Original Article

Efficacy of *Abroma augusta* on Biochemical and Histomorphological Features of Alloxan-Induced Diabetic Rabbits

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ABSTRACT

Background and Objectives: **Medicinal plants are well documented for possessing antidiabetic or antihyperglycaemic potential. *Abroma augusta* seems to have the same effect in treating diabetes mellitus. The aim of the present experiment was to report the efficacy of the aqueous extract of the leaves of *A. augusta* on biochemical and histological abnormalities of alloxan-induced diabetic rabbits.**

Materials and Methods: **An experiment was conducted to study the effect of aqueous extract of the leaves of *A. augusta* on biochemical values and histomorphological features of pancreas and kidneys of alloxan-induced (@ 80 mg/kg. body weight intraperitoneally) diabetic rabbits. The animals were divided into three groups viz, normal control, diabetic control and *A. augusta* treated diabetic rabbits. The extract was given by gavages at a dose of 2ml/kg body weight twice daily for a period of 21 days.**

Result: **Normal blood sugar ($P<0.01$), blood urea ($P<0.01$) and serum creatinine ($P<0.02$) levels were observed. Histological examination of the pancreas showed an increase in the number of beta cells in the *A. augusta* treated diabetic rabbits. Further, the extract was found to ameliorate the histological abnormalities of the kidneys in diabetic rabbits.**

Conclusion: **This experimental study indicates that *A. augusta* extract possess either antioxidant effect or regenerative ability in ameliorating the biochemical and histomorphological abnormalities in diabetic rabbits.**

***Keywords: Abroma augusta*, Diabetes mellitus, Rabbit**

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Introduction

In experimental diabetes the use of herbal medicine is widespread. More than 400 traditional plant treatments for diabetes mellitus have been recorded, but only small members of these have received scientific and medical evaluation to assess efficiency (1, 2). There are various medicinal plants in the world, which are the potential sources of the drugs and most of the herbs are reported to possess some degree of antidiabetic activity (3).

Abroma augusta has been used for the treatment of diabetes mellitus. Studies have shown that Abromine, the active constituent of the *A. augusta* identified as betaine is responsible for antihyperglycemic activity (4,5). The leaves contain octacosanol, taraxerol, β-sitosterol acetate, Lupeol, an aliphatic alcohol (C32H66O) and mixture of long chain fatty diols (5, 6).

According to Waguri *et al*. (7), the beta cell can present two types of regeneration: differentiation of the precursor cells from the pancreatic duct, or proliferation from existing or surviving mature beta cells.

The present experiment was designed to study the efficacy of the aqueous extract of the leaves of *A. augusta* on blood sugar, blood urea, serum creatinine and the subsequent regeneration of beta cells and kidney cells in alloxan-induced diabetic rabbits.

Materials and Methods

Experimental animals: New Zealand male white rabbits of almost uniform age were selected for the present study. The animals were fed on green vegetables and commercial pelleted diet, and received human care according to the guidelines outlined in the "Guidelines for the Care and Use of Animals in Scientific Research" prepared by the Indian Science Academy, New Delhi (8).

Induction of diabetes: The methods of Rastogi *et al*. (9) and Baqui *et al*. (10) were used for induction of diabetes mellitus in rabbits. The animals were made diabetic by intraperitoneal administration of alloxan (@ 80 mg/kg. body weight) dissolved in 0.1M freshly prepared citrate buffer (pH 4.5) following twelve hours fasting. An equal volume of isotonic saline without alloxan in similar manner was given to control rabbits. Fasting blood sugar level was checked at weekly intervals along with blood urea and serum creatinine levels for confirmation of diabetes mellitus. Animals with blood sugar more than 250mg/dl were used for the experiment. Treatment was started after seven weeks when diabetes was well established.

Plant material: The method of Islam *et al*. (11) was followed for preparing the plant extract. The leaves of *A. augusta* used in the present study were purchased commercially. After identification the dried leaves were powdered (25g) and extracted by soaking in 100ml of distilled water for two days at 40C with frequent stirring. The aqueous extract was kept airtight in a freeze until use.

Experimental Procedure

The animals were divided into three groups of six each.

Group I: Normal untreated rabbits

Group II: Diabetic control rabbits given 2 ml of saline solution orally for 21 days.

Group III: Diabetic rabbits given 2ml of aqueous extract of the leaves of *Abroma augusta* twice daily for a period of 21 days.

Biochemical parameters viz. blood glucose (Fasting) blood urea and serum creatinine were estimated on day 7th, 14th and 21st of treatment. At the end of 21 days the rabbits were sacrificed for histological examination of pancreas and kidneys.

Analytical Procedure

The blood sugar of rabbits was estimated by Glucometer Gx (Bayer Diagnostic India, Ltd.), blood urea by "DAM Method" and serum

creatinine by 'Alkaline Picrate Method" using commercially available kits. Histological examination was done by fixing pancreas and kidneys of rabbits in 10% formalin, processed and embedded in paraffin wax. Tissue blocks were sectioned 5 micron thick and stained with Harris Haematoxylin and Eosin (12). However, to demonstrate pancreatic islet cells, Modified Aldehyde Fuchsin stain (13) was used with a modification of substituting the lugol's iodine by equal parts of 0.5% KMNO4 and 0.5% sulphuric acid, and sodium thiosulphate by 2% sodium bisulphite respectively (14).

Statistical Analysis

All values were expressed as mean and analyzed using students 'T' test (15). '*P*' value was obtained from the distribution of '*t*' probability chart.

Results

The induction of diabetes mellitus by intraperitoneal administration of alloxan (@ 80mg/kg. body weight) was observed after first week by increased values of blood sugar (F), blood urea and serum creatinine. The blood sugar (F), blood urea and serum creatinine levels of alloxan-induced rabbits increased steadily upto sixth week reaching 292 ± 10.60 mg/dl, 53 ± 2.11 mg/dl and 3.32 ± 0.16 mg/dl respectively compared to the values of control rabbits which remained almost constant for these parameters till the end of the experiment.

An improvement in blood sugar, blood urea and serum creatinine in Group III rabbits following *A. augusta* treatment was observed in contrast to Group II rabbits (Table 1). The blood sugar of Group III rabbits decreased steadily up to 21st day reaching 111 ± 4.43 mg/dl in contrast to Group II rabbits. Further, a significant decrease of blood urea and serum creatinine from 49.75 ± 1.31 mg/dl and 3.10 ± 0.10 mg/dl to 22 ± 0.91 mg/dl and 1.75 ± 0.08 mg/dl respectively was observed up to 21st day in Group III rabbits in contrast to Group II rabbits.

Table 1- Effect of *A. augusta* on Blood Sugar (F), Blood Urea and Serum Creatinine of alloxan-induced diabetic rabbits

Factor Group	Initial Value			DAYS								
				7th			14th			21st		
	BS(F)	BU	SC	BS (F)	BU	SC	BS (F)	BU	SC	BS(F)	BU	SC
NC	94 ±6.06	19.5 ±0.64	1.25 ±0.20	99 ±5.95	19 ±1.46	1.05 ±0.20	105 ±4.66	21.03 ±1.58	1.29 ±0.25	103 ±3.19	19.75 ±0.84	1.21 ±0.04
DC	292 ±10.60	53 ±2.11	3.32 ±0.16	285 ±9.46	52.9 ±1.80	3.12 ±0.16	210 ±8.83	46.6 ±1.25	3.00 ±0.15	192 ±9.41	40.1 ±1.16	2.80 ±0.14
Abroma augusta treated	291 ±10.50	46.75 ±1.65	3.10 ±0.14	192 ±4.02	40 ±1.57	2.87 ±0.05	161 ±3.32	30.25 ±1.10	2.22 ±0.09	120 ±3.89**	24.75 ±0.62**	1.93 ±0.13*

Values represent mean ± SEM; Each experiment was performed on a group of four rabbits; BS(F): Blood Sugar (Fasting); BU: Blood Urea; SC: Serum Creatinine; NC: Non-diabetic control rabbits treated with normal saline; DC: Diabetic rabbits (Alloxanized) treated with normal saline; *$P < 0.02$, **$P < 0.01$, compared with DC.

Histomorphological Study

Pancreatic sections stained with Modified Aldehyde Fuchsin showed nuclear changes, karyolysis, disappearing of nucleus, rarefaction of nuclear contents and reduction in the number of beta cells (3.58%) in Group II rabbits in comparison to Group III rabbits where the number of beta cells (31.69%) was significant and almost comparable to Group I rabbits (63.5%).

H&E stained sections of kidneys of Group III rabbits showed amelioration of histomorphological changes in contrast to Group II rabbits where degenerative changes in cortex, subcapsular region, collecting tubules and tubular epithelium were observed.

Discussion

Alloxan-induced diabetes mellitus in rabbits was confirmed by elevated blood sugar (F) level on first week after intraperitoneal administration of alloxan, followed by persistent hyperglycemia during the entire period of the experiment. Keen and NgTang (16) reported that the minimum defining characteristic feature to identify diabetes mellitus is chronic and substantiated elevation of circulating glucose concentration. Establishment of diabetes mellitus in rabbits in the present study, induced by alloxan administration, might be attributable to specific irreversible toxic effects of alloxan on β cells of pancreas (17, 18). Fisher and Herman (19) reported that alloxan is rapidly reduced in the body forming dialuric acid, that undergoes auto-oxidation yielding detectable amounts of hydrogen peroxide, superoxide anion $(^{-}\cdot O_2)$ and hydroxyl free radicals $(^{-}\cdot H_2)$; the latter being produced by metal catalyzed Haber-Weiss reaction. These reduced species of oxygen particularly the extremely reactive OH radical, are believed to initiate alloxan based attack on β cells. The deleterious effects of alloxan causing hyperglycemia might be due to rapid inhibition of insulin secretary mechanism (20).

The normoglycemia in the rabbits observed in the present study by the administration of aqueous extract of the leaves of *A. augusta* might be due to the increased uptake of glucose peripherally and increased sensitivity of insulin (21). In a number of studies the antihyperglycemic activities of the *A. augusta* either alone or in combination with other drugs have been reported (5, 22). Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances (23), some may inhibit insulinas activity (24) and others may increase beta cells in the pancreas by activating regeneration of these cells (25, 26). Other studies have reported that administration of herbal products block the absorption of sugar molecules in the intestine and improve the body's ability to use sugar which would help to reduce blood sugar levels (27).

The significant increase in the number of beta cells in the islets of Langerhan's by the application of aqueous extract of the leaves of *A. augusta* in comparison to saline treated diabetic rabbits can be attributed to the regenerative effect of plants on pancreatic tissue (25, 26, 28). Increase in pancreatic beta cells mass may result from mitotic proliferation of pre-existing islet cells, or islets may bud off from the ductal system of the pancreas (29), or arise from transformation of the acini into new islets, or may even be derived from the centro-acinar cells (30). There is strong evidence that islet stem cells may exist in the pancreatic duct and that these ductal epithelial cells may be switched into a proliferative/regenerative phase leading to nesideoblastosis (neogenesis of islets) (31, 32). Lipsett and Finegood (33) reported beta cell neoformation from precursor cells in the pancreatic duct of diabetic animals. Schossler *et al*., (34) reported the regeneration of insulin producing cells in the pancreatic duct wall of *Syzgium cumini* treated alloxan-induced diabetic rats. Chakravarthy (28) reported that *Pterocarpus marsupium* Roxb. acts as hypoglycemic agent by a selective regeneration of beta cells of alloxan damaged pancreas and that its presence can protect the beta cells against the necrotic effect of subsequently administered alloxan. Such

evidences corroborate the suggestion that the aqueous extract of the leaves of *A. augusta* used in the present study possess the chemical substances that stimulate precursor cell differentiation causing regeneration of beta cells.

In the present study the improvement in blood urea and serum creatinine of diabetic rabbits following treatment therapy can be attributed to the recovery of renal function (35), which is explained by the regenerative capability of the renal tubules (36). The restoration of histopathological changes in diabetic rats has also been shown using herbal treatments (37). Good metabolic control is beneficial in slowing the progression of nephropathy in diabetes, and if the duration of diabetes is prolonged before reinstitution of normoglycemia, nephropathy is not easily reversed (38, 39). Tedong *et al*., (35) have reported that the normoglycemia in diabetic rats with treatment therapies could ameliorate the glomerular and tubular lesions that characterize diabetic nephropathy and subsequently recover renal morphology and function.

Conclusion

Diabetes mellitus is a multiple disorder that is associated with the changes in biochemistry and histomorphology of the affected individuals. Medicinal plants have great potential in limiting the extent of damage to the diabetic subjects. A medicinal plant namely *A. augusta* has been found to possess antidiabetic effects by ameliorating the biochemical and histomorphological changes in alloxan-induced diabetic rabbits.

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