ORIGINAL ARTICLE

Hypoglycemic effect of *Lodhradi Kashaya Ghanavati* in streptozotocin-induced hyperglycemia in rats

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Abstract

Objective: To evaluate the hypoglycemic effect of *Lodhradi Kashaya Ghanavati* (LKGV) in experimental animals, LKGV was prepared, containing *Lodhra* (*Symplocose racemosa*), *Haritaki* (*Terminalia chebula*), *Musta* (*Cyperus rotundus*), and *Katphal* (*Myrica esculanta*). **Materials and Methods:** LKGV was prepared by the standard procedure of *Kashaya Ghanavati*. Hyperglycemia was induced to create an equivalent to the diabetic state by giving streptozotocin (STZ) solution (intra-peritoneal [i.p.]) 65 mg/kg, 15 min after initial administration of 120 mg/kg nicotinamide i.p. After assessment of hyperglycemia as an approximate induction of diabetes, group of animals (III, IV, and V) were treated with dose titrations using 50, 150, and 275 mg/kg of LKGV. For treatment comparison, Group VI animals were treated with a standard hypoglycemic drug, glibenclamide 10 mg/kg. Blood sugar, the level was assessed by glucometer on the 7th, 14th, 21st, and 28th day. **Results:** LKGV extract produced a significantly reduction of fasting blood glucose with various doses in STZ-induced diabetic rats. In a 4-week study, LKGV produced a significant reduction in blood glucose compared to glibenclamide. **Conclusion:** LKGV and glibenclamide significantly reduced blood sugar level. The results were more significant with successive days in this *in vivo* comparative study.

Key words: *Lodhradi Kashaya Ghanavati, Prameha,* streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a well-known clinical syndrome since antiquity involving multiple organ systems and marked by chronic hyperglycemia. In Ayurveda, the chronic condition of high urine sugar and polyuria is known as *Prameha* and approximate to the modern medical diagnosis of DM. An ancient Ayurvedic texts first mention the role of diet in the prevention, etiology and treatment of diabetes under the heading of *Prameha* and *Madhumeha.*[1]

DM continues to be a global 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. Worldwide, the WHO projects death due to diabetes will be doubled between 2005 and 2030. The prevalence of diabetes for all age-groups worldwide was estimated in 2000 at 2.8% and is projected to be 4.4% in 2030.The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.[2,3] The three countries with the largest number of people with diabetes are India, china, and U.S. The International Diabetes Federation estimates the total number of people in India with diabetes to be around 50.8 million in 2010, rising to 87.0 million by 2030.[4]

Conventionally plenty of anti-diabetic agents are available. However, due to their adverse effects, cost, and decreasing efficacy over time, there is a search for safer, acceptable and effective anti-hyperglycemic agents, which do not

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Received: 10-02-2015
Revised: 09-07-2015
Accepted: 19-09-2015

destroy healthy tissues. In Ayurveda, the treatment of polyuria (*Prameha*) and a person with high sugar in their body (*Madhumeha*) is well described. Various plant-based medicines were developed and had been used for thousands of years, and are well-documented for their clinical effects in Ayurvedic classic books such as Charaka Samhita and Basavarajeeyam. In these texts, one of the classical poly-herbal formulations described for *Madhumeha* is *Lodhradi Kashaya Ghanavati* (LKGV). It contains *Lodhra* (*Symplocose racemosa*), *Haritaki* (*Terminalia chebula*), *Musta* (*Cyperus rotundus*), and *Katphal* (*Myrica esculanta*)[5] and is prescribed in decoction form according to Basavarajeeyam.

MATERIALS AND METHODS

Selection and Collection of Plant Materials

The components of LKGV were procured from the Ayurvedic Pharmacy Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, during the summer of 2012. The stem bark of *Lodhra* (*S. racemosa)*, the dry fruit pericarp of *Haritaki* (*T. chebula*), the rhizome of *Musta* (*C. rotundus),* and the stem bark of *Katphal* (*M. esculanta)*. The identification and authentication of each plant were confirmed by the *Dravyaguna* Department of the Faculty of Ayurveda, IMS, BHU, Varanasi.

Extract Preparation

The collected plant materials were cleaned and dried in the sunlight. The dried plant material was then ground into a moderately coarse powder (30-40 sieves) using a mechanical pulverizer. The coarse powdered drug was then boiled with tap water of 8 times its volume and reduced one-fourth part. It was then filtered while hot through a muslin cloth. The filtrate was evaporated on the gas stove, and the solidified extract was obtained.[6]

Chemicals

Streptozotocin (STZ) was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India, Batch number T-8361536, manufacturing date March 2012. Nicotinamide was purchased from SD Fine Chem., Mumbai, India. Glibenclamide was purchased from Emcure SANOFI, trade name Daonil (Manufacturing date April 2013 and Expiry date 2014) in India, for use as the standard anti-diabetic agent.

Animals

36 Charles Foster albino rats of either sex weighing between 150-180 ± 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, Sangali, supplied by Local Agency, Shri Rajan, Paramount Techno Chem., Praphool Nagar, Lanka, Varanasi) and *ad libitum* water during the study periods. Principles of laboratory animal care (NIH publication number 85-23, revised in 1985) guidelines were always followed and prior approval of Institutional Animal Ethical Committee (Reg. No. Dean/12-13/CAEC/34) of BHU was obtained before commencing experiments.[7]

Methods/Experimental Protocol

The experimental study was conducted at the Department of Pharmacology, IMS, BHU. 36 animals were divided into six 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), and the three test groups of LKGV in different doses (Groups III, IV, and V), with a diabetic standard group (Group VI).

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 prior to 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 with in 5 min after being dissolved.

15 min before administration of STZ, nicotinamide at 120 mg/kg were administered through insulin syringe by intra-peritoneal route. Hyperglycemia was induced (in-Group II to VI) by STZ solution intra-peritoneal (i.p.) using a dose of 65 mg/kg through insulin syringes. After 72 h estimation of blood sugar level by One Touch (Horizon) glucometer sponsor by Department of Rasa Shastra, IMS, BHU. 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 (Wu and Huan, 2008). NC and DC animals were treated accordingly with 10 ml/kg of 0.3% carboxymethylcellulose. On the 7th day after confirmation of hyperglycemia, animals of Groups III, IV, and V were treated with different doses of LKGV. Animals of Group VI 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 was collected every 7th day of duration for 4 weeks and compared among groups.

Dose Schedule

36 Charles Foster rats were divided into six groups, namely NC (I), DC (II), treated group with LKGV (III, IV, and V) in the dose of 50, 150, and 275 mg/kg body weight, respectively,

and standard group treated with glibenclamide in dose of 10 mg/kg body weight (VI). The test drugs LKGV and standard drug glibenclamide were administered according to the body weight of the animal by oral route with the help of intragastric tube using 2 ml 18-gauge rat gavaging needle with round tip made of steel. Prescribe dose of suspended drug was loaded in a syringe, and the tube was inserted into the esophagus and drugs was administered.

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 <0.001 were considered highly significant in the results of this study.

RESULTS

The base line value in all six 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. Nicotinamide 120 mg/kg was introduced before 15 min of administration of STZ i.p. A single dose (65 mg/kg) of STZ was administered to Groups II-VI, 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. A water-soluble extract of LKGV was given at the doses of 50, 150, and 275 mg/kg, to each of six rats in each of the three groups. Results are shown in Table 1, and demonstrated a dose-dependent reduction, with 150 mg/kg and 275 mg/kg showing the greatest reduction in blood glucose level. LKGV produced a maximum reduction of blood glucose of 38% ($P < 0.001$), 48% ($P < 0.001$), and 45% ($P < 0.001$) 1 h with doses of 50, 150, and 275 mg/kg, respectively [Figure 2].

The next task was to compare a dose of LKGV with a standard anti-diabetic drug, glibenclamide. In a 4-week study, LKGV 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 56% (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 LKGV and are probably mediated through enhanced secretion of insulin from β-cells of Langerhans or through an extra-pancreatic mechanism. LKGV extract produces significant effect with various doses in STZ-induced diabetic rats. In the LKGV treated group for 4 weeks, significant reduction of FBG level was observed.

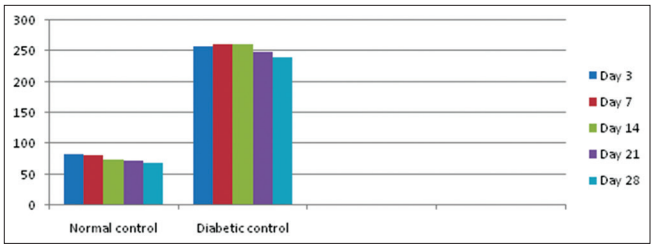

Figure 1: The effect on fasting blood sugar in streptozotocin-induced albino rats, where values are given as mean ± standard deviation (n = 6 in each group). Values are statistically significant at $P < 0.05$, $P < 0.001$ compared with normal control group

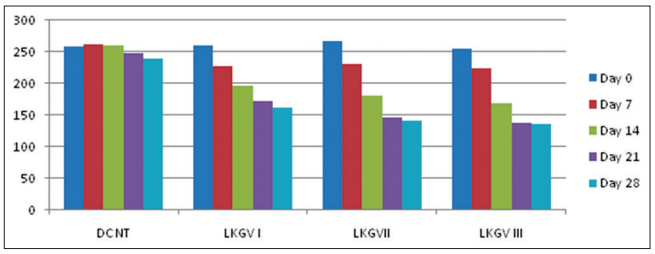

Figure 2: The effect of different dose of *Lodhradi Kashaya Ghanavati* on fasting blood sugar in streptozotocin-induced albino rats, where values are given as mean ± standard deviation (n = 6 in each group). Values are statistically significant at $P < 0.05$, $P < 0.001$ compared with diabetic control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

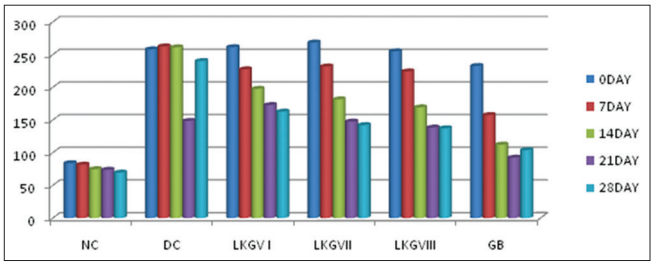

Figure 3: The effect of different dose of *Lodhradi Kashaya Ghanavati* and glibenclamide on fasting blood sugar in streptozotocin-induced albino rats, where values are given as mean ± standard deviation (n = 6 in each group). Values are statistically significant at $P < 0.05$, $P < 0.001$ compared with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

Mean and SD 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 compare to other groups. Mean sugar level decreased successively on different days. The inter group 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 were shown in Table 1.

DISCUSSION

LKGV is a poly-herbal formulation containing four herbs, each reported in the Ayurvedic classics to have the action of reducing *Prameha*. These herbs have also been studied in modern

Table 1: Effect of water extract of LKGV on blood sugar level (mg/dl)

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
NC	83.93±10.30	81.67±10.89	74.67±11.77	73.83±10.16	69.50±10.44
DC	258.00±16.49	262.50±17.89	261.00±20.00	248.50±11.52	240.17±20.28
LKGV I	261.33±16.51	227.50±12.59	197.67±12.70	172.83±9.23	162.83±8.03
LKGV II	268.50±24.87	231.8±17.75	181.50±15.68	147.50±19.93	142.17±16.80
LKGV III	255.00±25.63	224.33±12.86	169.33±16.35	138.33±15.79	137.33±11.69
GB	232.53±70.26	157.50±21.94	112.33±14.77	92.33±16.02	103.83±12.27
Between the group comparison one-way ANOVA	*F*=75.47 *P*<0.001	*F*=101.72 *P*<0.001	*F*=108.12 *P*<0.001	*F*=113.58 *P*<0.001	*F*=104.58 *P*<0.001
Post-hoc	(I II), (I III), (I IV), (I V), (I VI)	(II VI), (III VI), (VI VI), (V VI), (VI II), (VI III), (VI IV), (VI V)	(II III), (II IV), (II V), (II VI), (III II), (III VI), (VI III), (VI VI), (V II), (V VI)	(II III), (II IV), (II V), (II IV), (III IV), (III V), (III VI), (IV VI), (V II), (V VI), (VI V)	(II III), (II IV), (II V), (II VI), (III VI), (VI III)

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.
LKGV: *Lodhradi Kashaya Ghanavati*, NC: Normal control, DC: Diabetic control, SD: Standard deviation

science and show a significant reduction in blood glucose levels in DM animal models. 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 pathway involved in the pathogenesis of diabetic complications.[8]

Ahmad *et al*. found new phenolic glycosides of salirepin series in the n-butanol fraction of the bark of *Lodhra* (*S. racemosa*).[9] Choudhary *et al*. found phenolic glycosides that inhibited human nucleotide pyrophosphatase phosphodiesterase.[10] The preliminary phytochemical screening of different extracts of rhizome of *Musta* (*C. rotundus* Linn.) showed the presence of phenolic compounds, flavonoids, alkaloids and absence of triterpenoids, anthroquinones, and coumarins in all the extracts saponins and tannins were present in alcoholic and aqueous extracts.[11]

Haritaki (*T. chebula*) has long been used because a number of phytochemical constituents have been found to be associated with the plant extract that include mainly the different types of chebulic acid, gallic acid, ellagic acid, tannic acid, amino acids, flavonoids such as luteolin, rutins, and quercetin. These compounds found to be responsible for many of pharmacological action.[12] *T. chebula* fruit and seeds exhibited dose-dependent reduction in blood glucose of STZ-induced diabetic rats both in the short-term and long-term study and also had renoprotective activity.[13] Tannins, terpenoids of *Lodhra* stimulated secretion of insulin and possessed hypoglycemic activity,[14] respectively.

Besides this *T. chebula* was reported as an anti-diabetic agent acting through the extracellular inhibition of advanced glycation end (AGE) formation as well as intracellular reactive oxygen species scavenging in endothelial cells.[15] *C. rotundus* suppresses AGE formation and protein oxidation in a model of fructose-mediated protein glycoxidation.[16] Glibenclamide comes under sulfonylurea group, and this group reduces the blood glucose level by stimulating the release of insulin from the pancreatic β-cells and increases the sensitivity of the peripheral tissue to insulin.[17]

In our study, LKGV 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 anti-diabetic drug, glibenclamide in an animal model that replicated hyperglycemia. This study attempts to show that the mode of action of LKGV 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 causes selective destruction of insulin secreting beta cells and raised blood sugar level in animals.[18] STZ, N-(methylnitrocarbon)-D-glucosamine is a potent methylatning 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.[19]

CONCLUSION

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

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Source of Support: Nil. **Conflict of Interest:** None declared.