Research Paper

Polyherbal Ayurvedic Powder Effectively Reduces Blood Sugar in Streptozotocin-induced Diabetic Rats

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The polyherbal formulation *Madhumehantak churna* was studied for its efficacy at two doses using the hyperglycaemia animal model of low-dose streptozotocin-induced diabetic rats. The low-dose *Madhumehantak churna* group of 1080 mg/kg/day was extrapolated from the typical 4 g thrice daily human dose used in Ayurveda. The high-dose *Madhumehantak churna* group was 3× the low dose, equivalent to 36 g daily in humans. These groups were compared to a negative control group (non-streptozotocin-induced, non-treated), a positive control group (streptozotocin-induced, oral hypoglycemic agent-treated) and a diabetic control group (streptozotocin-induced, non-treated). Rats were fed for 28 consecutive days and monitored for plasma blood glucose. Results showed that rats with severe plasma glucose (438 and 325 mg/dl) in both low-dose *Madhumehantak churna* and high-dose *Madhumehantak churna* group re-attained almost-normal plasma glucose levels (118 and 128 mg/dl) in 28 d, reaching similar levels as the positive control, gold-standard treated group (120 mg/dl). *Madhumehantak churna* presents itself as a viable option for reversing hyperglycaemia, as shown in this *in vivo* animal experimental study, and as a potentially more practical option for patients than oral hypoglycemic agents.

Key words: Diabetes, polyherbal formulation, streptozotocin, Ayurveda, animal study

Numerous studies of polyherbal formulations[1-4] have demonstrated potential treatment options for type 2 diabetes mellitus (T2DM), a pandemic of chronic disease growing for the past 30 y. Defined as a group of disorders marked by sustained hyperglycaemia[5], several distinct subtypes of T2DM are classified and treated based on reduced insulin secretion, decreased glucose utilization, and increased glucose production. Without proper treatment, diabetes is associated with many complications, primarily due to toxicity in vasculature tissue exposed to sustained high blood sugar. The associated metabolic dysregulation when untreated causes secondary pathophysiologic changes that progress to end-stage renal disease (ESRD), non-traumatic lower extremity amputations associated with diabetic neuropathy and adult blindness from retinal damage. Each imposes a tremendous burden both on the individual with diabetes and on the health care system.

Unfortunately, India holds the dubious distinction as the capital of T2DM and the leading producer of preventable blindness primarily from diabetic retinopathy. Approximately 65.1 million people in India lived with T2DM in 2013, according to the International Diabetes Federation[6], up from 62.4 million in 2011. However, India is also a country with large biodiversity and a rich ancient tradition of the oldest continuously-practiced medical system on the planet, Ayurveda. Over half of the population uses some form of Ayurvedic practice, through herbs or lifestyle, or food combinations. Many of these herbs and foods lower blood sugar, thereby preventing complications until diabetes can be prevented. Therefore, while the successful treatment of diabetes remains still elusive, polyherbal formulations used by the masses and easily available to them may supplement the notable advancements of insulin use and hypoglycemic agents by using whole-practice medicine and providing patients significant practical benefits. In addition, the wider therapeutic window of most antidiabetic herbs

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and low cost of use provides added benefits, while decades of research using chemical compounds and theorizing multiple mechanisms continue.

In the present study, a formulation of eleven herbs recognized for its efficacy in improving diabetic symptoms, shown in Table 1, was investigated for its hypoglycemic effects in a pre-clinical animal study. The *Madhumehantak churna* (MMC) formulation is a composite of eleven individual raw plant parts, each of which is never heated or cooked (*paka*) to give any new chemical compounds. This formulation has been used only anecdotally in hundreds of patients in India, with no structured clinical documentation of its efficacy. Although each constituent has already been evaluated through ethnopharmacology to ensure safety, lack of toxicity and efficacy as an antidiabetic herb, modern pharmacologists may argue that it is still possible that a new compound to be formed in the composite, one that is less safe has arisen when these constituents have been compounded stored together. Earlier animal studies reported by Gupta *et al.*[2] in the same research group, used a very similar formula with 14 herbs, which revealed no toxicity and found to be safe; this formulation was a subset of 11 of those herbs.

Scientists advocate that animal research cannot be replaced by models such as cell cultures or biometric computer modeling because these cannot predict complex interactions that occur between molecules, cell transport and signaling in response to a host of factors, tissue interactions, organ-specific mass action and whole organisms in their environment[7]. In their classic chapter on pharmacometrics in 1964, Paget and Barnes[8] detailed the evaluation of drugs in animals and the steps that should be taken to obtain evidence of unexpected toxic effects. They recognized the need for humane supervision and proper regulation of animals and the potential for medical progress to actually be held back by misleading animal models that do not reliably predict effects in humans.

MATERIALS AND METHODS

The standard and well accepted biomarker used in biomedicine to evaluate diabetes is hyperglycaemia. Therefore, a standard protocol used widely as a model of hyperglycaemia in rats[9] was utilized for this experimental study of T2D. Streptozotocin (STZ) used in low concentrations produced animal models that mimic T2DM. STZ (1 g, batch no: 0000222052, Nov 2014, expiry: Feb 2017) was procured from Himedia Laboratories Private Limited, Dindori, Nashik, India. Glibenclamide, the standard antidiabetic agent was purchased as Daonil (manufactured: April 2015, expiry: 2016) from Emcure Sanofi. All other chemicals used were of analytical grade. The glucometer TRUEresult® was used for immediate biochemical measurement of random plasma glucose. Test strips were procured from Nipro Diagnostics, Florida, USA.

Plant materials-selection, collection and authentication:

Most components of this batch of MMC as shown in Table 1 were procured from Varanasi's ancient market, Gola Dinanath during the late summer of 2015. Onion seed (*palandu* or *pyaaz beej*) was obtained from a local provision store with cooking spices. Whole bitter gourd (*karela*) fruit was hand cut into 4-7 mm chips and dried in the hot season (June) at the Banaras Hindu University (BHU), Department of *Rasa Shastra*. Mango seed pulp was obtained from small unripe green mangos and separated from the fruit, then dried in peak season and stored.

The eleven raw ingredients were identified and authenticated according to traditional Ayurvedic use of organoleptic qualities by senior experts of Ayurvedic *dravyas* in BHU, experts in the BHU Ayurvedic Pharmacy, experts in the Department of *Dravya*

TABLE 1: COMPONENT HERBS OF *MADHUMEHANTAK CHURNA*

Sanskrit name	Botanical name	Common English/ *Hindi* reference
Amra beej majja	*M. indica*	Mango seed pulp
Karavella phali	*M. charantia*	Bitter gourd whole fruit/*karela*
Jambu beej	*S. cuminii*	Jamun seed
Nimba beej	*A. indica*	Neem seed
Palandu beej	*A. cepa*	Onion seed/*pyaaz beej*
Babbula phali	*A. nilotica*	Babul seed pod
Bala beej	*S. cordifolia*	Bala seed
Methika beej	*T. foenum-gracum*	Fenugreek seed Gurmar leaf and stem
Meshasrungi	*G. sylvestre*	Turmeric rhizome/ *haldi*
Haridra	*C. longa*	Hareda fruit pulp
Haritaki phala majja	*T. chebula*	

Herbs are given using several standard names utilized in Ayurvedic research: the *Sanskrit* name, by which the herbs are known in the ancient literature, and by which order (*varnamala*) the herbs are listed; the botanical name, by which the herbs are recognized by modern biology; the common English name, by which it is often cited in the nutrition literature; and/or the *Hindi* reference, by which the herbs were purchased in the locale of the study

Guna and Department of *Rasa Shastra*, Institute of Medical Sciences (IMS), BHU, Varanasi. Observations were supervised by the former Superintendent of BHU Ayurvedic Pharmacy. Analytical studies using chromatography with reference biomarkers and phytochemical analyses were also completed during the making of MMC to validate its authenticity.

Preparation of *churna*:

The collected plant materials were cleaned and dried, then each was ground separately into fine powder using a cyclone pulverizer. Equal proportions by weight of each of the eleven powders were mixed together to homogeneity then sieved through 80# mesh. The composite mix was packed immediately into large plastic bags and stored in a dry, dark room.

Standardization of dose:

Working in reverse from human use of MMC to test the powder in animals, we utilized the well-known table of relative surface-area ratios of some common laboratory species and man in the classic 1964 chapter by Paget and Barnes[8] in reverse to calculate an equivalent dosage in experimental animals and also used a dose of three times its strength to establish a preliminary therapeutic safety window according to dose-response relationship theory.

From the classic table of calculations, a 4 g standard dose as 4000 mg test dose for an average 70 kg patient. In mg/kg, this translates to a therapeutic dose equivalent of 57.14 mg/kg human. The absolute dose needed for a 200 g rat therefore is converted by taking the human dose and multiplying by a surface area conversion factor of 0.018. (extrapolation using the Paget-Barnes table: 4000×0.018 = 72). The dose to be given to a 200 g rat is 216 mg/d. The high dose is 3x the low dose or 648 mg/d. This dose corresponds closely with the reference given in the Ayurvedic Formulary of India for various *churnas*[10], in which the adult human dose of *churna* given orally is prescribed as 3-6 g.

Experimental animals:

Adult Charles Foster strain male[9] albino rats (150± 20 g) were used as experimental animals. They were obtained from the IMS, BHU, Varanasi. Rats were kept for six days to gain weight; rats in groups II-V received supplemental refined white bread pieces to increase carbohydrate intake in addition to pellet chow during which time they were acclimatized to laboratory conditions before using them for experiments. All rats were allowed to eat the same pellet chow (Amrut Laboratory Animal Feed, Pranav Agro Industries Limited, Sangali, India) and *ad libitum* clean RO (reverse-osmosis) water during the study period, except during the day of blood sampling when animals were subjected to an overnight fast. All animals were kept and maintained under the same laboratory conditions of temperature (25±2°), humidity (55±5 %) and a 12 h light/dark cycle, with free access to food and water. Once the study was started, cages were checked and cleaned every 2 d with wood straw to absorb dampness due to polyuria in diabetic animals. Rats were housed in conventional cages with ventilation and steel grate covers. They were checked regularly for skin and fur changes, injuries, movement and general well-being. The updated 2010 guidelines in Guide for the Care and Use of Laboratory Animals[11] were followed. Prior permission for the experimental protocols of the study was obtained from the Institutional Animal Ethics committee (No. Dean/2014-15/EC/1471), IMS, BHU.

Experimental protocol:

The experimental study was conducted in the animal room of the Department of Pharmacology, IMS, BHU with 30 animals. They were divided into five groups (Table 2) of six animals and kept under standard laboratory conditions for the entire duration of the study. To test efficacy of MMC, two groups (III and IV) were given equivalent of human dose (low dose) and high dose (3x low dose). Data of blood sugar levels

TABLE 2: STUDY DESIGN FOR EFFICACY OF MMC ON HYPERGLYCAEMIA

Group #	Name of group	Design purpose
Group I	Un-medicated non-diabetic control rat	Negative control
Group II	Disease control (un-medicated diabetic rat)	Disease control (STZ-induction model)
Group III	Diabetic rat+MMC	Experimental intervention for efficacy (low-dose) fed 216 mg daily×28 d
Group IV	Diabetic rat+MMC	Experimental intervention for efficacy (high-dose) fed 648 mg TID×28 d
Group V	Diabetic rat+oral hypoglycemic agent	Positive control standard drug intervention for hyperglycaemia (10 mg/kg/day, p.o. glibenclamide)

STZ= streptozotocin; MMC= *Madhumehantak churna*; TID= thrice daily; p.o= per oral, by mouth. The experimental study design is shown, including a negative control model, a positive control model, and a disease control of unmedicated, sustained diabetic model

was collected on days 0, 7, 14 and 28 and compared among groups.

Setup of controls:

A normal negative control (NC) group (I) served for baseline parameters, which was not induced for diabetes nor medicated. A diabetic non-treated disease control group (II) provided a baseline level. The efficacy of MMC vs. a gold standard for treatment was compared by keeping a diabetes-induced group (V) given glibenclamide 10 mg/kg/d, which provided the diabetic positive control (PC) group.

The PC group is a control group that is not exposed to the experimental treatment but that is exposed to a standard treatment (glibenclamide) that is known to produce the expected effect. Glibenclamide stimulated insulin secretion from healthy pancreatic β-cells[12] and also increased the sensitivity of peripheral tissues to insulin. The PC should give the expected gold standard result seen when a drug known to affect the condition is given. When the PC produced the expected result, it confirmed that the experimental procedure was conducted correctly. It can be compared to the experimental drug to establish efficacy.

Induction of chronic hyperglycaemia:

The night before irreversible chemical induction of hyperglycaemia by partial destruction of pancreatic cells, rats were kept in fasting mode. Only water *ad libitum* was permitted. STZ was prepared according to well-documented animal models[13-16] in which low-dose STZ[17-20] successfully led to partial destruction of ß-pancreatic cells. The following morning, immediately prior to injection, STZ was dissolved in 50 mg of cold sodium citrate buffer (pH 4.5) to a final concentration of 1 mg/ml. The STZ solution was freshly prepared for each rat and was injected within 5 min after being dissolved. Hyperglycaemia was induced in groups II, III, IV, and V by single injection of STZ solution into the peritoneum using a dose of 35 mg/kg using insulin syringes. After 72 h, blood sugar level was measured by Optimum Xceed glucometer (Abbott) using blood drawn through the tail central vein. Rats were allowed to rest overnight and checked regularly for lethargy or agitation. Sucrose water 10 % was provided alongside pure water to prevent hypoglycemic shock.

On experimental day 2, hyperglycaemia was confirmed by the elevated glucose level in the blood by glucometer, determined after 72 h. After confirmation of hyperglycemic state, 10 % sucrose water was replaced with normal drinking water. On experimental day 7, rats were fasted for 6-8 h (between 7 a.m. and 1-3 p.m.) and blood samples were collected to confirm sustained hyperglycemic state. Blood glucose level was measured by Optimum Xceed glucometer (Abbott) using blood drawn through the tail central vein. The animals with blood glucose concentration >250 mg/dl were used for the study. On day 14 after appropriate fasting, blood samples were collected from the retro-orbital venous plexus under light ether anesthesia using a glass capillary tube. Samples were analyzed for glucose using commercially available biochemical kits. On day 28 of treatment, blood samples were again collected from the retro-orbital venous plexus under light ether anesthesia using a glass capillary tube. Samples were analyzed for glucose using commercially available biochemical kits. The animals were then sacrificed by decapitation. Organs were collected for histological analysis.

Statistical analysis:

Basic statistical analysis was required in this experimental study. For continuous variables of blood glucose and body weight, where an average was meaningful, means and standard deviations (SD) were calculated within each group (n=6). The main intra-group comparison was temporal, before treatment (BT) and after treatment (AT). To test the significance of the mean of difference of paired observations (BT versus AT), the paired t test was applied. For inter-group comparisons between five independent groups (more than two) groups I-V, one-way Analysis of Variance (ANOVA) and the post-hoc test was utilized. $P<0.05$ was accepted as less than 5 % probability of results occurring by chance and thus considered statistically significant. Raw data from the two experimental groups, control groups are given in Table 3.

RESULTS AND DISCUSSION

The primary outcomes measure for this efficacy study was the ability of MMC to lower blood sugar levels in STZ-treated animals. In the two doses of MMC, shown in Table 4, similar results were found in reduction of extremely high blood glucose to near normal. These results were compared to the NC, which is expected to have sustained normal blood sugar (blue line). Results showed that both the low- and high-dose MMC resulted in lowering blood sugar levels towards the NC levels. The paired t test between BT and AT blood sugar levels in both low-dose (320±57.5) and high-dose MMC

TABLE 3: COMPARATIVE EFFECTS OF LOW AND HIGH DOSES OF MMC ON BLOOD GLUCOSE IN CONTROL AND DIABETIC RATS

	BT	AT			
Group (n=6 each)	**t=0 days**	**t=7**	**t=14**	**t=28**	**Paired t test (BT-AT)**
NC	107±4	113±5	114±4	104±5	2.8±5.7; t=1.22; p=0.276
DC	335±24	333±18	338±10	330±16	5.0±10.6; t=1.15; p=0.302
PC (glibenclamide)	327±17	231±27	176±19	120±8	207±20.7; t=24.5; p=0.000
Low-dose MMC (III)	438±64	328±142	152±58	118±24	320±57.5; t=19.29; p=0.00
High-dose MMC (IV)	325±44	284±45	156±36	128±21	197±45.7; t=14.92; p=0.00

Comparison of the effects of *Madhumehantak churna* (MMC) in low-dose (216 mg daily) and high-dose (648 mg daily) on blood glucose (mg/dl) in negative control (NC), positive control (PC) and diabetic control (DC) rats. BT is before treatment and AT is after treatment

TABLE 4: EFFECT OF LOW- AND HIGH-DOSE MMC ON BLOOD GLUCOSE

Group III	**t= 0 days**	**t= 7 days**	**t= 14 days**	**t= 28 days**
1	447	99	143	99
2	500	447	135	135
3	500	457	148	148
4	340	325	149	129
5	457	415	259	115
6	385	227	80	83
Average	438	328	152	118
Group IV	**t= 0 days**	**t= 7 days**	**t= 14 days**	**t= 28 days**
1	259	201	140	127
2	315	300	158	130
3	327	285	148	158
4	340	327	125	99
5	395	315	225	140
6	312	277	140	112
Average	325	284	156	128

MMC= *Madhumehantak churna*; Group III diabetic rats were tested for 28 d with low (216 mg daily for group III) and high-dose MMC (648 mg for group IV). *values are in mg/dl, the standard measurement used for blood glucose measurement

(197±45.7) showed a significant reduction in blood sugar levels to that of the PC group.

The PC was a group of animals treated with a pharmaceutical gold standard for diabetes. Results show both the low- and high-dose MMC perform well in lowering blood sugar similar to the PC levels indicating significant antihyperglycemic activity in MMC. The statistics show a significant ($p \leq 0.05$) difference between pre-treatment and post-treatment blood sugar levels, which is expected.

The paired t test shows no significant change between pre-treatment and post-treatment blood sugar levels in the NC and the diabetic control (DC) group, which is expected, as no significant treatment was given. The DC was a group of animals induced for diabetes but not treated, for which the high blood glucose remained sustained as expected.

MMC is a polyherbal formulation composed of eleven ingredients as shown in Table 1, each of which is detailed in classic texts of Ayurveda to be effective for *madhumeha* or *prameha*[3]. Using the theory of Ayurveda, herbs that are *tikta-kashaya*, roughly translated to bitter-astringent, reduce the *pitta* and *kapha* of the body and are discussed at length in a chapter devoted to gustatory phenomena and the classification of tastes[21]. *Pitta* correlates roughly with bio-physicochemical phenomena in the nature of the body that results in metabolism, heat production, production of acid, and fiery, fast-acting actions and reactions. *Kapha* correlates roughly with bio-physicochemical phenomena in the nature of the body that result in cytoskeletal stability, lubrication, and anabolism, production of fats and storage materials, and slow, deliberate actions of investment in the strength and constancy of the body. These Ayurvedic correlates hold up consistently and reliably when observing the human body, nature and most constructs of physiology. Bitter compounds have been described at length in the classic texts of Ayurveda as reducing fats and lightening the body; biomedical studies on bitter taste reflect the potential medicinal benefits of bitter and astringent taste-activating molecules as cancer protectants and antioxidants[22]. Ayurveda correlates the effects of bitter herbs therefore as helpful in the dysmetabolism seen in diabetes.

The results showed that the herbs in MMC appear to be acting in non-linear way, as both low- and high-dose MMC lower blood sugar levels to near-normal targets. The potential reason for the non-linear effect of multi-chemical herbal interventions such as MMC reflects the probable multiple targets contained in the polyherbal compound. Instead of working at one target tissue or exhibiting dose-dependent relationships pharmacologically as single drug agents will, this combination appears to have synergetic mechanisms of action influencing the multiple organs affected by the inflammation-mediated disease of diabetes. The

advantage is that the combination appears to create a synergy of targeted actions, all relevant to the pathophysiology of diabetes.

Several of the individual herb parts have been identified using biochemical markers and biomedical studies for their effectiveness in reducing blood glucose levels in hyperglycemic individuals and in DM animal models. The current theory of modern medicine attributes the ability to affect persistent hyperglycaemia to medicinal chemicals in plants such as phytosterols, alkaloids, terpenoids, flavonoids, saponins and phenolic compounds. These also affect oxidative stress and modulate various metabolic pathways involved in the pathogenesis of diabetic complications[23].

The most studied of the eleven ingredients is turmeric, whose current main biomarker curcumin has been investigated extensively. Using 974 references, Aggarwal[24] summarized its antiinflammatory activities for diseases like diabetes, emphasizing molecular targets in the inflammation cascade of the body. Curcumin down regulates the expression of various inflammatory cytokines, including TNF, IL-1, IL-6, IL-8 and chemokines and has been shown to inhibit the action of TNF, one of the most pro-inflammatory cytokines[25]. *Amra beej majja* is the seed pulp of the green mango (*Mangifera indica*). Sharma *et al.*[26] summarized the significant constituents of *M. indica* including mangiferin, a xanthone glycoside, that shows hepatoprotective, antiinflammatory activity, reduced glucose absorption, hypoglycemic activity and the ability to limit diabetic nephropathy.

Karela (*Momordica charantia*) is the fresh fruit known as bitter gourd, also known in *Sanskrit* as *Karavallaka*. It contained the alkaloid momoridicine, as well as several glycosides. Sekhar reviewed six studies that reported the protective and regenerative properties of *M. charantia* extracts on β-cells of islets of Langerhans. Each of these studies found reversal of elevated blood sugar levels in diabetic rats induced by STZ or alloxan[27]. In addition, various studies[28] also indicated that many different extracts and preparations of *M. charantia* resulted in the recovery and regeneration of scattered islet β-cells selectively. In addition, *M. charantia* showed preventive effects as well as delay in progression of the diabetic complications of nephropathy, neuropathy, gastroparesis, cataracts and insulin resistance in experimental animals[29].

The seeds of *jamun* or *jambu* (*Syzygium cuminii* or *Eugenia jambolana*) yielded antidiabetic phytochemicals as reported by Saravanamuttu and Sudarsanam, who employed a web-based software to predict protein-ligand interactions between the biomarker (4-propan-2-ylphenyl) methanol, also known as cuminyl alcohol, with the protein sirtuin-6. Molecular docking studies showed very high energy affinity and interactive surfaces between the molecules, predicting sirtuin-6 mediated cellular actions[30]. Using *jamun* extract, Kaushik *et al.* and Bansal *et al.* reported increase in cathepsin B activity, which assisted in proteolytic conversion of proinsulin to insulin[31,32].

Grover *et al.*[33] reported that administration of lyophilized powder of *E. jambolana* to STZ diabetic rats partially restored altered hepatic and skeletal muscle glycogen content and hepatic glucokinase, hexokinase, glucose-6-phosphate and phosphofructokinase levels. In 2001, Grover *et al.*[34] reported that *jamun* seed reduced plasma glucose concentrations, prevented polyuria and partially but significantly prevented renal hypertrophy. Ravi *et al.*[35] showed that the extract of seed and kernel of *jamun* fed to STZ diabetic rats produces prominent hypoglycemia.

The neem plant (*Azadirachta indica*) has also been studied for its myriad of therapeutic effects, after the isolation of azadirachtin from its seeds[36]. Sharma *et al.* reported reduction in blood glucose levels in rats[37], but with more pronounced effects in diabetic animals. Novotnik *et al.* reported a high content of trace elements in neem powder, including essential elements Cu, Se, Mo and Fe, as well as Zn and Cr[38]. Chromium (III) is an essential micronutrient for glucose and lipid metabolism. Onion has recently been recently reported to have effects on hyperglycaemia, though Ayurveda has been using *palandu* for ages in diabetes. Jung *et al.* showed that onion extracts reduced hyperglycaemia in STZ-induced rats and proposed a mechanism of insulin sensitizing and hypoglycemic effect owing to the presence of high quercetin levels found in onion peels[39].

Asad *et al.* showed the effect of *babbula* (*Acacia nilotica*) extract on lowering blood glucose levels and in increasing insulin levels in diabetic rats[40]. Kanth and Diwan showed that *bala* (*Sida cordifolia*) has strong hypoglycemic properties as well as antiinflammatory properties in animals[41]. *Methi* or fenugreek seeds as a part of polyherbal formulations (*Trigonella foenum-graecum*) can significantly improve glycemic status[42], most probably through powerful antioxidant activity[43]. Ahmed *et al.* showed that *gurmar* (*Gymnema sylvestre*)

increased serum insulin levels[44]. Saravanamuttu and Sudarsanam reported isolation of antidiabetic principles from *gurmar* that included gymnemic acids, gymnemagenin and gymnestrogenin[30].

Haritaki (*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[45]. Besides this *T. chebula* was reported as an antidiabetic agent acting through the extracellular inhibition of advanced glycation end formation as well as by scavenging the intracellular reactive oxygen species in endothelial cells[46].

Unlike chemical pharmaceutical studies that establish therapeutic dosages via logarithmic trials and pharmacokinetics, herbal formulations derived from Ayurveda utilize instructions given in classic texts. For this formulation, the dosage described in *Sharangdhar Samhita*[47] for fresh powders was utilized. It describes a daily dose of *churna* as one *karsa matra* or *pramana*, approximately 12 g, as the optimal dose.

In this study, both doses showed similar efficacy, indicating a wide therapeutic window of safety and efficacy. Logarithmic dosage studies may investigate a more precise therapeutic dosage curve. MMC is a viable and potentially alternate option to treatment with oral hypoglycemic agents for reversing hyperglycaemia, as shown in this *in vivo* animal experimental study. Efficacy studies conducted at low and high dose using the STZ-induced diabetes animal model for hyperglycaemia showed that MMC is effective in reducing blood sugar levels in a manner comparable to that of glibenclamide-treated PC and maintaining both PC (non-diabetic non-treated) and DC (diabetic, non-treated) groups for validation.

Future studies may be attempted to elucidate the mode of action of MMC that is described by *tikta-kashaya rasa* (the taste of bitter-astringent), and *kapha-pitta shamaka* (reduction of *doshas* that correspond to less sticky mucous, heaviness, acidity and heat in the body) according to Ayurveda. These studies should seek to reveal whether the herbs work to stimulate the pancreatic ß-cells directly or increase the sensitivity of the peripheral tissue to insulin, or whether they work through alternate mechanisms that reduce hyperglycaemia. The effects of MMC on specific organ systems, as well as actions of specific biomarkers on the body should be investigated. The antiinflammatory effect of this polyherbal compound needs to be elucidated as well, both using *in vitro* biochemical studies of suppression of inflammatory mediators, as well as quantification of the molecular agents in MMC thought to produce antioxidant or antiinflammatory effects.

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Conflicts of interest:

There are no conflicts of interest.

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