Evaluation of Antihyperglycemic and Hypoglycemic Activities of Shadguna Makaradhwaja and Guduchi Ghana in Swiss Albino Mice

Vaibhav A Charde¹, Kishor P Patel², Harmeet Kaur³, Chandrashekhar Jagtap⁴, Mukesh Nariya⁵, Biswajyoti Patgiri⁶, Soma N Murthy⁷, Pradeepkumar Prajapat⁸

ABSTRACT

Background: Diabetes mellitus (DM) is a group of metabolic disorders which share the common phenotype of hyperglycemia that occurs due to defects in insulin secretion, insulin action, or sometimes both. Nowadays DM is one of the leading causes of morbidity and mortality. Many herbomineral drugs possess the potential of lowering BSLs, and these are found effective when tested in experimental animal models. Makaradhwaja is one such well-known herbomineral preparation used by Ayurvedic fraternity due to its therapeutic properties to combat Madhumeha (DM). Makaradhwaja is a herbomineral drug prepared by Kupipakwa method. Shadguna Makaradhwaja (SM) is said to be superior to Owiguna and Triguna Makaradhwaja as Balijarana potentiate the therapeutic efficacy of the drug. Guduchi (Tinospora cordifolia L.) is reported to be a potent antidiabetic drug in Ayurveda. Guduchi, a well-known anti-hyperglycemic drug according to Ayurvedic classics, also acts as an antioxidant, immunomodulator, and a rejuvenator. The dried aqueous extract of Guduchi is used with Sindur Kalpana in DM. Different texts of Ayurved have mentioned antidiabetic properties of honey. Honey acts as a vehicle drug (Yogavahi) when mixed and used with herbomineral formulations. It is used as a vehicle drug with Makaradhwaja. For dose formation, honey is used as a liquid media to prepare suspension. It has been found effective in DM also.

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders, which share common phenotype of hyperglycemia that occurs due to defects in insulin secretion, insulin action, or sometimes both. Nowadays DM is one of the leading cause of morbidity and mortality. Many herbomineral drugs possess the potential of lowering BSLs, and these are found effective when tested in experimental animal models. Makaradhwaja is one such well-known herbomineral preparation used by Ayurvedic fraternity due to its therapeutic properties to combat Madhumeha (DM). Makaradhwaja is a herbomineral drug prepared by Kupipakwa method. Shadguna Makaradhwaja (SM) is said to be superior to Owiguna and Triguna Makaradhwaja as Balijarana potentiate the therapeutic efficacy of the drug. Guduchi (Tinospora cordifolia L.) is reported to be a potent antidiabetic drug in Ayurveda. Guduchi, a well-known anti-hyperglycemic drug according to Ayurvedic classics, also acts as an antioxidant, immunomodulator, and a rejuvenator. The dried aqueous extract of Guduchi is used with Sindur Kalpana in DM. Different texts of Ayurved have mentioned antidiabetic properties of honey. Honey acts as a vehicle drug (Yogavahi) when mixed and used with herbomineral formulations. It is used as a vehicle drug with Makaradhwaja. For dose formation, honey is used as a liquid media to prepare suspension. It has been found effective in DM also.

The Balijarana (addition of sulfur for digestion) and Ashtasamskaras (eight processes to purify mercury) are given much importance in the field of Rasashastra (iatrochemistry), but no work is reported till date with use of Talastha Swarna powder (bottom residue). Shadguna Makaradhwaja was prepared by two methods using SMV as well as SMR powder (residue at the bottom) form.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders, which share common phenotype of hyperglycemia that occurs due to...
The study may provide leads to assess the role of gold form in the preparation of SM used for DM. Keeping this in view, an attempt has been made to compare both the forms of SM, viz., SMV and SMR, for their antihyperglycemic and hypoglycemic activities.

Materials and Methods

Test Drugs

SMV and SMR were prepared in Rasa Shastra and Bhaishajya Kalpana department, IPGT and RA, Jamnagar, Gujarat, India, by adopting the standard manufacturing procedures. Two samples of SM, viz., one from SMV (thin foils of gold) and other prepared from SM powder (bottom residue) were prepared in the ratio of 1:8:48 [ratio of Swarna (Au), Parada (Hg), and Gandhaka (S)] and subjected to Kupipakwa through vertical electric muffle furnace for 36 hours.17 Honey which was used as a vehicle control was of Indian Honey marketed by Azad Khadi Gramodyoga Bhandar, approved by Government of India and was easily available.

Animals

Swiss albino mice of either sex weighing 30 ± 5 g were used in the experimental study. Animals were obtained from the animal house attached to Pharmacology Laboratory of Institute, IPGT and RA, Gujarat Ayurved University, Jamnagar, for experiments and maintained under standard experimental and husbandry conditions. The animals were housed in cage made of polypropylene with stainless steel top grill. The dry wheat (post hulled) waste was used as the bedding material and was changed every morning. The animals were exposed to 12-hour light and 12-hour dark cycle with the relative humidity of 50 to 70% and the ambient temperature during the period of experimentation was 22 ± 0.3°C. Animals were fed with Amrut brand rat pellet feed supplied by Pranav Agro Mills Pvt. Limited and drinking water ad libitum. Experiments were carried out after obtaining the permission from Institutional Animal Ethics Committee (IAEC/16/2014/04).

Dose Selection and Schedule

The classics of Rasashastra describe the dose of Makaradhwaja from 1 Ratti (125 mg) to 2 Ratti (250 mg).19 where the dose of Makaradhwaja comes as 14.63 mg. Shadguna Makaradhwaja has been given twice a day along with GG and honey as Anupana (vehicle).20 Hence, the final human dose of Makaradhwaja is 14.63 mg along with 235.37 mg of GG twice a day. Dose was calculated by extrapolating the human dose to animals based on the body surface area ratio9 which comes out to be 65 mg/kg body weight of mice. Test drugs were mixed in honey and distilled water in suitable concentration to make the suspension and administered to animals by oral route with gastric oral cannula according to their respective body weight. A total of 42 mice were selected for the experiment. Animals were divided randomly to relevant groups of six each after 7 days of acclimatization. Both activities were then evaluated in the test drug per the following protocols.

Antihyperglycemic Activity22

Swiss albino mice of either sex were randomly divided into five groups of six each (Table 1). Second group served as a standard control group to which GB (0.65 mg/kg) was administered. Animals were fasted overnight prior to experiment and in the morning the initial fasting BSL was measured with the help of One Touch EzGluco test strips per user guideline after anaesthetizing the animals with ether and collecting the blood sample from the tail vein following aseptic conditions. The vehicle control drug (VC) and GB were administered to the respective groups. The BSL was recorded after 1, 2, 3, and 5 hours of the test drug administration for assessing the hypoglycemic effect.

Antihyperglycemic Activity22

Swiss albino mice of either sex were randomly divided into five groups of six each. The animals were fasted overnight prior to experiment and the fasting initial BSL was measured. SMV, SMR, VC, water control group (WC), and GB were given to the respective group of animals per the body weight. After 1 hour of drug administration, glucose (5 g/kg) solution was administered to second, third, and fourth groups orally by dissolving it in distilled water. Thereafter, the BSL was recorded at 30, 60, 90, and 120 minutes of post-glucose overload for assessing the antihyperglycemic activity since glucose solution was given.

Statistical Analysis

The results were presented as mean ± standard error of the mean. Data generated during the study were subjected to Student’s “t” test for paired and unpaired data to assess the statistical significance, and the significance level was set at $p < 0.05$.

Results

The results of hypoglycemic study proved that GB showed highly significant decrease in blood glucose level (BGL) in overnight fasted animals at all time intervals in comparison to initial as well as WC mice. In hypoglycemic study, SMV showed 18.2, 27.23, 34.88, and 47.41% while SMR showed 4.85, 20.52, 29.70, and 44.3% reduction in BGL at all time intervals in comparison to the initial as well as control group of animals at all time intervals. The group treated with SMV produced significant decrease in BGL in overnight fasted animals at all time intervals in comparison to initial as well as control group of animals. The group treated with SMR produced significant decrease in BGL at all time intervals in comparison to initial values and produced significant decrease in fasting blood glucose level after 2 and 5 hours in comparison to the control group of animals. Overall, SMV produced pronounced hypoglycemic effect followed by SMR in normal mice. However, the BGL in drug-treated groups was still within the normal range (Tables 2 and 3).

The results of antihyperglycemic study indicated that GB produced marked and highly significant antihyperglycemic effect in comparison to its initial value as well as WC at all time intervals. SMV showed 81.73, 49.23, 14.8, and 0% and SMR showed 56.38, 42.23, 1.16, 21.58% reduction in BSL at 30, 60, 90, and 120 minutes, respectively. Both SMV and SMR produced significant increase in BGL.

Table 1: Grouping of animals

| Group | Animals                  |
|-------|--------------------------|
| I     | Water control group (10 mL/kg, po) |
| II    | Standard control group, glibenclamide (0.65 mg/kg, po) |
| III   | Vehicle control group received honey in distilled water |
| IV    | SM prepared from Swarna Varkha + GG (520 mg/kg, po) |
| V     | SM prepared from bottom residue + GG (520 mg/kg, po) |
Antihyperglycemic and Hypoglycemic Activities of Shadguna Makaradhwaja

Hypoglycemia is an abnormally diminished content of glucose in the blood. Recently drugs with α-glucosidase inhibitory effects have also been introduced. The basis for their development was the fact that in the intestine only the monosaccharides such as glucose

Discussion

Table 2: Effect of test drugs on blood sugar level in normal overnight fasted Swiss albino mice at 1- and 2-hour intervals

| Groups | Initial (mg/dL) | 1 hour | % decrease to initial | 2 hours | % change to initial |
|--------|----------------|--------|----------------------|---------|--------------------|
| WC     | 119.83 ± 2.24  | 116.00 ± 4.80 | 3.28 ± 3.20  | 99.50 ± 4.70** | 17.05 ± 3.06↓ |
| GB     | 103.16 ± 4.96  | 71.00 ± 13.68** | 31.45 ± 12.92** | 59.67 ± 8.61*** | 42.05 ± 8.16** |
| VC     | 103.0 ± 4.73   | 96.25 ± 9.63  | 7.17 ± 3.65↓  | 83.75 ± 5.07** | 18.63 ± 8.12↓ |
| SMV    | 115.33 ± 6.72  | 93.00 ± 6.154** | 18.20 ± 6.98** | 82.67 ± 1.26** | 27.23 ± 3.31↑ |
| SMR    | 100.00 ± 5.92  | 95.00 ± 10.5* | 4.85 ± 5.2↑   | 77.80 ± 8.55*** | 20.52 ± 5.80↓ |

Data: mean ± SEM; ↓ decrease; *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001 when compared to respective initial values (paired ‘t’ test); $p < 0.05, ##p < 0.02, ###p < 0.01, ####p < 0.001 when compared to control group (unpaired ‘t’ test)

Table 3: Effect of test drugs on blood sugar level in normal overnight fasted Swiss albino mice at 3- and 5-hour intervals

| Groups | Initial (mg/dL) | 3 hours | % change to initial | 5 hours | % change to initial |
|--------|----------------|---------|---------------------|---------|--------------------|
| WC     | 119.83 ± 2.24  | 88.83 ± 4.90** | 25.71 ± 4.42↑ | 78.83 ± 2.85** | 34.11 ± 3.08↑ |
| GB     | 103.16 ± 4.96  | 47.16 ± 5.90*** | 53.88 ± 5.95↑ | 51.33 ± 5.21*** | 50.05 ± 4.93↑ |
| VC     | 103.0 ± 4.73   | 78.00 ± 7.80* | 24.38 ± 5.21↑ | 67.00 ± 6.56** | 35.37 ± 3.06↑ |
| SMV    | 115.33 ± 6.72  | 74.0 ± 3.87** | 34.88 ± 3.91↑ | 59.83 ± 1.58*** | 47.41 ± 2.20** |
| SMR    | 100.00 ± 5.92  | 71.83 ± 11.23* | 29.70 ± 6.83↑ | 55.83 ± 5.95*** | 44.37 ± 3.30↓ |

Data: mean ± SEM; ↓ decrease; *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001 when compared to respective initial values (paired ‘t’ test); $p < 0.05, ##p < 0.02, ###p < 0.01, ####p < 0.001 when compared to control group (unpaired ‘t’ test)

Table 4: Effect of test drugs on blood sugar level in glucose overloaded Swiss albino mice at 30- and 60-minutes intervals

| Groups | Initial (mg/dL) | 30 minutes (mg/dL) | % change to initial | 60 minutes (mg/dL) | % change to initial |
|--------|----------------|--------------------|---------------------|--------------------|---------------------|
| WC     | 97.00 ± 4.21   | 276.20 ± 7.78***** | 85.90 ± 8.75↑ | 155.40 ± 12.45↑ | 92.36 ± 8.71↑ |
| GB     | 88.33 ± 3.70   | 120.0 ± 3.36***   | 7.10 ± 1.47↑      | 76.66 ± 3.03***   | 13.10 ± 1.33↓   |
| VC     | 122.33 ± 8.54  | 243.75 ± 41.55*   | 97.06 ± 23.73↑    | 168.25 ± 15.22*   | 37.97 ± 7.36↑   |
| SMV    | 94.00 ± 5.52   | 169.75 ± 8.80***  | 81.73 ± 10.73↑    | 137.75 ± 7.42↑    | 49.23 ± 15.78↑  |
| SMR    | 116.60 ± 6.79  | 181.80 ± 17.42*** | 56.38 ± 13.79↑    | 166.80 ± 15.30*   | 42.23 ± 6.20↑   |

Data: mean ± SEM; ↑ increase; ↓ decrease; *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001 when compared to respective initial values (paired ‘t’ test); $p < 0.05, ##p < 0.02, ###p < 0.01, ####p < 0.001 when compared to control group (unpaired ‘t’ test)

Table 5: Effect of test drugs on blood sugar level in glucose overloaded Swiss albino mice at 90- and 120-minutes intervals

| Groups | Initial (mg/dL) | 90 minutes (mg/dL) | % change to initial | 120 minutes (mg/dL) | % change to initial |
|--------|----------------|--------------------|---------------------|--------------------|---------------------|
| WC     | 97.00 ± 4.21   | 117.60 ± 3.43*     | 22.57 ± 8.07↑      | 106.80 ± 2.76      | 10.92 ± 5.53↑     |
| GB     | 88.33 ± 3.70   | 65.33 ± 1.82****   | 25.65 ± 2.41↑      | 60.00 ± 1.71*****  | 31.64 ± 2.66↓     |
| VC     | 122.33 ± 8.54  | 125.25 ± 1.56      | 4.52 ± 8.84↑      | 106.25 ± 3.09*     | 12.01 ± 4.41↓     |
| SMV    | 94.00 ± 5.52   | 106.50 ± 7.71      | 14.80 ± 11.81↑    | 93.50 ± 5.31       | –                  |
| SMR    | 116.60 ± 6.79  | 117.20 ± 4.65      | 1.16 ± 3.65↑      | 91.40 ± 8.25       | 21.58 ± 5.31↓     |

Data: mean ± SEM; ↑ increase; ↓ decrease; *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001 when compared to respective initial values (paired ‘t’ test); $p < 0.05, ##p < 0.02, ###p < 0.01, ####p < 0.001 when compared to control group (unpaired ‘t’ test)
and fructose can be transported from the intestinal lumen into the blood stream. Hence, complexes such as starches, oligosaccharides, and disaccharides must be first converted to monosaccharide molecules before they are absorbed from the intestine. The enteric enzymes α-amylase and α-glycosidase that are attached to the intestinal brush border catalyze the breakdown of the complex sugars to monosaccharose. If their activity is inhibited, the intestinal absorption of the carbohydrates would be decreased. This will lead to fall in the BSL. This will result in insulin sparing effect.26 The test drug needs to be assessed for this effect.

It is obvious from data that metformin (GB) is a better hypoglycemic agent as compared to both the Ayurvedic preparations. Overall, SMR produced pronounced antihyperglycemic effect followed by SMV in glucose overloaded hyperglycemic mice. However, the BGL in drug-treated groups reached normal range after 90 minutes and remained in normal range even after 120 minutes. Both the drugs produced hypoglycemic and antihyperglycemic effect in mice. SMV showed more hypoglycemic effect, while SMR showed more antihyperglycemic effect statistically. It infers that the drugs may show high glycemic lowering activity in empty stomach. Ayurvedic classics also advocate using antidiabetic drugs in empty stomach. It is quoted that if drug is taken empty stomach (before meal), the therapeutic effect of drug increases.27 Contemporary evidences also support these ancient claims.28 Honey also has been mentioned as a novel anti-diabetic agent,29 but nowadays due to adulteration, it is not prescribed for patients with glucose intolerance. Evidence suggests that fructose consumption prolongs gastric emptying,30 which may slow down the rate of intestinal absorption.31 In addition to fructose, oligosaccharides such as palatinose (isomaltulose) present in honey have been reported to delay digestion and intestinal absorption of glucose resulting in reduced glycemia.32,33 In case of Makaradhwaja, it is chemically sulfides of mercury (HgS) which are well absorbed in stomach in the presence of gastric juice in very minute quantity. But no evidence of mercury was found as traces in the body fluids after absorption of these sulfides. It means only sulfur contents are absorbed in stomach and not mercury, which is excreted with feces.34 Mercury is said to be a bioenhancer in Ayurvedic pharmaceutical science. In addition to maintaining its own activity, it increases the therapeutic activity of the other substances by many folds.35 Although the mechanism of its absorption and excretion is still not clearly understood, the exact mechanism needs to be established further. Likewise the action of sulfurylurea group of oral hypoglycemic agents, Makaradhwaja, in which sulfur is processed by mercury and gold, may stimulate the beta cells of pancreas which in turn stimulates the secretion of insulin and thus lowers the BSL.15 Pronounced hypoglycemic effect of Varkha might be due to more gold content in the sublimed product, while the anti-hyperglycemic effect may be due to repeated heating as SMR was prepared from residue. It is understood that repeated heating causes stability, which affects the absorption and assimilation of the drug in the body. This fact is also evident by the analytical studies.

CONCLUSION

Study provided definite evidence for the presence of hypoglycemic and antihyperglycemic activity in SM. However, among two samples, SM prepared with Varkha demonstrated better hypoglycemic activity while SM prepared with residue possesses better antihyperglycemic action.

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हिंदी सारांश
स्विस एल्बिनो माइस में छड़गुण मकरध्वज एवं गुड्डी घन की एंटीहाइपरग्लाइसेमिक और हाइपोग्लाइसेमिक गतिविधियों का मूल्यांकन

पृष्ठभूति: डायबिटीज मेलिटस चयापचय संबंधी विकारों का एक समूह है जो हाइपरग्लाइसेमिक के सामान्य फेनोटाइप को शेयर करता है जो इंसुलिन साव, इंसुलिन कार्य या कभी-कभी दोनों में दोषों के कारण होता है। हीरोस्मिनरल्स सॉर्टेंटिक ऑर्डर हाइपरग्लाइसेमिक एंजीटों के लिए उनकी क्षमता, उपयुक्तता और नगण्य दुष्प्रभावों के कारण वैकल्पिक हो सकता है। मकरध्वज आयुर्वेद में प्रयुक्त शक्तिशाली मधुमेह रोधी (एंटीडायबिटिक) औषधियों में से एक है।

उद्देश्य: स्विस एल्बिनो माइस में गुड्डी घन (जीजी) के साथ अपक्षक तलस्थ घवर्ण गॉडर (एसएमआर) के अवशेष से और घवर्ण वर्ख दवाओं का योग छड़गुण मकरध्वज (एसएम) की हाइपरग्लाइसेमिक और एंटीहाइपरग्लाइसेमिक गतिविधियों का मूल्यांकन करना।

सामग्री और विधियां: ओरल ग्लुकोज टॉर्लेंस परीक्षण और 18 घंटे फास्टेड माइस के मॉडल का प्रयोग किया गया। 65 मिग्राफिक्रिया के एक खुराक में शहद के साथ जीजी सहित छड़गुण मकरध्वज (5.85 : 94.15 अनुपात में) दिया गया। मानक औषधि के रूप में विलबेनकल्येमाइड (जीजी; 0.65 मिग्राफिक्रिया) का उपयोग किया गया।

परिणाम: हाइपरग्लाइसेमिक अध्ययन में 1,2,3 और 6 मिनट और क्रमशः एसएमआर ने 18.2, 27.83, 34.88 और 47.41% जबकि एसएमआर ने 4.85, 20.52, 29.90 और 44.3% पाइ गई। एंटीहाइपरग्लाइसेमिक अध्ययन में 30, 60, 90 और 120 मिनट क्रमशः एसएमआर ने 81.73, 49.23, 14.8 और 0% और एसएमआर ने 56.38, 42.23, 1.16 और 21.58% पाइ गई। निर्यात समूह की तुलना में दोनों परिणाम सांद्रितिक रूप से महत्वपूर्ण थे।

निष्कर्ष: निर्यात समूह की तुलना में परीक्षण औषधियों ने महत्वपूर्ण हाइपरग्लाइसेमिक और एंटीहाइपरग्लाइसेमिक प्रभाव दर्शाए। घवर्ण वर्ख ने अधिक हाइपरग्लाइसेमिक प्रभाव जबकि एसएमआर ने एंटीहाइपरग्लाइसेमिक प्रभाव दर्शाए।

मुख्य शब्द: आयुर्वेद, डायबिटीज मेलिटस, हीरोस्मिनरल्स, मकरध्वज, रसशारस्त्र।