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EVALUATION OF ANTI-DIABETIC ACTIVITY OF *POLYALTHIA LONGIFOLIA* EXTRACT IN RATS MODELS

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ABSTRACT

Herbal traditional plants are considered as resources of ingredients which can be used in drug development pharmacopoeial, non- pharmacopoeial or many types of synthetic drugs. *Polyalthia longifolia* is used by tribal people for treatment of diabetes. The aim of present study was to evaluate the potency of antdiabetic activity of leaves extract. The *P. longifolia* leaf extract was administered orally in Streptozotocin (STZ)-induced diabetic rats. When the extract was administrated, blood glucose levels were monitored at specific intervals and it was found that they were significant lowered. The effect of *P. longifolia* on induced hyperlipidemia was analyzed where the significantly lowered the elevated total cholesterol, triglycerides (TGL) and low density lipoprotein (LDL) level while increased the High density lipoprotein (HDL). Standard drug glibenclamide was used at a dose of 0.50 mg/kg body weight. Moreover, the extract treated rats exhibited the significant rise in serum insulin level compared with streptozotocin- induced diabetic rats. The present results showed that extract isolated from *Polyalthia longifolia* has significant antidiabetic activity in streptozotocin-induced rats compared to standard drug.

KEYWORDS

Antidiabetic, *Polyalthia longifolia*, Streptozotocin, Total cholesterol and Glibenclamide.

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INTRODUCTON

Diabetes is a disease in which are blood glucose, or blood sugar, levels are too high. Glucose comes from the foods are eat. *Diabetes* is a group of metabolic diseases in which there are high blood sugars levels over a prolonged period (Brown, 2013)[1]. High blood sugar symptoms are include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications (Kitabchi *et al.*, 2009)[2]. Acute complications can include diabetic ketoacidosis,

nonketotic hyperosmolar coma, or death (Ripsin *et al*., 2009)[3]. The long-term complications of Diabetes include heart disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes. The main reason of cause diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. There are three main types of *diabetes mellitus* (Santaguida *et al*., 2008)[4]. International Diabetes Federation documented that India has the highest number diabetic patient, and India is considered to be diabetes capital of the world. It is deliberated that the diabetes is the third most life-threatening disease whose death rate is just after cancer and cardiovascular disease. Now in prior basis, we applied effort to control the diabetes, because it is assuming that more than 400 million people of the world will be effected from diabetes by 2030 (Lin, *et al.,* 2007[5], Xiaoming, *et al.,* 2011)[6]. Today synthetic drug namely acarbose, miglitol and voglibiose, sulphonylureas, biguanides etc are used for the ailment of hyperglycemia. The synthetic drugs are associated with various side effects such as causing hypoglycemia at higher doses, dermatological reaction, liver problems, nausea and vomiting, generalized hypersensitivity reactions, lactic acidosis and diarrhea (Mukherjee, *et al.,* 2006[7], Sivagnanam, *et al.,* 2013)[8]. The secondary complication of synthetic drugs leads to limitation in its use; and creating major medical issues in management of diabetes. Hence, health-care professionals are considering alternative medicines system, comprising many herbal medicines for the ailment of diabetes. Now the demand of herbal drugs has increased because of their effectiveness, less side effects and relatively low cost. Therefore more researches are required to develop new antihyperglycemic agents with high efficiency and low toxicity from traditional medicines.

Polyalthia longifolia is a lofty evergreen tree, native to India, commonly planted due to its effectiveness in alleviating noise pollution. It exhibits the symmetrical pyramidal growth with willowy weeping pendulous branches and long narrow lanceolate leaves with undulate margins. The tree is known as Ashoka in Hindi and grows over 30 feet in height. The name of plant *Polyalthia* is derived from a combination of Greek words meaning 'many cures' with reference to the medicinal properties of the tree while *Longifolia*, in Latin, refers to the length of its leaves *Polyalthia longifolia* is sometimes incorrectly identified as the Ashoka tree because of the close resemblance of both trees. It as a tree with effectively no branches, but in fact a *Polyalthia* allowed growing naturally grows into a normal large tree with plenty of shade. The trunk of *Polyalthia longifolia* has grey bark. Trunk and the bark are used in manufacturing of fiber. Timber is used for making boxes, pencils and long masts that are why it is also known as the mast tree. India and Sri Lanka, where the mast tree is held in high esteem, its leaves are used in religious ceremonies and for decorating arches and doorways. Sometimes *Polyalthia longifolia* is incorrectly identified as Ashoka tree because of closely resembling leaves of both species. (Buenz *et al*., 2004)[9] other names of the mast tree are Asupala or the Buddha tree. In India and Sri Lanka, it is introduced in gardens in many tropical countries around the world. It is, for example, widely used in parts of Jakarta in Indonesia (Chen *et al.,* 2000)[10]. The leaves extract of *Polyalthia longifolia* was further analyzed for antihyperglycemic activity in Rats models.

MATERIAL AND METHODS

Collection and identification of plant material

The leaves of *Polyalthia longifolia* were collected from the local herbal garden of Chhattisgarh, India. The plant was authenticated by well-known botanist. After authentication, plant material was dried at room temperature until it was free from the moisture. Finally leaves were subjected to size reduction to get coarse powder.

Preparation of *Polyalthia longifolia* of hydro-ethanol extracts

The powder of the leaves of *Polyalthia longifolia*, was packed separately in the Soxhlet apparatus and extracted with water and ethanol, until the completion of the extraction. The extract was filtered while hot, and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, and later dried in a desiccators. After that hydro-ethanol extract of bark was kept in air tight container for further study.

The Effect of *Polyalthia longifolia extract on* Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed in overnight fasted (18 hours) normal rats. The experimental rats were divided into four groups (n = 6). Group I served as normal control rats, administered drinking water daily; Group II had glucose control rats; Group III rats were administered standard drug Glibenclamide (0.5 mg/kg); Group IV rats were administered leaves extract (50 mg/kg). Glucose (2 g/kg) was fed to rats of Group II to Group IV, 30 minutes prior to the administration of the extracts and standard drug. Blood was withdrawn from the retro-orbital sinus after 0, 30, and 90 minutes of extract and standard drug administration, and the plasma obtained after centrifugation at 3000 rpm was estimated for fasting plasma glucose levels using a glucose oxidase–peroxidase glucose estimation kit (Singh, *et al.,* 2011[11], Verma, *et. al.,* 2013)[12].

Induction of non-insulin dependent diabetes mellitus (NIDDM)

Non-insulin dependent diabetes mellitus was induced (Singh, *et al.,* 2011[11]; Verma, *et. al.,* 2013)[12] in overnight fasted adult Wistar strain albino male rats weighing 60-90 g by a single intraperitoneal injection, at the dose of 60 mg/kg Streptozotocin, 15 minutes after i.p. administration of 120 mg/kg of nicotinamide. Streptozotocin (STZ) was dissolved in a citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hours and then on day 7, after injection. The fasting plasma value of glucose to diagnose diabetes was taken as > 126 mg/dl. The rats which were found these values to have permanent NIDDM were used for the study.

Evaluation of antidiabetic activity of Polyalthia longifolia Extract

The experimental animals were divided into four groups of six rats each. The extract was administered for 28 days. Group I served as normal control rats, administered drinking water daily for 28 days; Group II had diabetic control rats, administered drinking water daily for 28 days; Group III diabetic rats were administered standard drug Glibenclamide (0.5 mg/kg) and Group IV diabetic rats were administered *Polyalthia longifolia leaves extract* (50 mg/kg) The fasting glucose levels were determined on days 0, 7th, 14th and 28th of extract administration. During the experimental period, the rats were weighed daily and the mean change in body weight was calculated (Singh, *et al.,* 2011)[11].

Estimation of biochemical parameters

Estimation of biochemical test was determined on day 12 after the animals were sacrificed by cervical dislocation. Total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), were determined by the glucose oxidase method, using an auto-analyzer (Singh, *et al.,* 2011)[11].

RESULTS

Oral glucose tolerance effects of *Polyalthia longifolia* Extract

The Oral glucose tolerance effects of *P. longifolia* extract on the plasma glucose level are shown in Table No.1. When the administrated of glucose in rats the rise in glucose level was observed in glucose control, extract treated and standard group. In rats treated with *P. longifolia* leaves extract, there was a significant reduction in plasma glucose level, while in glucose control rats the plasma glucose level increased. The same results were also observed in glibenclamide treated group.

Effect of *Polyalthia longifolia* Extract on non-insulin dependent diabetes mellitus

Showed the diabetes in experimental rats was confirmed by the presence of a high fasting plasma glucose level. Serum glucose levels of normal and Streptozotocin-induced rats by *Polyalthia longifolia* are shown in Table No.2. In the Group - II, animals treated with streptozotocin, a significant increase in serum glucose level was observed on 0, 7th, 14th and 28th day when compared with normal group rats (Group I). The glibenclamide (0.5 mg/kg p.o.) as group - III, showed significant decrease in serum glucose level when compared with diabetic control rats. After the oral administration of *Polyalthia longifolia* extract in diabetic control rats, a significant reduction in blood glucose level was observed when compared with diabetic control rats. After the administration of *Polyalthia longifolia* extract in diabetic control rats, also significantly

decreased the serum glucose level compared with diabetic control rats. The result showed that plant extract at dose of 50 mg/kg body weight significantly decreases the blood glucose level of diabetic rats on 7th day.

Anti-hyperlipidaemic activity of *Polyalthia longifolia Extract*

The results of lipid profiles in control and experimental rats are exhibited in Table No.3. The experimental animals of diabetic control showed significant increase in serum TGL, total cholesterol and LDL while increase in HDL when compared with normal. The groups treated with glibenclamide also reduced TGL, total cholesterol, LDL, and increased HDL when compared with diabetic control group. The *P. longifolia* extract showed significant decrease in total cholesterol, LDL, Triglycerides and significant increase in HDL when compared with diabetic control group. All these effects were observed on day 28th. The result indicated that plant extract exhibited a potent blood glucose lowering properties in STZ diabetic rats.

Effect on body weight by *Polyalthia longifolia Extract*

During the study, the body weights of rats before and after induction of diabetes, and after treatment were measured (Table No.4). The results exhibited that decreased in body weight of rats after induction of diabetes, and increased in body weight of rats after treatment with extract.

DISCUSSIONS AND SUMMARY

Streptozotocin, a monofunctional nitrosourea derivative, derives diabetogenic activity due to its ability to induce oxidative stress and damage in β-cells. Streptozotocin can selectively attack pancreatic β-cells by producing free radicals of oxygen, nitrogen monoxide, and reducing intracellular NAD and NADP, which are crucial for the electron delivery and energy metabolism in β-cells.

Traditional plant products are being used as a source of medicine since long. According to the World Health Organization, more than 80% of the world's population, mostly in poor and less developed countries depend on traditional plant-based medicines for their primary health care needs. Traditional herbal plants are considered as resources of ingredients which can be used in drug development pharmacopoeial, non-pharmacopoeial or synthetic drugs. Number of medicinal plants play a critical role in the development of human cultures around the whole world moreover, some plant are considered as important source of natural compound and they are useful for their therapeutic values. Traditionally many drugs stated to process anti-diabetic activity in the Ayurveda system of medicine of India (Kumar *et al*., 2011)[13]. The inorganic part of medicinal parts of medicinal plants containing mainly mineral, plays a contributory role in enhancing hypoglycemic activity and their indirect role in diabetes management is increasingly recognized. The antidiabetic potential of leave *Polyalthia longifolia* may to be due to the significant presence of Mg and Ca in the extract. *Polyalthia longifolia* is a large genus of shrub and trees distributed in tropics and subtropics. *Polyalthia longifolia* is a tall handsome, evergreen tree with a straight trunk and horizontal branches and is a native of Srilanka and cultivated all over Indo-Pakistan sub-continent (Annan *et al*., 2013)[14]. It is locally known ashok. Branches about 1-2 m long, glabrous, and pendulous. Leaves estipulate, distichous, mildly aromatic, 7.5-23 by 1.5-3.8 cm, shining, glabrous, narrowly lanceolate, tapering to a fine acuminate apex, margin markedly undulate, pinnately veined, leathery or sub coriaceous, shortly petiolate; petiole about 6 mm long. Flowers, non-fragrant, 2.5-3.5 cm across, yellowish to green, in fascicles or shortly pedunculated umbels; petals 6, 2 seriate, flat, from a broad base, lanceolate, long accumulate, spreading; and sepals 3, broad, short, triangular, the tips reflexed. Stamens many, cuneate; connective truncately dilated beyond the cells. Ovaries indefinite; ovules 1-2; style oblong Ripe fruits ovoid, 1.8-2 cm long, numerous, stalked, glabrous, 1 seeded; stalk 1.3 cm long, short, glabrous. Seeds smooth, shining. Flowering and fruiting: February-June (Yadav and Sardesai, 2000)[15].

Diabetes also called as blood sugar, is too high. Blood glucose is your main source of energy and comes from the food you eat. Pancreas produce the insulin hormone, helps glucose from food get into your cells to be used for energy. Sometime human body doesn't make enough or any insulin or doesn't

use insulin well. Due to glucose then stays in your blood and doesn't reach your cells. At the regional level, the number of people with diabetes is expected to increase by 71% between 2007 and 2025 (WHO). There are two main types of *diabetes mellitus*, Type 1 or insulin dependent *diabetes mellitus* (IDDM) and Type 2 or non-insulin dependent mellitus (NIDDM). Numbers of active compounds have been isolated from the plant and herb species of India. These active principles are alkaloid, amino acid, steroid, Saponins, flavonoids, and others. These have produced potent hypoglycemic, anti-hypoglycemic properties activities (Gabrielian *et al*., 2004)[16]. *Polyalthia longifolia* is abundant in leave and can easily isolate from the crude plant extracts as crystalline solid (Ahsan *et al*., 1996)[17].

The effects of *Polyalthia longifolia* leaves extract on the plasma glucose level are shown in Table No.1. After administration of glucose in rats the rise in glucose level was observed in glucose control, extract treated and standard group. In rats treated with leaves extract of *Polyalthia longifolia*, there was a significant reduction in plasma glucose level, while in glucose control rats the plasma glucose level increased. Meanwhile almost same results were observed in glibenclamide treated group.

Induction of diabetes in experimental rats was confirmed by the presence of a high fasting plasma glucose level. The effect of *Polyalthia longifolia extarct*, on serum glucose levels of normal and Streptozotocin-induced rats are shown in Table No.2. The animals treated with streptozotocin namely group II, a significant increase in serum glucose level was observed on 0, 7th, 14th and 28th day when compared with normal group rats (Group I). The group III received glibenclamide (0.5 mg/kg p.o.) showed significant decrease in serum glucose level when compared with diabetic control rats. After the oral administration of *Polyalthia longifolia* extract in diabetic control rats, a significant reduction in blood glucose level was observed when compared with diabetic control rats. Moreover the administration of extract in diabetic control rats, also significantly decreased the serum glucose level compared with diabetic control rats. The outcomes exhibited that plant extract at dose of 50 mg/kg body weight significantly decreases the blood glucose level of diabetic rats on 7th day.

The outcomes of lipid profiles in control and experimental rats are exhibited in Table No.3. The rats of diabetic control showed significant increase in serum TGL, total cholesterol and LDL while increase in HDL when compared with normal. The rat treated with glibenclamide also reduced TGL, total cholesterol, LDL, and increased HDL when compared with diabetic control group. The plant extract showed significant decrease in total cholesterol, LDL, Triglycerides and significant increase in HDL when compared with diabetic control group. All these effects were observed on day 28th. The present experimental result indicated that plant extract exhibited a potent blood glucose lowering properties in STZ diabetic rats.

During the study, the body weights of rats before and after induction of diabetes, and after treatment were measured (Table No.4). The results exhibited that decreased in body weight of rats after induction of diabetes, and increased in body weight of rats after treatment with extract. The leaf extract of *Polyalthia longifolia* produced glucose lowering activity. However, the extracts did not modify any of the biochemical parameter significantly (Nair, *et al.,* 2007)[18]. The antidiabetic effect of the extract may be due to the presence of one or more than one Antihyperglycemic principle and there synergistic properties *Polyalthia longifolia* contains flavonoid, steroid and polyphenols as the major bioactive components.

Thus, the significant antidiabetic activity of *Polyalthia longifolia* observed in the present study may be attributed to the presence of steroidal lactones in addition to glycaemic minerals. Since the extract is effective against STZ induced SD mice therefore it suggests that it suggests that it will be helpful for type 2 diabetic patients in controlling their BGL. Further investigations are underway to elucidate its impact on hyperlipidaemia caused by diabetes and mechanism of action responsible for it anti-diabetic effect.

Table No.1: The Effect of *Polyalthia longifolia* on Blood Glucose Levels in Diabetic Mice

S.No	Group	Plasma Glucose concentration (mg/dl)			
		Day 0	Day 6	Day 12	Day 18
1	Normal control	76.66 ±1.85	72.33 ±0.87	76.00 ±1.15	74.66 ±0.87
2	Glucose control	146.66 ±1.19	178.0 ±1.15	195.33±1.73	210.33 ±2.90
3	Glibenclamide Positive Control	98.25 ± 3.56	108.75±9.49	130.5±10.6	81.75 ± 4.40
4	*P. longifolia* extract treated (50 mg/kg)	140.66 ±1.76	111.0 ±1.52	94.0±1.15	87.66 ±0.87

Table No.2: Effect of *Polyalthia longifolia* extract on fasting plasma glucose level in rats

S.No	Group	Fasting plasma glucose concentration (mg/dl)			
		Day 0	Day 7th	Day 14th	Day 28th
1	Normal Control	78.00 ± 1.29	76.00 ± 1.63	78.66 ± 2.03	77.00 ± 0.57
2	Glucose Control	146.16 ± 3.66	185.66 ± 4.21	225.75 ± 1.65	258.00 ± 1.15
3	Glucose + Glibenclamide (0.5 mg/kg)	142.16 ± 1.17	101.33 ± 1.08	86.16 ± 1.40	80.80 ± 0.67
4	*P. longifolia* extract treated (50 mg/kg)	141.59 ± 0.57	139.19 ± 1.23	121.10 ± 1.20	91.50 ± 0.98

Table No.3: Determination of biochemical parameters after treatment with *Polyalthia longifolia Extract*

S.No	Group	Lipid Profile (mg/dl)			
		Triglyceride	Total Cholesterol	HDL	LDL
1	Normal Control	86.9 ± 3.28	83.4±1.73	54.3±1.17	43.2±2.21
2	Glucose Control	177.3± 1.20	154.00 ± 1.12	38.3 ± 0.23	165.00 ± 0.87
3	Glucose + Glibenclamide (0.5 mg/kg)	82.00 ± 1.01	89.90 ± 0.75	57.20 ± 0.98	69.10 ± 1.10
4	*P. longifolia* extract treated (50 mg/kg)	102.10 ± 1.98	98.90 ± 1.10	48.90 ± 1.86	110.00 ± 1.90

Table No.4: The Effect of *Polyalthia longifolia* Extract on Body Weight (B/W)

S.No	Group	Before Induction	After Treatment
1	Normal control	56.5 ± 4.31	60.37 ± 4.33
2	Glucose control	57.12 ± 4.29	65.0 ± 5.11
3	Glibenclamide (standard drug)	57.25 ± 2.75	58.0 ± 4.04
4	*Polyalthia longifolia* extract treated	54.75 ± 6.85	58.0 ± 4.94

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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