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AN EXPERIMENTAL APPRAISAL OF THE SAFETY AND EFFICACY OF AN AYURVEDIC HERBOMINERAL COMPOUND MEHAKULANTHAKA RASA

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ABSTRACT

Mehakulanthaka Rasa is a herbo mineral compound used in the management of Diabetes mellitus. The formulation is mentioned in one of the latest classics, Bhaishajyaratnavali. It is a combination of Abhraka Bhasma, Vanga Bhasma, Parada, Gandhaka, Shilajatu and around 15 herbal ingredients. Though herbo minerals are highly acclaimed for their therapeutic efficacy, at present there are apprehensions regarding the safety and efficacy of the above said preparations. Need of the hour is to revalidate the safety and efficacy of these formulations in a more meticulous manner, to improve the global acceptance of Ayurveda. In the present study an attempt was made to evaluate the acute toxicity and the anti diabetic property of Mehakulanthaka Rasa. The anti diabetic potential was evaluated in streptozotocin induced diabetic rat model. The test drug was given in two different doses to the experimental diabetic rats for a duration of 28 days. Biochemical parameters like blood sugar level, SGOT, SGPT, lipid profile and total protein were evaluated. A significant reduction in blood glucose level was observed in the diabetic rats along with reduction in other biochemical parameters also. The study concluded that Ayurvedic formulations are safe and effective when proper guidelines are followed during the manufacturing of these formulations.

KEYWORDS: Mehakulanthaka Rasa, safety, efficacy, streptozotocin.

INTRODUCTION

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease.[1] The chronicity of disease is associated with many co morbidities like obesity, depression, cardiovascular problems and serious micro vascular complications like blindness, renal failures etc. The pre diabetic stages also carry high risk for cardiovascular diseases (CVDs) and clustering of the cardiovascular risk factors or the metabolic syndrome.[2] The increased prevalence of DM is predicted to occur virtually for every nation, while the greatest increase is expected in developing countries.[3] As a proportion of the population, diabetics make up about 8% of India's total - less than the U.S. figure of 10.3%, according to the International Diabetes Federation.[4] Research on diabetes in India has meanwhile been scant, making it hard to build up a true picture of the crisis and, in turn, formulate an adequate national response. A recent report, estimated that there were 62.4 million diabetics across the country. The study also hypothesized that an additional 77.2 million Indians could be pre diabetic and that by 2030, 100 million Indians could suffer from the disease.[5]

Although lifestyle changes remain the corner stone of Diabetes management, individually they are insufficient to enable the patients to maintain normal blood sugar level. Oral hypoglycemic agents form a primary part of treatment but prominent side effects of such drugs are the main reason for a number of people seeking alternative therapies that may have less side effects. It is important to note that multiple defects in pathophysiology of diabetes in modern medicine is still being unraveled.[6] The importance of oxidative stress and deficiency of micro elements in the pathophysiology of diabetes is a latest addition.[7]

Rasa Yogas, the organometallic formulations of Ayurveda have been in use in the treatment of diabetes for centuries. Mehakulantaka Rasa is one such excellent herbomineral preparation described in the Pramehadhikara of one of the prominent Rasashastra texts of 19th century; Bhaishajya Ratnavali.[8] The mineral part of compound includes Abhraka bhasma, Vanga bhasma, Parada, Gandhaka and Shilajatu while herbal components are Triphala, Trikatu, Dadima, Bilwa etc. There is accumulating evidence that metabolism of several micro elements is altered in diabetes.[9] The effectiveness of herbomineral drugs has been well established and well documented in the form of classics attributed to them. However in the wake of safety and efficacy issues' presenting a challenge to the tradition system it is

necessary to allay the prevailing notions regarding safety and efficacy of Rasoushadis. Considering these facts an attempt was made to revalidate the safety and efficacy of the herbomineral compound Mehakulanthaka rasa(MKR).

MATERIALS AND METHODS

Test Drug

Mehakulanthaka Rasa (MKR) was prepared in the Dept. of Rasashastra and Bhaishajya Kalpana, N.I.A. Jaipur as explained in the pharmaceutical study.

Chemicals

1. Streptozotocin (STZ) was purchased from Himedia Ltd., Mumbai.
2. Glibenclamide was purchased from R.N Medical store, Jaipur
3. Other chemicals from Metro Trading Corporation, Jaipur.

Selection Of Animals And Animal Care

The study was conducted on mature Wistar strain male albino rats, weighing 150 -200g. Animals were acclimatized for a period of seven days in laboratory conditions prior to the experiments. Rats were housed in poly propylene cages (six rats per cage), at an ambient temperature of 25 ± 2°C with 12 h light: 12 h dark cycle in the animal house of NIMS, Jaipur. The animals were provided with standard pellet diet and water ad libitum. Ethical clearance was obtained from Institutional Animal Ethics Committee, before conducting the experiment (**IAEC Clearance no: *NU/NIP/IAEC/12/003***).

Dose Selection And Administration Of Drug

In the present study the dose of Streptozotocin was fixed as 45mg/kg body weight by intra peritoneal injection for one day for induction of diabetes in rats. Dose fixation was done as per the earlier studies.[10] Dose of the reference drug Glibenclamide was fixed as 0.5 mg/kg body weight orally;[11] daily for 28 days. The drug was dissolved in a suspension of 5% gum acacia solution and was administered orally according to the body weight of the animals with the help of feeding needle attached to a disposable 1ml syringe. Two doses of the test drug Mehakulanthaka Rasa was fixed from the acute toxic study as 200mg/kg and 400mg/kg body weight orally for 28 days .Sample of test drug was taken in requisite quantity in porcelain mortar and 5% gum acacia suspension was added, the formed mixture was further ground for 5 minutes and the volume was made up with distilled water, so as to contain the required dose in solution.

Induction of Diabetes Mellitus

Rats were fasted overnight before inducing diabetes with Streptozotocin. A freshly prepared solution of Streptozotocin dissolved in sodium citrate buffer (pH-4.5) was administered intra-peritoneal at a dose of 45mg/kg body weight. Diabetes was established in STZ treated rats over a period of 48 hours. After 2 days the animals with blood sugar level >250mg/dl were considered as diabetic and included in the study.

Acute oral toxicity study

In the present study observation of acute toxicity was carried out according to OECD guideline 423.[12] Healthy young adult Wistar strain female albino rats weighing 150-200g, were employed for the study. The animals were randomly selected, marked to permit individual identification, and were kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions .Three animals were used for each step. The dose level to be used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. Animals were observed for their gross behavior and mortality.

Drug Efficacy Study

Table .1 Grouping of rats

Group	No of rats	Treatment
Group I: Normal Control	6	5% gum acacia suspension orally for 28 days
Group II: Diabetic Control	6	5% gum acacia suspension orally for 28 days
Group III: Diabetic + *Test drug*	6	MKR(200 mg/kg b.wt.) for 28 days
Group IV: Diabetic + *Test drug*	6	MKR(400 mg/kg b.wt.) for 28 days
Group V: Diabetic + Standard drug	6	Glibenclamide (0.5mg/kg b.wt.) for 28 days

Test drug and vehicles were administered to respective groups at morning hours and continued for 28 days. Blood glucose level was further recorded from all the animals of all groups at 7days interval (7th day, 14th day, 21st day and 28th day). Blood samples were obtained through puncture tail vein and collected blood samples were analyzed by One touch Glucometer (*Elegance*, Germany).Weight of rats were also recorded weekly during the study period of 28 days. At the end of the 28 days study, the rats were anesthetized with diethyl ether following a fast of 12 hrs. Blood was drawn by retro orbital puncture, allowed to clot and then centrifuged at 3000 rpm for 20 minutes. Sera sample was collected and used for various biochemical estimations like serum total protein, serum total cholesterol, serum triglycerides, SGOT and SGPT.

Statistical Analysis: Values are given as mean + SEM (standard error of the mean) and were compared using one way ANOVA with Tukey-Kramer multiple comparison test, to judge the difference among various groups. Values of $P<0.05$ were considered statistically significant.

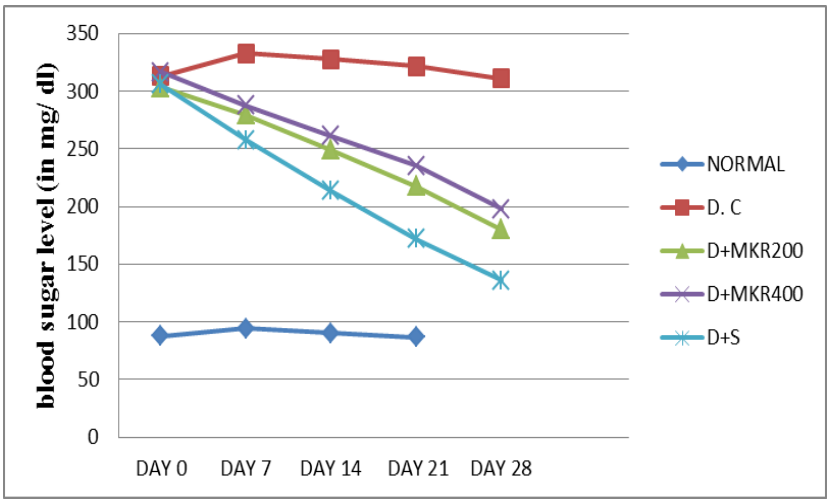
OBSERVATIONS AND RESULTS

1. Effect on body weight

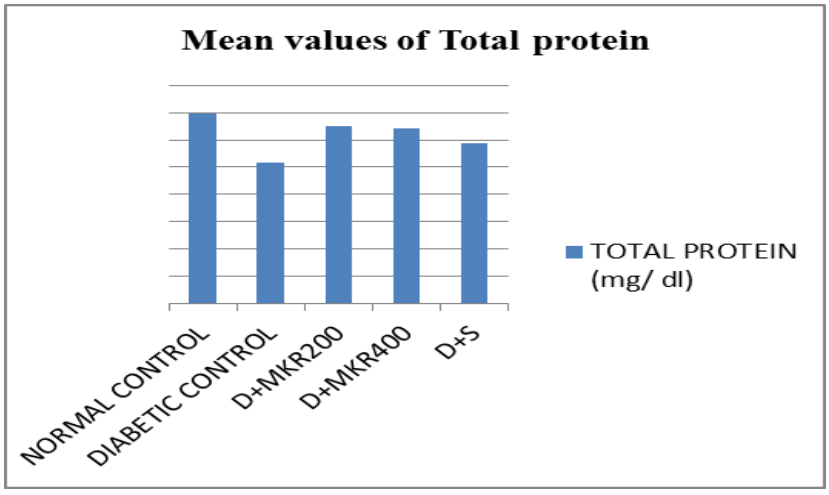
Table 2: Initial and final body weights of rats.

Group	Body weight (g)			
	Initial	Final	Change in wt.	% Change
Normal control (NORMAL)	182±1.96	197.8±2.08	15.83 ± 1.19 ↑	8.69
Diabetic control (D.S)	177.6±2.29	163.5±2.81	14.16 ±1.94 ↓	7.97
Diabetic+MKR200 (D+MKR200)	160±3.33	164.3±3.43	3.66 ±0.516 ↑	2.25
Diabetic+MKR400 (D+MKR400)	157.6±2.74	162.6±2.55	5.0 ± 0.36 ↑	3.17
Diabetic+ standard (D+S)	162.6±2.74	174.8±3 2.75	12.1± 0.94 ↑	7.4

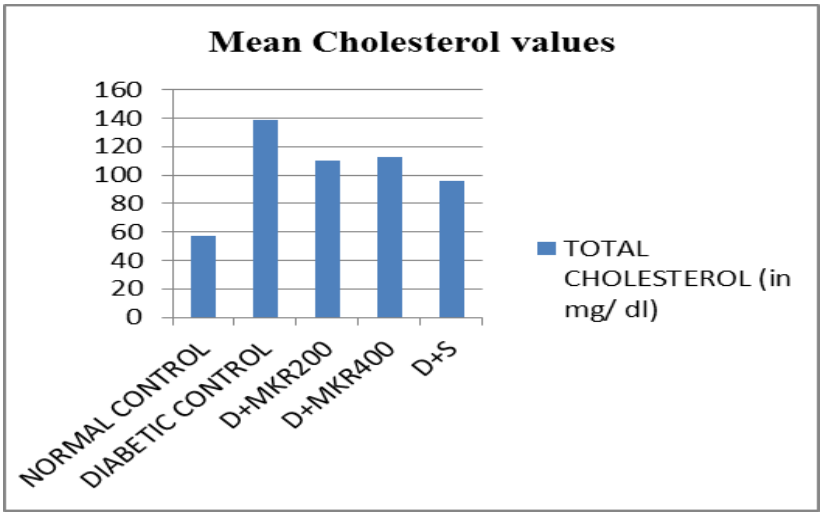
2. Effect on blood sugar level



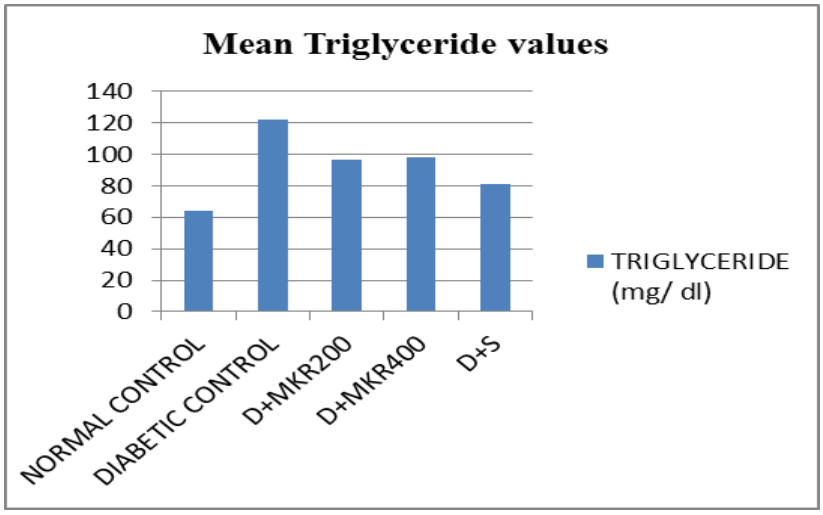
Graph.1: Blood glucose levels of 5 groups on day 0,7,14,21 and 28



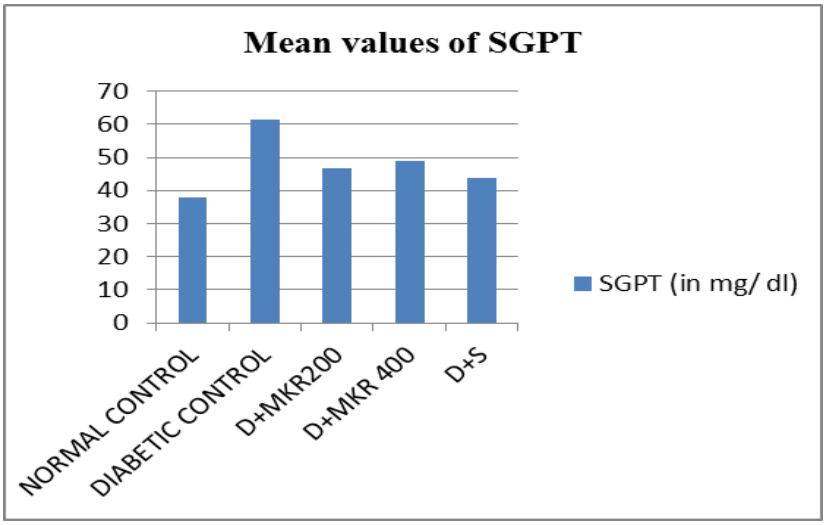
Graph 2: Mean values of total protein



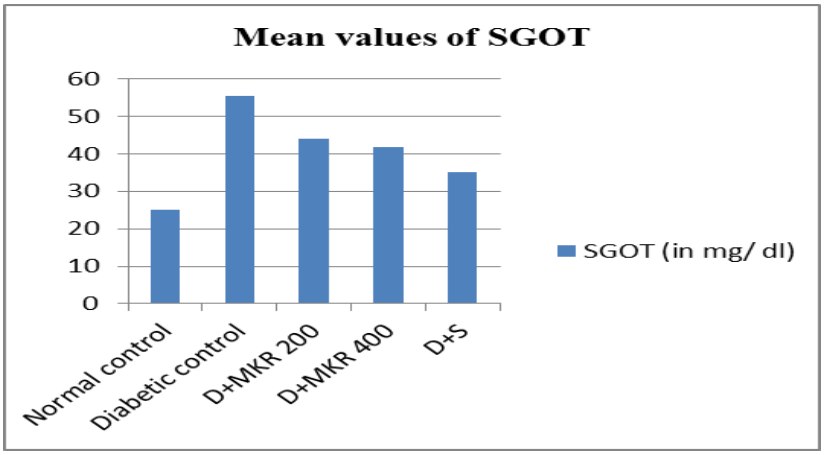
Graph 3: Mean values of total cholesterol



Graph 4: Mean triglyceride values



Graph 5: Mean values of SGPT



Graph 6: Mean values of SGOT

DISCUSSION

Mehakulantaka Rasa is an excellent herbomineral preparation described in the classical text of Rasashastra for the treatment of Prameha .The present study was designed to evaluate the acute oral toxicity as well as the antidiabetic property of the drug. No gross changes in behavior could be observed in the acute toxicity study. MKR was found to be safe at the maximum dose of 2000mg/kg. Following the study, one tenth and one fifth of the maximum dose of MKR tested for acute toxicity study was selected for evaluation in Streptozotocin induced diabetes.

For evaluating antidiabetic activity Streptozotocin induced diabetic rat model was chosen. Glibenclamide was taken as standard drug. Streptozotocin is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitroso urea. It

causes hyperglycaemia mainly by its direct cytotoxic action on the pancreatic beta cells.[13] STZ is a preferred agent to induce experimental diabetes since it has some advantages over Alloxan such as, relatively longer half-life (15 min), sustained hyperglycaemia for longer duration and the development of well characterized diabetic complications with fewer incidences of ketosis as well as mortality., insulinotropic and other hypoglycaemic/ antihyperglycaemic activities.[14]

Analysing the results of the present study it was found out that the diabetic rats showed a weight loss of around 14g .Administration of test drug improved the weight of animals where the maximum weight gain was found in the standard drug treated group with 7.4% followed by 3.17 and 2.25 respectively in MKR400 and MKR200 groups. Regarding blood sugar levels the activity profile indicated a persistent lowering in blood glucose level by all the treated groups. Highest % of decrease was shown by Glibenclamide treated group followed by MKR200 and MKR 400 treated groups respectively. Even though MKR200 showed a greater decrease in the blood sugar level it was not statistically significant when compared toMKR400 treated group.

Induction of STZ diabetes lead to significant elevation in SGOT and SGPT values. The STZ induced SGOT elevation was significantly reversed by all the three treated groups with a greater reduction shown in standard treated group, showing good correlation with its anti-hyperglycemic activity. Here also no significant difference were noted when comparison between two different dosage groups were done. In the case of SGPT no significant decrease was shown by the test drug, while standard treated group showed significant decrease.

Significant decrease in the level of Total proteins were noted after STZ induction in rats. Significant increase was shown by MKR 400 and standard treated group. While MKR 200 raised the level, the result was not statistically significant when compared to diabetic contropl group.

Lipids play an important role in the pathogenesis of diabetes mellitus and the level of serum lipids is usually raised in diabetes and such an elevation represents risk factors for coronary heart disease. Induction of STZ diabetes lead to significant elevation in serum cholesterol and triglycerides level. A highly significant decrease was shown by both MKR200 and MKR400 treated group. Extremely significant decrease in cholesterol and triglycerides were noted in standard treated group. Thus correlation between anti-hyperglycemic and lipid

elevation attenuation was observed in all the three treated groups with varying level of significance.

This anti diabetic potential of the drug can be attributed to all of its ingredients. The trial drug Mehakulantaka Rasa is formulated in such a way each and every ingredient possess the antidiabetic potential and all of them have acted synergistically to each other. Classically, the ingredients like Vanga and Shilajatu are acclaimed as the choice drugs in treating diabetes. Apart from the classical claims various researchers have worked on each ingredient for their antidiabetic potential and reported the study. Salil et al, 2005, reported Shilajatu attenuates streptozotocin induced diabetes mellitus and decrease in pancreatic islet superoxide dismutase activity in rats.[15]

Insulin contain a large amount of sulphur(3.31%) which is in the form of cysteine .It has been claimed that venous blood of pancreas contain more total neutral sulphur than the arterial blood entering the organ. Large amount of sulphur in insulin has suggested that SH groups are a part of fundamental enzymatic systems in these cell and are concerned with the production of insulin. The selected formulation Mehakulanthaka Rasa comprises sulphur as one of the ingredients and the response it showed against the lowering of blood sugar, based on these facts, can be attributed to the presence of sulphur .Numerous studies published in the second half of last century evaluating the role of trace elements in diabetes have reported the deficiency of the same in diabetic individuals. Several studies have reported that the imbalance of some essential metals might adversely affect pancreatic islet and cause development of diabetes. It is also speculated that some reactive oxygen species (ROS) are produced during diabetes due to imbalance of essential metals. The presence of various elements in the trial drug is thus justified in their role in diabetes.

CONCLUSION

The drug Mehakulanthaka Rasa is having moderate anti diabetic activity when compared to the standard drug Glibenclamide. It can be concluded that Ayurvedic formulations are safe and effective when proper guidelines are followed during the manufacturing of these formulations.

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