

Hypoglycemic effect of *Talapotaka Churna* in streptozotocin-induced hyperglycemia in rats

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Abstract

Objective: To evaluate the antihyperglycemic effect of *Talapotaka Churna* in experimental animals, *Talapotaka Churna* was prepared, containing *Avartaki* (*Cassia auriculata*), *Amalaki* (*Emblica officinalis*), *Haridra* (*Curcuma longa*), and *Daruharidra* (*Berberis aristata*). **Materials and Methods:** *Talapotaka Churna* was prepared by the standard procedure of *Churna Kalpana*. Hyperglycemia was induced to create an equivalent to the diabetic state by giving streptozotocin (STZ) solution (intraperitoneal) 35 mg/kg. After assessment of hyperglycemia as an approximate induction of diabetes, group of animals (IV and V) were treated with doses 300 and 600 mg/kg of *Talapotaka Churna*. For treatment comparison, Group III animals were treated with a standard hypoglycemic drug, glibenclamide 10 mg/kg. Blood sugar, the level was estimated by glucometer on the 7th, 14th, 21st, and 28th day. **Results:** *Talapotaka Churna* produced a significantly lowering of fasting blood glucose with various doses in STZ-induced diabetic rats. In a 4-week study, *Talapotaka Churna* produced a significant reduction in blood glucose compared to glibenclamide. **Conclusion:** *Talapotaka Churna* and glibenclamide significantly lowered blood glucose level. The results were found more significant with respect to treatment days in this *in vivo* comparative study.

Key words: Prameha, Streptozotocin, *Talapotaka Churna*

INTRODUCTION

Diabetes mellitus (DM) is a leading metabolic disorder with clinical syndrome since antiquity involving various organ systems and marked by chronic hyperglycemia. DM continues to be a worldwide health problem and is a major health-care puzzle, as it becomes a serious threat to humankind due to its secondary complications of kidney failure, blindness, and neuropathy, with its associated high health-care costs and challenge for all physicians. The prevalence of diabetes for all age groups worldwide was estimated 8.3% (387 million) in the 21st century and is estimated to be 592 million by 2035.^[1,2] In *Ayurveda*, *Prameha/Madhumeha* can be considered as DM by different perspectives based on clinical symptoms and attempts have been made by *Ayurvedic* physicians and researchers to treat these two entities using classical formulations mentioned in *Prameha Chikitsa*.^[3]

Worldwide, people are successfully using and trusting herbal medicine for the treatment of various health problems. Many of the diabetic patients are getting side effect due to allopathic medication, so now patients are relying on alternative therapies with antihyperglycemic effects. This comes as no surprise since alternative treatments have been most widely used in chronic diseases, which may be only partially alleviated by conventional treatment. Herbal medications are the most commonly used alternative therapy for lowering blood sugar. The interest in herbal drug research continues with an expectation that someday or other, we would be able to bring a safer, efficacious, and more effective compound

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with all the desired parameters of a drug that could replace the synthetic medicines. The plant has definite promises in the management of diabetes. Isolation and identification of active chemical principle from plant and preparation of standardized dosage can play a key role in the management of diabetes. Besides this for the global acceptance, the herbal remedies are very easily accepted by the society.

Talapotaka Churna has mentioned by *Acharya Vallabhacharya* in his text *Vaidya Chintamani* in *Prameha Prakarana* (20th chapter). The four ingredients of *Talapotaka Churna* have already proven antidiabetic activity in ancient *Ayurvedic* literature. *Talapotaka Churna* has been prescribed as *Sarva-mehahara* from the period of *Vaidya Chintamani* (15th century).^[4]

MATERIALS AND METHODS

Selection and Collection of Plant Materials

The formulation contains 4 ingredients. Among these, *Avartaki* was collected from the peripheral region of Satara district, Maharashtra, India. The rest 3 raw drug samples were procured from Gola Dinanath (Raw drug market), Varanasi, Uttar Pradesh, India. All the collected samples were pharmacognostically identified and confirmed in the Department of Dravyaguna, Faculty of Ayurveda, IMS, Banaras Hindu University, Varanasi.

Churna Preparation

The collected plant materials of *Talapotaka Churna* were cleaned and dried in the sunlight. The dried plant material was then ground into a fine powder using a mechanical pulverizer in Ayurvedic Pharmacy, Banaras Hindu University, Varanasi, India. This sample was used for antidiabetic study.

Chemicals

Streptozotocin (STZ) was sponsored by the Department of Rasa Shastra, Faculty of Ayurveda, IMS, BHU, Varanasi, which was purchased from Himedia Laboratories Pvt. Ltd., Dindori, Nashik, India. Batch number was 0000222052; manufacturing date was November 2014, and Expiry date is February 2017. Glibenclamide was purchased from Emcure SANOFI, trade name Daonil (Manufacturing date April 2015 and Expiry date 2016) in India, for use as the standard antidiabetic agent.

Animals

A total of 30 Charles Foster albino rats of either sex weighing between 180 ± 30 g were used for the experimental study. The animals were obtained from the Central Animal

House, Institute of Medical Sciences, Banaras Hindu University, Varanasi. The animals were freely allowed to eat pellet chow (Amrut Laboratory Animal Feed, Pranav Agro Industries Limited, Sangli) and *ad-libitum* water during the study periods. Principles of laboratory animal care as per NIH guidelines were always followed, and prior approval of Institutional Animal Ethical Committee (Reg. No. Dean/2014-15/EC/1057) of BHU was obtained before commencing experiments.^[5,6]

Experimental Protocol

The experimental study was conducted at the Department of Pharmacology, IMS, BHU. 30 animals were divided into five groups and were kept under standard laboratory condition during the study. The groups were named normal control (NC) (Group I), diabetic control (DC), non-treated (Group II), diabetic standard group (Group III), and the two test groups of *Talapotaka Churna* in different doses Groups IV (300 mg) and V (600 mg).

On day $t = -1$, before induction of hyperglycemia as an approximate induction of DM, the rats were kept fasting from all food; only water was given. Immediately before injection, STZ was dissolved in 50 mg of sodium citrate buffer (pH 4.5) to a final concentration of 10 mg/ml. The STZ solution was freshly prepared for each rat and was injected within 5 min after being dissolved.

Hyperglycemia was induced (in-Group II to V) by STZ solution intraperitoneal using a dose of 35 mg/kg through insulin syringes. After 72 h, blood sugar level was measured by optimum exceed glucometer (Abbott). For investigation of blood glucose, blood of rats was withdrawn through a tail central vein. Hyperglycemia was confirmed by the elevated glucose level in the blood by glucometer, determined after 72 h.^[7] On the 7th day after confirmation of hyperglycemia, animals of Groups IV and V were treated with different doses of *Talapotaka Churna*. Animals of Group III were treated with hypoglycemic drug glibenclamide 10 mg/kg. Glibenclamide stimulates the pancreatic beta cells of the pancreas and increasing the sensitivity of the peripheral tissue to insulin. Data of blood sugar were collected every 7th day of duration for 4 weeks and compared among groups.

Dose Schedule

About 30 Charles Foster rats were divided into five groups, namely, NC (Group I), DC (Group II), standard group treated with glibenclamide in dose of 10 mg/kg body weight (Group III), and treated group with *Talapotaka Churna* (Group IV and Group V) in the dose of 300 mg/kg and 600 mg/kg body weight, respectively. The test drugs *Talapotaka Churna* and standard drug glibenclamide were administered according to the body weight of the animal by oral route with the help of an intragastric tube.

Statistical Analysis

Statistical analysis of data was performed using SPSS 16.0 and one-way analysis of variance. Results were expressed as mean ± standard deviation from six rats in each group. *P* < 0.05 was considered statistically significant, and *P* < 0.001 was considered highly statistically significant in the results of this study.

RESULTS

The baseline value in all five groups of albino rats showed that the fasting blood glucose (FBG) levels were within normal limits. The first task was to create an experimental model of hyperglycemia. A single dose (35 mg/kg) of STZ was administered to Groups II-V, leaving Group I as an NC. Hyperglycemia was significantly induced compared to NC FBG after 72 h and was confirmed on the 7th day following STZ administration [Figure 1].

The second task was to evaluate dose-dependence on reduction in blood glucose of experimentally-hyperglycemic rats in our diabetic model. The stock solution was prepared freshly by the adequate quantity of distilled water with the samples of *Talapotaka Churna* was given at the doses of 300 mg/kg and 600 mg/kg, to each of six rats in each of the two groups. Results are shown in Table 1, and demonstrated a dose-dependent reduction, with 300 mg/kg and 600 mg/kg showing the greatest reduction in blood glucose level. *Talapotaka Churna* produced a maximum reduction of blood glucose of 55% (*P* < 0.001) and 53% (*P* < 0.001) 1 h with doses of 300 and 600 mg/kg, respectively [Figure 2].

The next task was to compare a dose of *Talapotaka Churna* with a standard antidiabetic drug, glibenclamide. In a 4-week study, *Talapotaka Churna* produced a significant reduction in blood glucose compared to glibenclamide as shown in

Table 1. Glibenclamide (10 mg/kg) produced a maximum reduction of FBG 63% (1 h, *P* < 0.001) compared to DC Group II [Figure 3].

The results indicate a prolonged action in a reduction in blood glucose by *Talapotaka Churna* and are probably mediated through enhanced secretion of insulin from β-cells

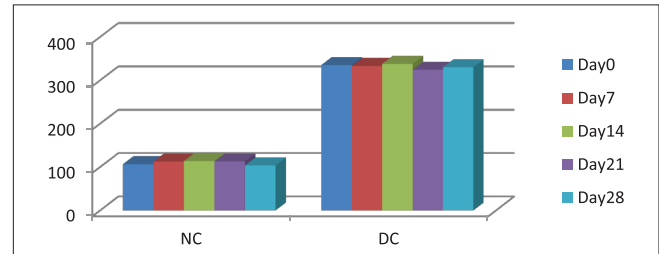


Figure 1: The effect on fasting blood sugar in streptozotocin-induced albino rats, where values are given as mean ± standard deviation (*n* = 6 in each group). Values are statistically significant at *P* < 0.05, *P* < 0.001 compared with normal control group

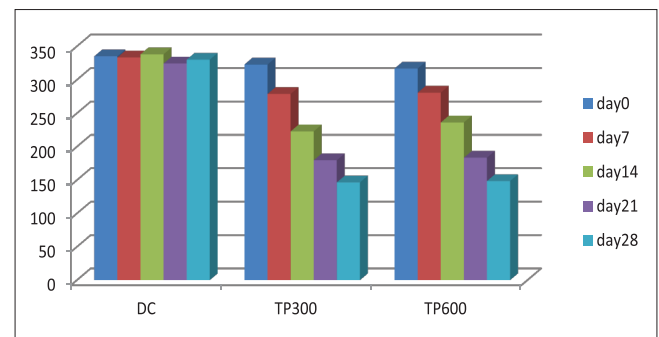


Figure 2: The effect of two different doses of *Talapotaka Churna* on fasting blood sugar in streptozotocin-induced albino rats, where values are given as mean ± standard deviation (*n*=6 in each group). Values are statistically significant at *P* < 0.05, *P* < 0.001 compared with diabetic control group

Table 1: Effect of *Talapotaka Churna* on blood sugar level (mg/dl)

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
NC	106.50±4.46	112.67±5.164	114.0±3.79	113.0±4.14	103.67±4.63
DC	335.0±23.62	333.33±17.51	338.0±10.04	324.33±11.82	330.0±16.44
GB	326.67±17.28	231.0±26.79**	175.50±19.26**	150.0±10.19**	119.67±7.52**
TP300	322.67±17.51	278.67±20.65**	222.83±14.59**	179.67±12.61**	146.50±9.54**
TP600	316.67±21.45	280.67±19.65**	236.17±22.99**	183.50±12.29**	148.50±7.58**
One-way ANOVA	<i>F</i> =175.50 <i>P</i> =0.000	<i>F</i> =112.20 <i>P</i> =0.000	<i>F</i> =167.15 <i>P</i> =0.000	<i>F</i> =337.73 <i>P</i> =0.000	<i>F</i> =506.11 <i>P</i> =0.000
Post hoc	(I II), (I III), (I IV), (I V)	(I II), (I III), (I IV), (I V), (II III), (II IV), (II V), (III IV), (III V)	(I II), (I III), (I IV), (I V), (II III), (II IV), (II V), (III IV), (III V)	(I II), (I III), (I IV), (I V), (II III), (II IV), (II V), (III IV), (III V)	(I II), (I IV), (I V), (II III), (II IV), (II V), (III IV), (III V)

Values are given as mean±SD (*n*=6 in each group). Values are statistically significant at **P*<0.05, ***P*<0.001 compared with control group. NC: Normal control, DC: Diabetic control, GB: diabetic standard group (Glibenclamide treated), TP 300: *Talapotak Churna* in 300 mg dose, TP 600: *Talapotak Churna* in 600 mg dose, SD: Standard deviation

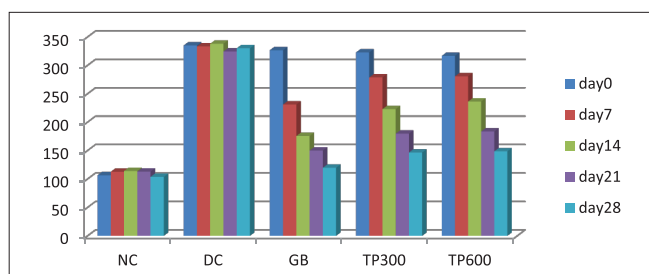


Figure 3: The effect of two different doses of *Talapotaka Churna* and glibenclamide on fasting blood sugar in streptozotocin-induced albino rats, where values are given as mean \pm standard deviation ($n = 6$ in each group). Values are statistically significant at $P < 0.05$, $P < 0.001$ compared with control group

of Langerhans or through an extrapancreatic mechanism. *Talapotaka Churna* produces a significant effect with various doses in STZ-induced diabetic rats. In the treated group for 4 weeks, significant reduction of FBG level was observed.

Mean and standard deviation of sugar of normal group and various treatment groups on day 0, 7, 14, 21, and 28 was determined and show in Table 1. Mean sugar in NC group was much smaller as compared to other groups. Mean sugar level decreased successively on different days. The intergroup comparison was statistically highly significant on each 7th day observation. *Post-hoc* test for pair wise group comparison was also applied, and pairs were found statistically significant as shown in Table 1.

DISCUSSION

Talapotaka Churna is a poly-herbal formulation containing four herbs, each reported in the *Ayurvedic* classics to have the action of reducing *Prameha*.^[8-11] These herbs have also been studied in modern science and showed a significant reduction in blood glucose levels in DM animal models. Gupta *et al.* (2010) found *Cassia auriculata* leaf extract has insulinogenic action in STZ-induced diabetic rats.^[12] Latha *et al.* (2003) found *C. auriculata* L. flower extract suppresses enhanced gluconeogenesis and enhances utilization of glucose through increased glycolysis in STZ-induced diabetic rats.^[13] Abesundara *et al.* (2004) showed that *C. auriculata* flower extract exerts a strong antihyperglycemic effect in rats comparable to the therapeutic drug acarbose.^[14] Patel *et al.* (2011) found fruit juice of *Emblica officinalis* showed decreased glucose level by enhancing insulin sensitivity and inhibit the production of reactive oxygen species by elevating the levels of antioxidant enzymes in diabetic heart.^[15] Kumar *et al.* (2012) found fruit juice of *E. officinalis* (mixed with fresh bitter gourd juice) stimulate the islets of Langerhans.^[16] Kumar *et al.* (2013) showed antidiabetic effects of aqueous extract of *Curcuma longa* rhizome in alloxan-induced diabetic rats.^[17] Krishnaswamy (2008) found *C. longa* has increased plasma insulin and hepatic glucokinase activity

levels in STZ-induced diabetic rats.^[18] Rezaq (2014) found that curcumin has pancreatic islet regeneration capacity in STZ-induced diabetic rats.^[19] Singh *et al.* (2010) showed berberine reduces blood sugar by inhibiting absorption of sugars from the intestine. Furthermore, it enhances production of insulin. Mall *et al.* (2013) found that root bark powder of *Berberis aristata* stimulates pancreas to secrete insulin.^[20]

All ingredients of *Talapotaka Churna* have different phytochemicals.^[21-24] It is believed that the basis of the chemical constitution of different herbal drugs and various medicinal/plant extracts contain active flavonoids, alkaloids, phenolic compounds, terpenoids, saponins, and phytosterol type chemical constituents that are effective in the management of diabetic complications. This effect might be attributed to the amelioration of persistent hyperglycemia, oxidative stress, and modulations of the various metabolic pathways involved in the pathogenesis of diabetic complications.^[25]

In our study, *Talapotaka Churna* showed a significant decrease in blood sugar level both compared to a diabetic non-treated control group and to a group treated with a standard antidiabetic drug, glibenclamide in an animal model that replicated hyperglycemia. This study attempts to show that the mode of action of *Talapotaka Churna* may be similar to the mode of action of glibenclamide, i.e., by stimulating the pancreatic beta cells of the pancreas and increasing the sensitivity of the peripheral tissue to insulin. STZ caused selective destruction of insulin-secreting beta cells and raised blood sugar level in animals.^[26] STZ, N-(methyl nitrocarbon)-D-glucosamine is a potent methylating agent for DNA and acts as nitric oxide donor in pancreatic β -cell, and thus β -cells are more sensitive to damage by nitric oxide and free radical scavenging enzyme.^[27]

CONCLUSION

Talapotaka Churna significantly decreases blood sugar level in experimental animals induced by STZ. *Talapotaka Churna* reduced blood sugar level gradually. The results suggest a protective role of *Talapotaka Churna* in STZ-induced hyperglycemia.

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