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ANTI DIABETIC EFFECT OF *ALSTONIA SCHOLARIS LINN* BARK IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is among the most common disorder in developed and developing countries, and the disease is increasing rapidly in most parts of the world. It has been estimated that up to one-third of patients with diabetes mellitus use some form of complementary and alternative medicine. *Alstonia scholaris* is a plant of family Apocynaceae and has a great medicinal importance. It is widely used by tribal people to treat various diseases and ailments. The present communication deals with the organoleptic and preliminary physico-phytochemical studies of the stem bark of the plant. The organoleptic study was done according to the W.H.O. guidelines for medicinal plants. *Alstonia scholaris* is a plant that has been used in popular medicine for the treatment of the diabetes. It is native to the Indian subcontinent, Indomalaya, Malaysia, and Australasia. This has been investigated based on amerolative properties of bioactive compounds of *Alstonia scholaris* stem bark extract up on alloxan induced diabetic rats. The blood glucose levels were increased significantly. Ethanolic stem bark extract of *A. scholaris* was given to the diabetic rats in daily dose of 450mg/ kg of body weight (21 days). In diabetic rats of blood glucose levels decreased highly significant ($p<0.005$). The reduction in blood glucose can be used as a marker in the evaluating the severity of diabetes.

INTRODUCTION:

For centuries, medicinal herbs have been used to treat all types of health maladies. In fact, modern medicine is essentially based on herbal medicine. Even today in the times of advanced technology and medical science still depend on plants for their healing. These medicinal plants consider as a rich source of ingredients which can be used in drug development and synthesis. Medicinal plants exhibit phytotherapeutic effects caused by biologically active compounds specific secondary metabolites.

The plants have been utilized for basic and curative health care since time immemorial. The use of plants as food and medicines started ever since man started life on the planet. The plant kingdom is a virtual goldmine of potential drug targets and other active drug molecules waiting to be discovered. During the last decade, use of traditional medicine has expanded globally and gained popularity. Plant based drugs are having a revived interest now-a-days because of awareness of deleterious effects of modern synthetic drugs. Natural products can play a

very crucial role in pharmaceutical industry as drug them or as drug carrier or bio-enhancers or excipients. The importance of herbal/plant medicines is well documented in Vedas, which proved to be the ancient literature. The properties of the plants and their remedies are given in detail and in fact Ayurveda is the very principle root for the emergence of Ancient medical science in India that gave origin to branches like Sushruta and Charka Samhita. In order to set up quality in production and products, research documentation is mandatory to supply to international requirements. By referring global standards and international pharmacopoeia like Herbal B.P, China, Japanese Herbal, Ayurvedic Formulary of India, WHO Guidelines on Herbal Medicines, this could be met with. If the Indian herbal industry, is to survive in the domestic and international markets steps have to be taken to establish a good quality control mechanism, for which the government should consider assisting the standardization of drugs to meet International requirements in the coming years. It is also necessary to integrate modern knowledge with traditional knowledge. The drugs and products of the industry are working on the scientifically defined techniques and explained with modern biological and chemical definitions and tools, and that alone will give a therapeutically active herbal original drug available for health care worldwide.

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis, with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Diabetes mellitus is represented by hyperglycemia, lipidaemia, and oxidative stress; it predisposes affected individuals to longterm complications affecting the eyes, skin, kidneys, nerves, and blood vessels. Diabetesis prevalent in all parts of the world and rapidly increasing worldwide. The estimated number of adults living with diabetes has soared to more than 371 million, having a prevalence of 8.3%. India has the more than 63 million of diabetic persons. Despite considerable progress in the treatment of diabetes by oral hypoglycaemic agents, search for newer drugs continues because the existing synthetic drugs have several limitationsand harmful effects. Therefore, managing diabetes without any side effects is still a challenging task for health care providers. Hence, the studies are being conducted for finding more efficient, safer, and less expensive hypoglycaemic agents. Herbal medicines have ever been used and claimed as antidiabetic agents but very less are available on commercially formulated forms. Ethnomedicine is a promising field of research in Kashmir, as the valley grows varied medicinal and aromatic plants including those used in curing various diseases. It has been reported that there are 220 medicinal plant species, belonging to 178 genera distributed over 77 families being used in Kashmir and there are many plants which are not being paid due attention. Medicinal plants have been used for years in daily life to treat diseases all over the world. Interest in medicinal plants reflects the recognition of the validity of many traditional claims regarding the value of natural products in healthcare. Numerous useful drugs have been discovered from higher plants by following up ethnomedical practices. *Alstonia scholaris* is an important medicinal plant in folklore medicine. The plant belongs to family Apocynaceae and is native to India. It grows throughout India, in deciduous and ever-green forests and in plains. The plant possesses valuable medicinal properties but most of the advantages are still confined to tribal areas because of raw knowledge and absence of proper scientific standardization. For the useful application of the plant parts in modern medicine, physico-chemical and phytochemical standardization is very important so that the medical benefits of the plant may be used properly and scientifically and reach to the larger populations of the world.

Plant *Alstonia scholaris* (*Linn.)* belonging to family apocynaceae is commonly known as Saptaparna. The bark is useful in malarial fevers, abdominal disorders, dyspepsia and in skin diseases5. The bark is bitter, astringent, digestive, laxative, anthelmintic, antipyretic, stomachic, cardiotonic and tonic. The bark extract has been reported to posses antiplasmodial, immunostimulant , anticancer effect and is also hepatoprotective. In Ayurveda, it is reported that the bark of the plant when soaked in water overnight, can reduce the blood glucose level after oral administration however

no much characterization of this activity has been done on scientific basis. We therefore subjected the ethanol extract of bark of *Alstonia scholaris L.* to preliminary phytochemical investigation which showed presence of alkaloids, tannins, flavonoids, saponins, glycosides and triterpenoids. The phytochemicals are indicative of its potential in the treatment of diabetes mellitus hence we undertook the present work to study the antidiabetic effect of the bark extract in healthy and alloxan diabetic rats with the objective to focus on mechanism underlying the activity.

Alstonia scholaris is known to be a rich source of alkaloids and there is interest among the scientist to use this for therapeutic purposes. Amongst the chemical classes present in medicinal plant species, alkaloids stand as a class of major importance in the development of newer drugs because alkaloids possess a great variety of chemical structures and have been identified as responsible for pharmacological properties of medicinal plants. However, of the large variety of the alkaloids (about 180 alkaloids) isolated, so far only few have been assessed for biological activities. Almost all the parts of plant (bark, flower, root) are found to contain active principles. The species A.scholaris is used in commercial formulation Ayush.

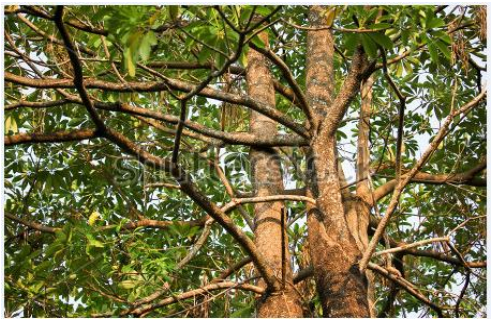

Fig. No. 1: *Alstonia scholaris* plant

Fig. No. 2: *Alstonia scholaris* stem

MATERIALS AND METHODS

Plant material:

Dried bark of *Alstonia scholaris* were procured from local market of medchal dist.

Preparation of extract

The stem bark of *A. scholaris* was collected, washed with running tap water, shade-dried at room temperature and grounded in a manual mill to get a coarse powder of 60 mesh. Powdered plant materials of *A. scholaris* was extracted with 80% ethanol in a soxhlet apparatus at 40 ^{0}C. Extraction was done with solvent until the supernatant in the soxhlet apparatus became transparent (for 48 hours). The extracts were filtered through a Buchner funnel with Whatman filter paper no. 1. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator at 40 ^{0}C. The crude extract was stored at 4 ^{0}C in airtight bottles until used in the diabetic studies.

Preliminary Phytochemical Screening:

The different chemical tests were performed for establishing profile of the extract for its chemical composition; the following chemical tests for various phytoconstituents in the ethanol extract was carried out as described below.

(A) Test for alkaloids:

i) Dragendroff's Test: In a test tube containing 1ml of extract, few drops of Dragendroff's reagent was added and the color developed was noticed. Appearance of orange color indicates the presence of alkaloids.

ii) Wagner's Test: To the extract, 2 ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicates the presence of alkaloids.

ii) Mayer's Test: To the extract, 2 ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

iv) Hager's Test: To the extract, 2 ml of Hager's reagent was added; the formation of yellow precipitate confirmed the presence of alkaloids.

(B) Test for terpenoids:

i) Salkowski test: To 1 ml of extract, tin (one bit) and thionyl chloride were added. Appearance of pink color indicates the presence of terpenoids.

ii) Hirshonn reaction: When the substance was heated with trichloroacetic acid, red to purple colour was observed.

(C)Test for steroids:

i) Liebermann Burchard Test: To 1ml of extract, 1ml of glacial acetic acid and 1ml of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution become red, then blue and finally bluish green indicates the presence of steroids.

(D)Test for coumarins: To 1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

(E)Test for tannins:

i) To few mg of extract, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

ii) The extract was mixed with basic lead acetate solution; formation of white precipitate indicated the presence of tannins.

(F)Test for saponins:

To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

(G)Test for flavones:

i) Shinoda Test: To the extract, a few magnesium turnings and 2 drops of concentrated hydrochloric acid were added,

ii) To the extract, 10% sodium hydroxide or ammonia was added; dark yellow color shows the presence of flavones.

(H) Test for Fixed Oils and Fats:

i) Spot Test: A small quantity of extract was pressed between two filter papers. Oil stains on the paper indicates the presence of fixed oils and fats.

Selection of experimental animals

The anti diabetic activity of ethanol extract of *Alstonia scholaris* bark was assessed in normal, glucose loaded and alloxan induced diabetic rats. In all studies, the animals were fasted overnight for 16 hrs with free access to water throughout the duration of the experiment. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions as per the guidelines of CPCSEA.

Methodology

1. Alloxan – induced diabetes mellitus

Thirty Male Wistar rats weighing 200-250gm were randomly divided into 4 groups of 5 each and kept in their cages for 10 days prior dosing to allow for acclimatization to the laboratory conditions. Group 1 served as normal control; group 2, 3, 4, received alloxan 10mg/kg/day subcutaneously for 10 days; on day 11, after overnight fasting, retro-orbital puncture was performed to obtain blood sample for estimation of lasting and postprandial blood sugar. Only those rats whose fasting and postprandial blood glucose levels were higher than those of the normal controls were utilized for further study. From day 11 to day 20, group 2, 3, 4, continued to receive alloxan 10mg/kg/day subcutaneously. Group 3 received 100mg/kg/day of *Alstonia scholaris* plant bark powder in 1ml of distilled water per oral, in addition to alloxan in addition to dexamethasone. Group 4 received Glibenclamide 500µg/kg per oral, in addition to alloxan. On the 20th day, after overnight fasting retro orbital puncture was done on the left eye to obtain blood for estimation of fasting blood glucose using autoanalyser. Immediately after this, a glucose load for estimation of postprandial blood glucose levels.

Group - 1: Administered vehicle serves as Normal control

Group- 2: Administered alloxan (10mg/kg s.c.) serves as diabetic control

Group- 3: Diabetic rats treated with alloxan (10mg/kg, s.c. once daily)

[Alloxan (10mg/kg, s.c.) + *Alstonia scholaris* 100mg/kg p.o.]

Group- 4: Administered Reference standard, Glibenclamide (500µg/kg, p.o. once daily) [Alloxan (10mg/kg, s.c.) + Glibenclamide (500µg/kg, p.o)]

Biochemical analysis

On the last day all the animals were weighed. Blood samples were collected and plasma and serum separated for estimation of glucose and triglyceride, respectively. Biochemical estimation of plasma glucose and serum triglyceride was done by glucose oxidase GOD/POD and glycerol-3-phospate oxidase (GOD)/PAD methods, respectively using standard diagnostic kits.

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM) and statistically analyzed by ANOVA followed by dunnett test, with level of significance set at $p<0.05$ and $p< 0.01$.

RESULTS

The organoleptic studies were done in fresh and moist stem bark strips of the plant *A. scholaris* as mentioned in Table 1. The outer surface of the bark was grayish brown in colour while the inner surface was yellowish brown. No specific odour was present in the bark. Surfaces were rough, spongy, unevenly fissured and exude milky sap when cut. Fracture was short and smooth. These characteristics are in agreement of some previous literature about the plant. The phytochemical screening of ethanol extract of bark was performed and reported in Table 2.

Diabetes is attributed to the diminished production of insulin or mounting resistance to its action. Chronic hyperglycemia during diabetes causes glycation of body proteins, which in turn leads to secondary complications affecting the eyes, kidneys, nervous system and arteries (Sharma & Misra, 1993). In this experiment aqueous alloxan monohydrate in acetate buffer (0.15 M, pH 4.5) injected by intraperitonial (i.p). Then the diabetic mice were used to assess the effects of *A. scholaris* bark extracts for a period of 21 days in alloxan –induced diabetic rats. These diabetic mice were treated after 48 hours of alloxan injection (i.p). In this period no detectable irritation or restlessness was observed after drug administration.

Blood glucose levels

The blood glucose levels decreased after feeding ethanolic bark extract of *A. Scholaris* was highly significant ($p<0.005$) which indicates this antihyperglycemic effect of the ethanolic extracts. And control rats are treated with bark extract showed the decrease in blood glucose level which indicates the hypoglycemic effect of the ethanolic extract (Table 3).

DISCUSSION

Diabetes mellitus patients in India are increasing day by day probably due to change in lifestyle, change in food pattern i.e. from traditional fiber rich diet to sugary fast food diet and also because of genetic basis. The disorder being chronic in nature needs long term treatment to prevent the complications arising due to persistent high blood glucose level. Pharmacotherapy available for the treatment of diabetes in modern healthcare system includes insulin and oral hypoglycemic drugs. However due to economic constraints, it is not possible for majority of the diabetic patients in developing countries like India to use these drugs on regular basis. Moreover these synthetic antidiabetic drugs are associated with large number of adverse effects. Hence there is increase in the trend to use traditional indigenous plants widely available in India for the treatment of diabetes mellitus. Over 150 plant extract and some of their active principles including flavonoids, tannins, alkaloids etc are used for the treatment of diabetes. However very few of these plants have been screened pharmacologically. Alloxan induced diabetes in experimental animals is a valuable model for induction of diabetes mellitus. Diabetes mellitus induced by alloxan may be due to pancreatic beta cell destruction. However the animals survived without insulin treatment and showed improvement by glibenclamide which act by stimulating residual beta cells of the pancreas indicate incomplete destruction of pancreatic beta cells of the diabetic rats in the present study. The model can be therefore be considered as poorly controlled type I diabetic model showing symptoms like hyperglycemia, glycosuria, polyuria, loss of body weight inspite of polyphagia. In the present study alloxan -diabetic rats exhibited significant increase in blood glucose level. Chronic treatment with aqueous extract of *Alstonia scholaris L.* bark reduced blood glucose level throughout the experimental period in duration dependent manner indicating its anti diabetic activity.

Table 1: Organoleptic evaluation of plant material

Features	Observation
Condition	Moist
Shape of Pieces	Flat Strips
Colour	Outer surface Grayish Brown, inner surface yellowish brown
Odour	No odour
Surface Texture	Rough, spongy
Fracture	Short and Smooth

Table no. 2: Preliminary phytochemical screening of *Alstonia scholaris* bark

Constituents	Ethanol extract
Terpenoids	++
Saponins	+
Steroids	-
Carbohyrates	++
Flavonoids	+
Alkaloids	++
Quinones	-
Tannins	+
Fixed oils and fats	-
Phenols	+

(++) - Strong Presence, (+) Present, (-) Absent

Table no. 3: Effect of administration of *Alstonia scholaris* bark extract (100 mg/kg, p.o) + glibenclamide (500 mg/kg, p.o) for 21 days on blood glucose levels in diabetic rats

Group	Treatment	Glucose level at 11th day	Glucose level at 16th day	Glucose level at 21st day
Group – 1	Control (Normal control)	106 ±1.62	99.86 ± 1.57	95.58 ± 1.33
Group – 2	Diabetic control	287.53 ±5.52*	274.93± 9.31**	270.6 ± 9.16**
Group – 3	*Alstonia scholaris* bark extract (100 mg/kg, p.o)	294.13 ±15.43*	199.73±36.50**	150.06±4.49**
Group – 4	glibenclamide (500 mg/kg, p.o) (reference standard)	285.43 ±5.49*	189.65±34.66**	145.58±5.49**

Value expressed in MEAN ±SEM, n=6 Experimental groups statically compared with control groups where significant *p<0.05, moderately significant **p<0.01. All the values are compared with the alloxan control group.

However blood glucose levels were not altered in normoglycemic rats further strengthening the antidiabetogenic potential of the extract. In diabetes mellitus, body cells are unable to utilize glucose as a source of energy due to which proteins are spared as energy source. In the present study alloxan diabetic rats show decrease in body weight throughout the experimental period. Oral treatment with aqueous extract of *Alstonia scholaris L.* bark significantly improved the body weight loss in diabetic rats as compared to diabetic control indicating possible role of the extract in restoration of protein metabolism. From our Studies it is evident that these bioactive compounds in group (or) individually from *A. scholaris* extract exerted potential mechanism of action with speculating against anti hyperglycemic activity. The *A. scholaris* bark extract, possesses stimulatory effect against

anti- diabetic activities in alloxan diabetic rats and showed a consistent effect on the alloxan induced changes in the blood sugar level and the β-cells population in the pancreas. It is conclude that the bark extract of *A. scholaris* extract exhibited significant anti-hyperglycemic activity and can be used for the treatment of insulin dependent diabetes mellitus**.**

CONCLUSION

The present study showed that ethanol extract of *Alstonia scholaris* significantly reduced elevated blood glucose level in alloxan diabetic rats without showing any hypoglycemic effect in normal rats. Since alloxan effectively destroys pancreatic beta cells and causes persistent hyperglycemia, the mechanism of action of *Alstonia scholaris* might involve actions other than pancreatic beta cells insulin release or secretion. The antidiabetic effect of the bark extract could be due to increased utilization of glucose by peripheral tissues, improved sensitivity of target tissues for insulin or it may be due to improved metabolic regulation of glucose. Thus the present study showed that bark of *Alstonia scholaris L.* possesses anti diabetic effect in alloxan diabetic rats.

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