 7: Effect of B.A on Glycosylated Hb. At the 28th day test drug B.A shows significant result as compared to diabetic control group. "***" represents significant (P<0.001) change as compared to diabetic control group.

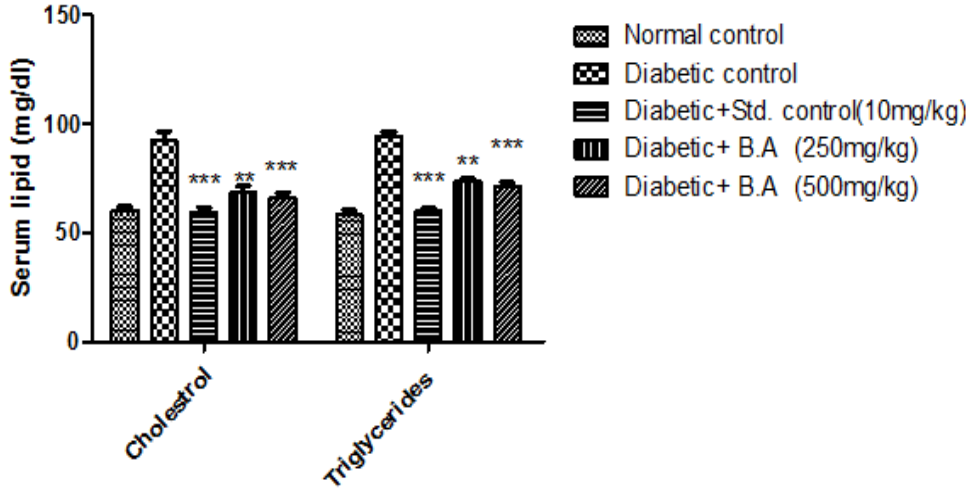

Fig 8: Effect of B.A on lipid profile. On the 28th day test drug B.A shows significant result as compared to diabetic control group. "***" represents significant (P<0.001) and "**" represents (P<0.01) change as compared to diabetic control group.

Table 6: Effect of *B. axillaris* on creatinine, urea and total protein

Groups	**Creatinine (mg/dl)**	**Urea (mg/dl)**	**Total protein (g/dl)**
Normal control	0.910 ± 0.048	24.820 ± 1.151	7.033 ± 0.334***
Diabetic control	6.442 ± 0.782	54.913 ± 2.740	3.052 ± 0.216
Diabetic + Std. control (10mg/kg)	1.203 ± 0.256***	28.607 ± 0.933***	5.858 ± 0.196***
Diabetic + B.A (250mg/kg)	2.980 ± 0.191***	37.232 ± 1.299***	4.935 ± 0.309***
Diabetic + B.A (500mg/kg)	2.287 ± 0.257***	34.045 ± 1.242***	5.312 ± 0.143***

Values are expressed as mean±SEM, (n=6), *** P<0.001 compared to the diabetic group, using One way ANOVA followed by Dunnet's multiple comparison test.

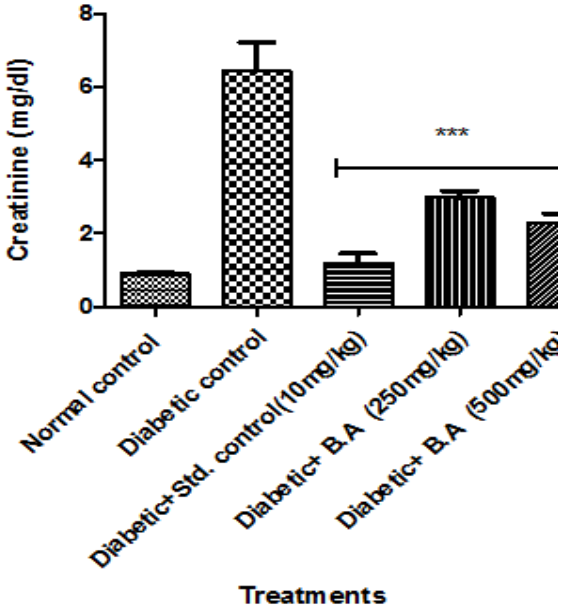

Fig 9: Effect of *B. axillaris* on creatinine

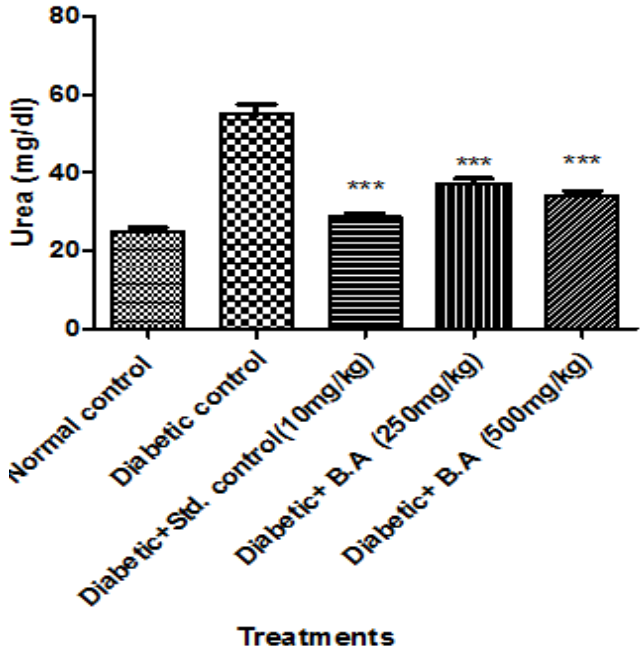

Fig 10: Effect of *B. axillaris* on urea

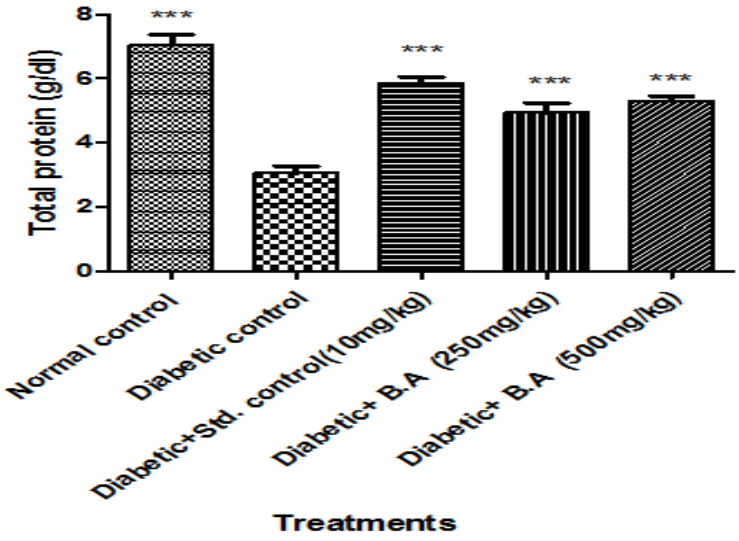

Fig 11: Effect of *B. axillaris* on total protein

Above figures shows the effect of B.A creatinine, urea and total protein. At the 28th day test drug B.A shows significant result as compared to diabetic control group. "***" represents significant ($P<0.001$) change as compared to diabetic control group.

Table 7: Effect of *B. axillaris* on TBARS, GSH and CAT

Groups	**TBARS (mM)**	**GSH (µmol/g)**	**CAT (µg/mg)**
Normal control	0.655 ± 0.035***	8.695 ± 0.125	42.295 ± 0.345
Diabetic control	1.895 ± 0.005	2.260 ± 0.080	18.950 ± 0.280
Diabetic + Std. control (10 mg/kg)	0.780 ± 0.040***	7.370 ± 0.160***	37.830 ± 0.590***
Diabetic + B.A (250mg/kg)	1.375 ± 0.035***	4.845 ± 0.125***	33.050 ± 0.470***
Diabetic + B.A (500mg/kg)	1.140 ± 0.020***	5,790 ± 0.100***	36.490 ± 0.000***

Values are expressed as mean±SEM, (n=6), *** P<0.001 compared to the diabetic group, using One way ANOVA followed by Dunnet's multiple comparison test.

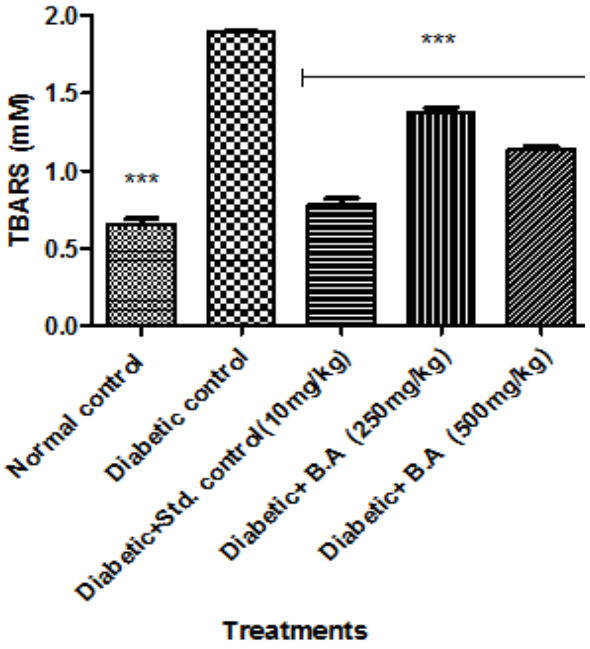

Fig 12: Effect of *B. axillaris* on TBARS

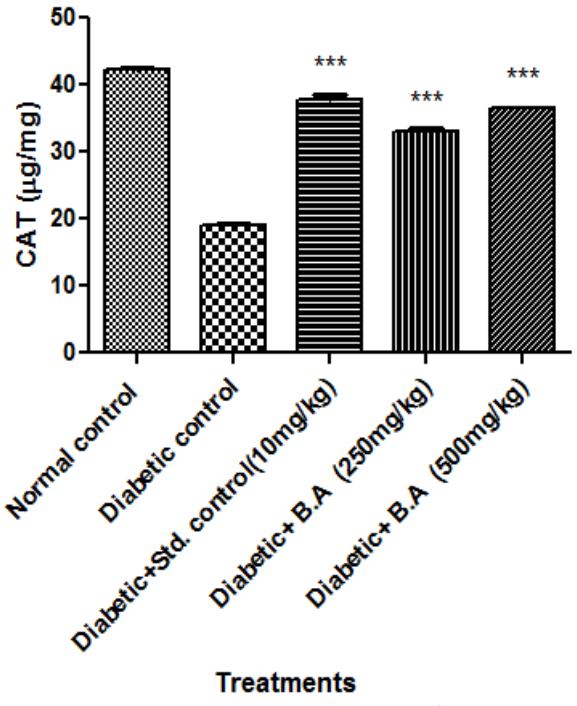

Fig 13: Effect of *B. axillaris* on CAT

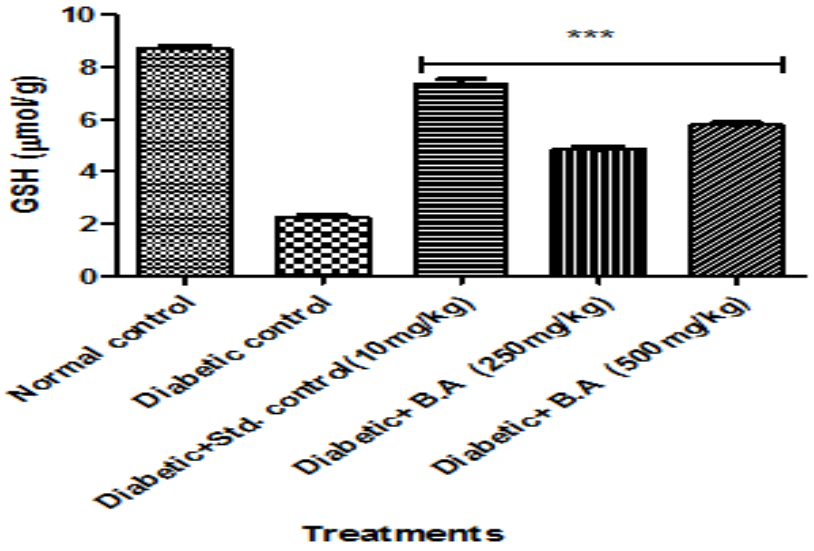

Fig 14: Effect of *B. axillaris* on CAT.

Above figures shows that, test drug B.A shows significant result as compared to diabetic control group. "***" represents significant (P<0.001) change as compared to diabetic control group.

HISTOPATHOLOGY OF PANCREAS

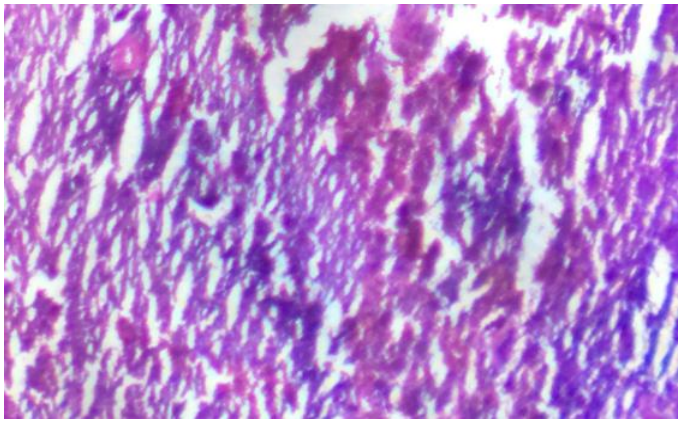

Fig 15: A photomicrograph of rat pancreatic tissue of the normal group showing islet appeared lightly stained in the surrounding cells (H&E, 45X)

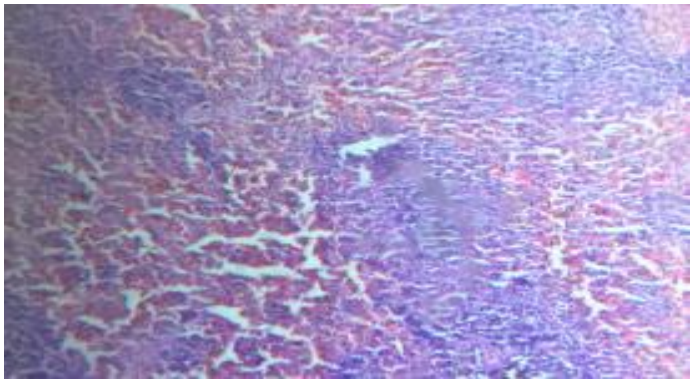

Fig 16: A photomicrograph of rat pancreatic tissue of the diabetic control group showing pathological changes, degeneration and necrosis in cellular components and inflammatory cells are also appeared. The β-cells almost lost in STZ induced rats (H&E, 45X).

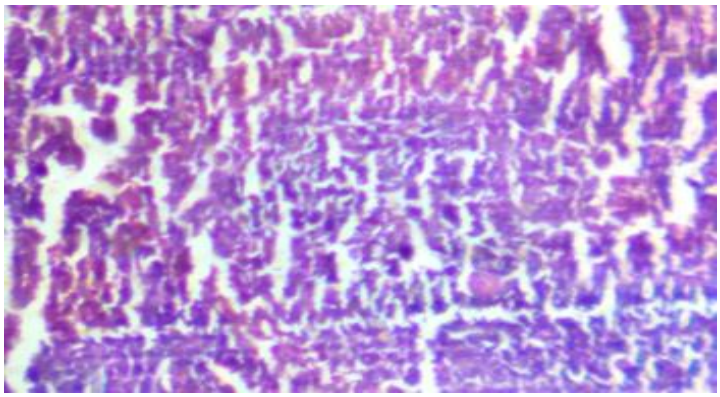

Fig 17: A photomicrograph of rat pancreatic tissue in standard control group showing a wider intralobular duct inside the cells and islets appear normal (H&E, 45X).

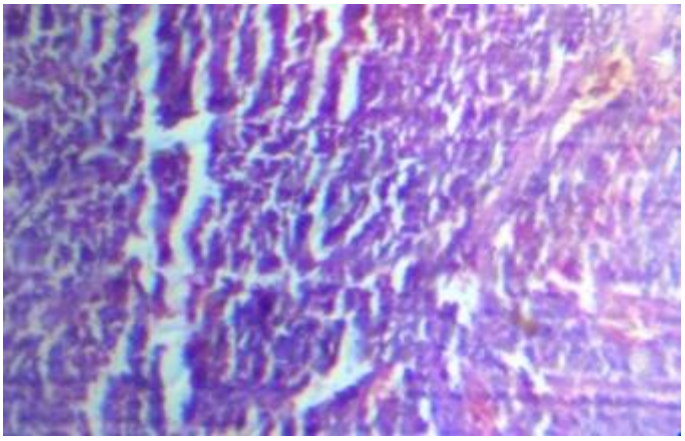

Fig 18: A photomicrograph of rat pancreatic tissue in the B.A (250 mg/kg) treated group showing wider interlobular and intralobular duct with the regeneration of islets (H&E, 45X).

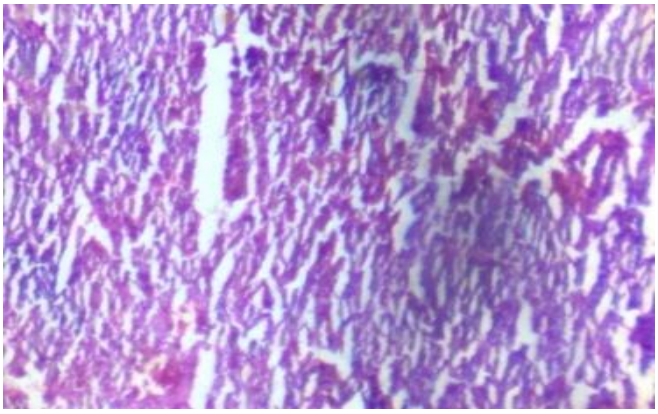

Fig 19: A photomicrograph of rat pancreatic tissue in the B.A (500 mg/kg) treated group showing regeneration of islets with the increase in the cell density and islet appear structure nearly normal (H&E, 45X).

DISCUSSION

Experimental diabetes mellitus has been induced in laboratory animals by various methods that have been used to analyze the hormonal, biochemical and morphological changes during the disease period [12].

It is well stated that STZ destroys β- cells of the pancreas and causes hyperglycemia in rats. It is commonly employed for the induction of type I and type II diabetes. Hyperglycemia is also responsible for oxidative stress and produces reactive oxygen species. These ROS helps in the development of various diabetic complications.

In the 28 day experimental period, alcoholic extract of *Buchanania axillaris* Desr. Bark shows a significant reduction in blood glucose. Glimepiride was taken as a reference drug which shows a significant reduction in blood glucose level in dose-dependent manner. The extract treated diabetic rats show a decrease in blood glucose level may be due to the presence of phytochemicals like flavanoids, carbohydrates, alkaloids, steroids, proteins and glycosides.

The present study showed an elevated level of lipids and other biochemical parameters in diabetic rats. The significant control in the level of serum lipids and other parameters in the extract treated group. STZ also causes weight loss in the diabetic group but there is also a reversal of weight loss in the extract treated diabetic rats.

Free radical generation plays a major role in the progression of the disease [13]. STZ produces oxygen radicals in the body which causes lipid peroxide mediated pancreatic injury. The alcoholic extract of B.A was found to decrease the lipid peroxide level in the pancreas of extract treated rats,

which can be determined by the level of TBARS, GSH and CAT. There is also a reduction in the glycosylated Hb level as compared to the diabetic control group.

From histopathology studies, it has also been seen that regeneration of islets in extract treated group and also a reduction in inflammatory cells.

The optimum dose of B.A gives a prominent effect and shows maximum efficacy in different biochemical parameters. There was no toxic effect seen during the 28 day study period. So, *B. axillaris* showed hypoglycemic, hypolipidemic and antioxidant property and minimizing the risk factors which are associated with diabetes.

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