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A Study on the *In-Vivo* Evaluations of Anti-Hyperglycemic Activity using Various Medicinal Plants and its Antidiabetic Effects

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Abstract - Disturbances in glucose, lipid, and protein metabolism are hallmarks of diabetes mellitus, a devastating illness. A diabetic hallmark is a persistent hyperglycemia, which plays a major role in diabetes' vascular consequences. The similarity of human hyperglycemic nonketonic diabetes mellitus with streptozotocin-induced diabetes in the animals has been extensively studied. In these models, histopathological modification of pancreatic islet β cells was facilitated through a reduction of β cell nicotinamide adenine dinucleotide. The aim is to study the evaluations of anti-hyperglycemic activity using various medicinal plants and its antidiabetic effects

Keywords - In-vivo evaluations, Anti-hyperglycemic activity, medicinal plants, antidiabetic effects

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INTRODUCTION

Over 140 million individuals have been diagnosed with diabetes mellitus globally, and the number is rising. In 1999, around 30 million Indians suffered from diabetes mellitus; by 2025, experts expect that figure would rise to 80 million. As its incidence rises continuously, the illness has become a serious threat to public health in emerging nations (1, 2).

Promoting physical exercise, nutritious nutrition, and a decrease in obesity may prevent a large number of cases of the most common form of diabetes. Effective health care and education may significantly minimize the risk of long-term consequences for persons with diabetes, therefore protecting their quality of life (3).

Several millions of people around the world suffer from diabetes mellitus, a group of metabolic disorders characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both in conjunction with gross abnormal Uties in glucose homeostasis and lipid metabolism. Polyuria, polydipsia, polyphagia, pruritis, and sudden weight loss are all classic signs of diabetes (4).

The prevalence and severity of diabetes make it a major public health problem across the world. Diabetes mellitus is caused by insulin's inability to properly stimulate its target tissues. Inadequate insulin production and/or diminished tissue responses to insulin at different sites along the hormone activity pathways lead to insulin insufficiency. The world's largest public health crisis, it is the most common metabolic condition (5).

Diabetes gets its name from a Greek word for "*syphon*" since the fluid doesn't accumulate in the body but rather uses it as a bladder to exit. In - 5 AD, two Indian doctors named Charaka and Sushruta were credited in Sanskrit literature for making the connection between polyuria and a sweet taste in the bladder. Because the fluid doesn't remain in the body but instead utilizes it as a bladder to leave, Areteus of Cappadocia explains, "the illness is called diabetes from the Greek term which means a direct" (- 150 AD). It wasn't until 1650 that Thomas Willis, an English physician, anatomist, and physiologist, made this finding public. William Cullen, a Scottish physician, first used the term "mellitus" to describe the illness in 1750. (a Greek word meaning honey-like). Late in the nineteenth century, researchers finally pinpointed what was triggering diabetes mellitus. Paul Langerbans identified two distinct types of pancreatic cells in 1869. Josef von Mering and Oskar Minkowski discovered, 20 years later, that removing a dog's pancreas resulted in diabetes, suggesting that the pancreatic played a role in blood sugar management (6-8).

In the past, Type 1 diabetes was referred to as juvenile-onset diabetes or insulin-dependent diabetes mellitus (IDDM). Unfortunately, the only cells in the body that produce insulin are under attack: pancreatic beta cells. Adolescents and young adults are disproportionately affected by this

kind of diabetes, although it may strike anybody at any time. Currently, there is no proven method for avoiding the onset of type 1 diabetes. There are several diabetes treatment studies now recruiting participants or in the planning phases (9).

A shortage of insulin or a reduction in target cell responsiveness brought on by a change in insulin receptors is what defines insulin resistance. Muscle glucose uptake is reduced, and glucose accumulates in the liver as a consequence. It runs in families, but the risk rises with obesity and a sedentary lifestyle. People over the age of 40 and those with excess body fat are at increased risk for developing Type 2 diabetes (10).

In the United States, type 2 diabetes accounts for over 90% of all cases of diabetes. The remaining population has Tier 1 diabetes. Epidemiological and clinical research has shown that hyperglycemia is a major cause of problems. Clinical manifestations of diabetic nephropathy include glomerulosclerosis, glomerular nephropathy, the buildup of immunoglobulin G (IgG), and alburninuria, making it the most serious consequence of diabetes (11).

Patients and their loved ones may experience severe psychological distress due to the disease's mental and social repercussions and the treatment's associated obligations. Although the beneficial benefits of these medications on glycemic levels have been widely documented in western medicine, they have shown only modest and ineffective results in preventing the progression of diabetes and associated macro and microvascular consequences. Several different types of oral diabetic medications, including insulin, sulfonylureas, metformin, a-glucosidase inhibitors, and troglitazone, are on the market. The glycemic balance may be improved by using any one of these medicines alone or a combination of them. The aforementioned oral medicines all come with a host of potentially fatal adverse effects (12-14).

Medicinal herbs having hypoglycemic characteristics have been proven to be effective in the management of diabetes mellitus, according to several studies. These plants have properties that may help in avoiding diabetes and restoring metabolic equilibrium. Several hypoglycemic plants extracts now in clinical trials have shown stronger anti-diabetic potency than standard oral hypoglycemic medicines (15).

MATERIAL AND METHODS

Collection and Authentication of Plant Materials

The plants were harvested in the month of October from the Chattarpur region of Madhya Pradesh and authenticated by the Ayurvedic Hospital and Research centre. Albino adult In vivo (in the animal) tests for hypoglycemia effects were conducted using Wistar rats.

Animals

Adull albinos In vivo tests for hypoglycemic effects were performed on 6-week-old Wistar rats weighing between 150 and 200 grammes. The rats lived in clean polypropylene cages in a climate- and humidity-controlled animal facility with a constant 12-hour light/dark cycle. The rats had free access to water and pelleted food. During this period, the animals were monitored by the vet to ensure they were healthy enough to participate in the study. All procedures involving animal care and experimentation were conducted in accordance with the OECD's guidelines for the protection and welfare of animals in scientific research after receiving appropriate evaluation and permission from an ethical review board**.**

Acute oral Toxicity

Following recommendations from the Organization for Economic Co-operation and Development (OECD), Wistar albino rats were used to test the acute oral toxicity of hydro alcoholic extracts of a polyherbal formulation. Acute oral toxicity experiments were conducted on a total of 16 female Wister rats that were around 6 weeks old. Cage labels and individual animals were marked with identifying information such as group number, cage number, gender, and individual number as shown below. Literature review and a dosage range finding study informed the selection of the doses used in the research.

In this experiment, we split the animals into four groups of two. Group 1 served as the control and got simply 0.9% salt water, while Groups 2, 3, and 4 were given polyherbal extracts diluted at a ratio of 1:1:1 in 0.9% salt water. After an overnight fast during which the animals were given only water, a single oral administration of the hydro alcoholic extract (herbal formulation) was given to each animal at a fixed dose of 5 mg/kg, 50 mg/kg, 300 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight, with blank (water) given at a rate of 2 ml/100 mg bw. There was a 4-hour period following medication delivery during which no food nor drink were provided. Each animal was monitored for the emergence of any pathological symptoms or unusual alterations in behaviour. The animals were humanely put down if they were in obvious pain or discomfort. On day 14, assessments of sensory reactivity to auditory, visual, and proprioceptive stimuli were made.

Rats' reactions to clicker sounds, placed at a distance of about 30 cm, were used to test their responses to auditory stimuli, while those to visual stimuli were measured by shining a pen light into the eye of the rat and positioning a blunt object close to the rat's eye. To a normal degree, animals in both the control and medication treatment groups reacted to these three activities. For 14 days, every animal was checked on once in the morning and again in the afternoon to check for signs of sickness or death

at the approximate 1, 3, and 4 hours post-dose on the day of dosing.

Experimental induction of diabetes mellitus

The rats were divided into five different groups and kept for overnight fasting (n=6). Both the nicotinamide and sterptozotocin were dissolved in a citrate buffer at pH 4.5. Overnight starved rats were injected intraperitoneally with nicotinamide (120 mg/kg) and then given a single intraperitoneal dose of streptozotocin (60 mg/kg) 15 minutes later to develop non-insulin dependent diabetes mellitus. At 72 hours, a glucose metre (GlucocardTM 01-mini, Arkray Factory, Inc., Japan) proved the existence of hyperglycemia due to the raised blood glucose levels. The research used animals with blood glucose levels over 250 mg/dl.

Experimental design for oral glucose tolerance test (OGTT)

Rats that had fasted all night were split into five groups so that the hypoglycemic activity of polyherbal extracts could be tested. Both the control and normal groups were given simply glucose (vehicle), whereas the standard group was given 1 ml of Glibenclamide (10 mg/kg, p.o.) suspended in the vehicle. After 30 minutes, the extracts (250 and 500 mg/kg, p.o.) and glucose (2 g/kg, p.o.) were given to groups four and five. Before dosing, at 30, 60, 90, and 120 minutes after glucose injection, tail vein blood samples were taken. Reactive glucose-oxidase-peroxidase strips were used to measure the fasting blood glucose level (Accu-chek, Roche Diagnostics, GmbH, Germany).

Study design for evaluation of anti-hyperglycemic activity

The animals were split into 5 groups and the experiment lasted 28 days:

- Group 1: Control (only normal saline)
- Group 2: Only Streptozotocin 60 mg/kg/b.w. (IP) +Nicotinamide 120mg/kg (po) Group3: Streptozotocin (60 mg/kg) + Nicotinamide 120mg/kg (po) rats treated with Glibenclamide 20 mg/kg (po)
- Group 4: Streptozotocin (60 mg/kg) + Nicotinamide 120mg/kg (po) rats treated with 250mg/kg of polyherbal extract.
- Group 5: Streptozotocin (60 mg/kg) + Nicotinamide 120mg/kg (po) rats treated with 500mg/kg of polyherbal extract.

For 28 days, animals in each group received either a vehicle (normal saline), polyherbal extract, or glibenclamide. Throughout the course of the trial, the polyherbal extract and glibenclamide were kept in a fresh propylene glycol suspension before injection. On days 0th, 7 th, 10 th, 15 th, and 28 th, the animals' body weight and blood glucose level were measured while they fasted.

RESULTS

In the present study, the antidiabetic polyherbal extract was administered at different doses of 5 mg/kg, 50 mg/kg, 300 mg/kg, 1000 mg/kg and 2000 mg/kg to rats and observed for consecutive 14 days. Doses were selected based on the data obtained during literature review. All animals were observed daily once for any abnormal clinical signs, food consumption and weekly body weight. No mortality was observed during the entire period of the study and data obtained during the study have not indicated any physical and behavioural signs of toxicity due to administration of antidiabetic polyherbalextract.

At the 14th day, all animals were observed for functional and behavioral examination. The physical and behavioral examination, home cage activity and hand held activities were observed. From acute toxicity study, it was observed that the administration of polyherbal extract to the rats at a dose upto 2000 mg/kg did not produce any drug-related toxicity and mortality. Therefore, the No-Observed-Adverse-Effect- Level (NOAEL) of antidiabetic polyherbal extract was determined as 2000 mg/kg.

The physical and behavioral examinations of Wistar rats upon administration of polyherbal extract were observed as normal. Food consumption of all treated animals was found to be normal as compared to control group. Home cage activities like body position, respiration, clonic involuntary movement, tonic involuntary movement, palpebral closure, approach response, touch response, pinna reflex, sound responses and tail pinch response was observed and found to be normal. Hand held activities like reactivity, handling, palpebral closure, lacrimation, salivation, piloercetion, papillary reflex, abdominal tone and limb tone was observed and found to be normal.

The results of oral glucose tolerance test that showed the Effect of polyherbal extract on OGTT are presented in the following Figure 1.

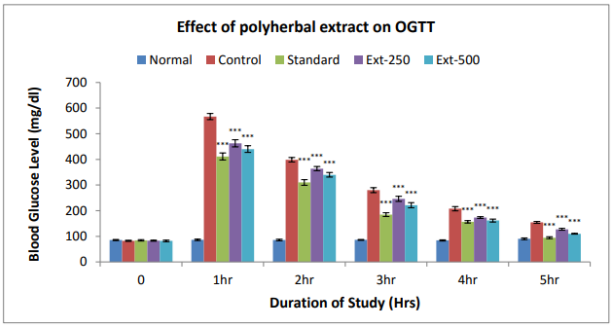

Figure 1: Effect of polyherbal extract on OGTT

Values are expressed as mean ± S.D (N=6). Statistical significance (p) was calculated by one way ANOVA followed by Tukeys multiple comparison test. ***P<0.001, **P<0.01, *P<0.05 were considered significant as compared to control

The antidiabetic effect of polyherbal extract on the body weights of Streptozotocin induced diabetic rats was evaluated and presented in Figure 2. No significant change in body weight was observed when compared with the standard glibenclamide.

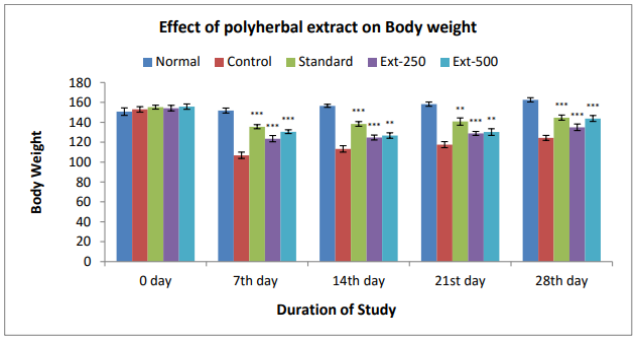

Figure 2: Effect of polyherbal extract on Body weight

Values are expressed as mean ± S.D (N=6). Statistical significance (p) was calculated by oneway ANOVA followed by Tukeys multiple comparison test. ***P<0.001, ***P<0.01 were considered significant as compared to control.

Streptozotocin induced diabetic rats have exhibited decreased body weight associated with decrease in endogenous insulin and hyperglycemia. Treatment with phytochemical formulation to diabetic rats increased body weight and decreased the elevated blood sugar level. The results demonstrated that the test formulation decreases fasting blood glucose level in diabetic rats. Administration of polyherbal formulation increases the body weight in STZ induced diabetic rats.

The ability of formulation to protect body weight loss is because of its ability to reduce hyperglycemia. Glibenclamide is known to encourage weight gain as a result of its effect to raise insulin levels and cause improved exploitation of glucose.

As presented in Figure 3, body weight of the normal rats that took the vehicle was normal; however, the body weight of diabetic control rats have showed a decrease in their body weight (i.e. 153.0±2.82 to 124.3±2.50 g) after four weeks. No significant change in body weight was noticed for standard glibenclamide and polyherbal extract (500 mg/Kg) treated rats. The *in-vivo* results has shown no significant changes in blood glucose levels of rats in control group while significant reduction was observed in both test groups (250 mg/Kg and 500 mg/Kg of polyherbal extract). The results of both test groups were also comparable with the marketed formulation (Glibenclamide) and more similarity was observed for test group with highest drug concentration (500 mg/Kg) when compared with standard formulation.

In present study, weight loss of 18.75% was noticed in streptozotocin induced diabetic control rats as compared with normal rats. Continuous treatment with polyherbal extract (500 mg) for 28 days showed an increase of 11.15% in body weight as compared with streptozotocin induced diabetic control rats. Glibenclamide treated rats showed a body weight gain of 11.95% at the end of the study.

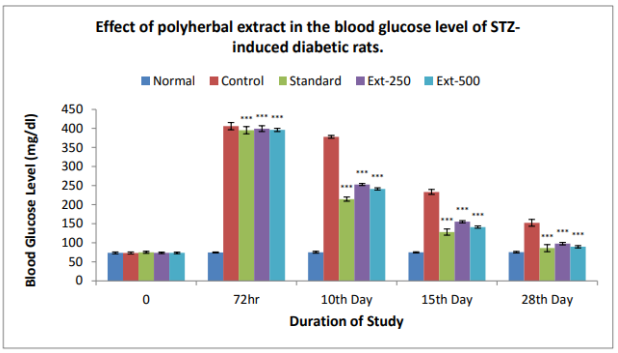

Figure 3: Effect of polyherbal extract in the blood glucose level of STZ-induceddiabetic rats

Values are expressed as mean ± S.D (N=6). Statistical significance (p) was calculated by one way ANOVA followed by Tukeys multiple comparison test. ***P<0.001 were considered significant as compared to control.

The effect of glibenclamide and antidiabetic polyherbal extract on fasting blood glucose level was evaluated after completion of 28 days study period. Both polyherbal and glibenclamide treatments have shown good hypoglycemic activity (P<0.05) on comparison with diabetic control. Polyherbal extract (500 mg) treated diabetic rats showed 41.2% reduction in fasting blood glucose levels while glibenclamide treated diabetic rats showed 43.6% reduction in fasting blood glucose level on comparisonwith diabetic control.

The results of present study have shown better results for polyhebal extract treatment in rats on comparison with above mentioned studies.

The present study showed that the blood glucose levels are maintained below 100 mg/dl in streptozotocin-induced diabetic rats after administration of polyherbal extracts (97.67 ±2.73 mg/dl for 250 mg and 89.50 ± 2.88 mg/dl for 500 mg) and standard drug, glibenclamide (85.83±2.78 mg/dl) at the end of 28 days treatment.

CONCLUSION

Several medicinal plants have been shown to have anti-diabetic and anti-hyperglycemic effects. Despite polyherbal formulation, there are currently various

herbal medicinal therapies on the market that include the same plant ingredients. The polyherbal extract (both 250mg/Kg and 500mg/Kg) had antidiabetic action that was on par with the gold standard medication Glibenclamide in in-vivo anti-hyperglycemic tests.

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