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Research Article

AN EXPERIMENTAL STUDY OF *SWARNA MAKSHIKA BHASMA* AS ANTI DIABETIC MEDICINE

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ABSTRACT

Present research was taken in consideration to see the effect of *Swarna Makshika bhasma* (SMB) on serum sugar level, total cholesterol and triglyceride level. Healthy Charles foster albino rats having weight of 180- 200gm bred in the Central Animal Facility were used for the present study. Diabetes was induced by injecting intraperitoneally freshly prepared solution of Streptozotocin. Experiment was carried out in 7 groups and for total duration of 27 days. The effect of graded dose of SMB solution (5.85mg/kg, 11.25mg/kg and22.5mg/kg), standard drug glibenclamide and a combination of standard drug and medium dose of SMB was observed on Blood sugar level, total cholesterol and triglyceride level. SMB showed marked decrease in blood sugar level from 7th day onwards of treatment. The results with experimental drug SMB was comparable with standard drug glibenclamide.

Keywords : *Swarna Makshika Bhasma*, diabetes, blood sugar, total cholesterol, triglyceride

INTRODUCTION

In therapy of Ayurveda, *Rasaoushadhies* are used as quick savior of ailments . *Swarna Makshika Bhasma* (SMB) is one of these treasures of Ayurveda .The contemporary scientist always prefer reproducible and revalidated data to accept any molecule/substance as medicine . Therefore to generate facts and figures of efficacy and safety of *Swarna Makhika Bhasma* on diabetes we planned and executed this study.

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin[1]. Several drugs such as biguanides and sulfonylureas are presently available in allopathic system of medicine to reduce hyperglycemia in diabetes mellitus. Several new molecules are being developed with new progressive researches but none of these are free from any other unwarranted effect in body.

Therefore, it was felt that why not we should think out for an effective antidiabetic drug with other therapeutic effects. So out of the herbal drug phenomenon of Ayurveda we thought out of Ayurvedic minerallic compound which is required in a minimal dose and have higher shelf life and wide therapeutic range.

In our classics of Rasa Shastra, Swarna Makshika bhasma has been indicated as a solitary drug or in combination of other herbal/ metallic/ mercurial/ minerallic medicines, in the form of a compound to treat diabetes since last so many years.

MATERIALS AND METHODS

Animals

Healthy Charles foster albino rats (both male and female) having weight 0f 180- 200gm bred in the Central Animal Facility of IMS, BHU were used for the present study. These animals were acclimatized for two weeks. They were provided with adequate pellet diet and drinking water *ad libitum*. Rats were maintained in a well ventilated room with temperature at 28±5^{0}C. They lived in natural source of light and dark cycle (12:12) and housed in polypropylene cages with paddy husk bed which were changed on every 4-5 days. 'Principles of laboratory animal care' (NIH publication no. 82-23, revised 1985) guidelines were followed.

Drugs and Chemicals

1. Drugs
   a. *Swarna Makshika bhasma* (SMB) prepared by authors as research drug was used in the experiment.
   b. Glibenclamide (Daonil)
2. Kits

Kit	Company
Glucose Kit	Accurex Biomedicals Pvt. Ltd., Mumbai
Cholesterol Kit	Span Diagnostics Ltd., Surat
Triglyceride Kit	Accurex Biomedicals Pvt. Ltd., Mumbai

3. Chemicals
Streptozotocin

Induction of Diabetes

A freshly prepared solution of streptozotocin (45mg/Kg) in .1 M Citrate buffer(prepared by dissolving 105.07mg citric acid in 50ml distilled water and 147.05mg sodium citrate in 50ml distilled water and mixing the two liquids to make 100ml of citrate buffer), pH 4.5 was injected intraperitoneally in a volume of 1ml. After 48 hours of streptozotocin administration, rats having hyperglycemia (i.e. blood glucose level of 200- 300mg/dl) were taken for the experiment. Drugs were administered for a period of 7 days. On the 7th day drug was given one hour before the experiment and blood samples was collected from the retro-orbital plexus of the rats and serum was separated by centrifugation at 3000rpm for three minutes. Various biochemical parameters were estimated in the unhemolysed serum by kits.

Schedule of the experiment

In the experiment a total of 42 rats were taken. The rats were divided into 7 groups of 6 rats each. The details of the groups are as follows:

Group I: Normal untreated rats. Animals were maintained in experimental condition with no drug treatment and no induced diabetes. Animals were provided with .5% CMC solution.

Group II: Diabetic untreated rats. Diabetes was induced in this group and animals were provided with only .5% CMC solution and no drug treatment.

Group III: Diabetic rats treated with high dose of drug SMB (22.5mg/kg)

Group IV: Diabetic rats treated with medium dose of drug SMB (11.25mg/kg)

Group V: Diabetic rats treated with low dose of drug SMB (5.85mg/kg)

Group VI: Diabetic rats treated with standard drug Glibenclamide (600µg/kg)

Group VII: Diabetic rats treated with Glibenclamide plus medium dose of drug (SMB)

Treatment Protocol

The referred dose of Swarna Makshika bhasma in classics (Rasa Tarangini) is ½ Ratti- 2 Ratti. Therefore three graded doses of drug were taken to study the antidiabetic action. Drug solution was prepared in .5% CMC solution in distilled water. The three drug solutions of SMB and standard oral hypoglycemic drug glibenclamide were administered orally once daily. Control rats received .5% CMC only. The animals received the drug orally with the help of an orogastric tube in the volume of 1.0ml/100gm body weight.

The effect of Swarna makshika bhasma and standard drug Glibenclamide was observed on various biochemical parameters like blood glucose, total cholesterol and triglyceride. Total experiment was carried out in 27 days. Streptozotocin was administered on day one. On day third blood glucose level was estimated and mild diabetic (blood sugar > 200 mg/dL) rats were started with treatment. Treatment was continued and the biochemical parameters were estimated on day 10, 17 and 27.

Dose calculation & Preparation of Swarna Makshika Bhasma Solution

As discussed earlier three doses (65mg, 125mg, 250mg) of the drug Swarna Makshika bhasma was taken for study. As from the formula for calculation of animal dose

Total clinical dose (a) x 0.018 = animal dose / 200gms of rat
Lower Dose: 65mg x 0.018 = 1.17mg/200gm of rat = 5.85mg/kg
Medium Dose: 125mg x 0.018 = 2.25mg/200gm of rat = 11.25mg/kg
High Dose: 250mg x 0.018 = 4.5 mg/ 200gm of rat = 22.5mg/kg
[Conversion formula by Paget and Branes (1964)]

Tests applied

Data was analyzed by using SPSS software package. Paired sample t test and one way ANOVA were tests applied.

RESULTS

Moderate diabetes was produced by intraperitoneal administration of streptozotocin (STZ, 45mg/kg stat) to adult rats of either sex. The rats were put on fast for 18hrs before experimentations.

The effect of graded dose of SMB solution (5.85mg/kg, 11.25mg/kg and22.5mg/kg) was studied on Blood glucose level, total cholesterol and triglyceride level. SMB was administered once daily from 4th day of STZ administration. Glibenclamide (GLC) was given in the dose of 600µg/kg orally. GLC showed decrease in blood glucose level in diabetic rats. The experimental drug SMB showed marked decrease in blood glucose level from 7th day onwards. The results with experimental drug SMB was comparable with standard drug glibenclamide.

Table 1: Effect of SMB and glibenclamide on blood glucose level after 3rd, 10th, 17th and 27th day of treatment

		Blood Sugar Level (mg/dl)				
Group	**Treatment**	**Day 1**	**Day 3**	**Day 10**	**Day 17**	**Day 27**
I (NC)	.5%CMC	88.83±2.47	90.00±1.15	90.67±1.85	90.67±.95	90.50±.85
II(DC)	.5%CMC	89.33±1.47	278.67±7.74	280.33±7.60	275±7.80	273.33±4.09
III	SMB 22.5mg/Kg	88.17±1.30	280.33±3.16	239.17±4.05	209±2.63	200±1.80*
IV	SMB 11.25mg/Kg	89.50±1.98	278.67±4.62	236±4.48	209±3.84	199±3.02*
V	SMB 5.85mg/kg	86.83±2.07	273.67±4.18	205.17±1.68	172.67±2.95	164.00±2.17*
VI	GLC 600µg/kg	87.83±2.77	274.67±7.52	210.50±2.59	169.33±3.05	97.17±1.99*
VII	SMB 11.25mg/ Kg+GLC600µg/kg	84.67±2.24	272.17±5.75	212.00±2.20	169.33±2.68	101.50±3.14*

Results are mean±SEM of 6 rats in each group
* P value < .001 compared to Day 3
The level of significance of blood lowering effect of drug was observed by applying paired t test. The study was done between Day 3 and Day 27. All the treated groups show significant level of decrease in blood sugar level. Diabetic control group show a rise in blood sugar level on Day 3rd and this level is maintained till completion of the study. Similarly in normal control group there was non significant change in BSL during the whole study. From the table it can be concluded that the experimental drug SMB is decreasing the level of BSL but not up to the normal level like GLC. The study can be prolonged for longer duration to see whether it brings the BSL to normal level or not. It was also marked that the sugar level drops sharply on initial 7 days of treatment and further the value decreases slowly. There was no mortality observed in the whole study.

Table 2: Effect of SMB and glibenclamide on total cholesterol level after 3rd, 10th, 17th and 27th day of treatment

		Total Cholesterol Level (mg/dl)				
Group	**Treatment**	**Day 1**	**Day 3**	**Day 10**	**Day 17**	**Day 27**
I (NC)	.5%CMC	78.50±1.12	78.67±.88	76.17±1.17	79.17±.95	80.83±1.30
II(DC)	.5%CMC	81.17±1.35	131.33±1.05	141.67±3.39	144.50±1.18	144.83±.95
III	SMB 22.5mg/Kg	81.00±1.63	133.50±1.82	137.33±.76	124±1.39	89.83±.31*
IV	SMB 11.25mg/Kg	79.67±.99	135.67±1.8	137.67±.67	130±.36	92.17±.79*
V	SMB 5.85mg/kg	80.50±1.23	135.67±1.76	135.17±.94	125.17±.94	101.50±.76*
VI	GLC 600µg/kg	78.5±.76	136±2.17	125±1.31	114.17±1.01	85.17±.94*
VII	SMB 11.25mg/ Kg+GLC600µg/kg	78.5±1.52	135.67±1.8	121.5±.76	112.33±.88	80.17±.60*

Results are mean±SEM of 6 rats in each group
* P value < .001 compared to Day 3
The total cholesterol lowering effect of drug was observed by comparing the initial and final level of cholesterol i.e. before and after treatment at day 3rd and day 27th by applying paired sample t test. From the above table it is visible that the experimental drug SMB is significantly lowering the total cholesterol level to normal from day 3rd and this effect is comparable to standard drug glibenclamide.

Table 3: Effect of SMB and glibenclamide on triglyceride level after 3rd, 10th, 17th and 27th day of treatment

		Triglyceride Level (mg/dl)				
Group	**Treatment**	**Day 1**	**Day 3**	**Day 10**	**Day 17**	**Day 27**
I (NC)	.5%CMC	75.17±1.58	70.83±.1.45	72.00±.93	72.00±1.55	71.83±.65
II(DC)	.5%CMC	77.50±2.8	115.67±1.11	136.17±1.42	153±.73	162.50±.43*
III	SMB 22.5mg/Kg	74.50±2.74	113.33±1.45	127.83±.48	107.17±1.49	87.67±.2.90*
IV	SMB 11.25mg/Kg	77.33±.2.96	116.83±1.94	107±19.01	100±.73	83.83±.95*
V	SMB 5.85mg/kg	78±2.70	120.33±.68	122.17±.87	104.50±1.48	81.67±.615*
VI	GLC 600µg/kg	77.33±2.44	117.3±1.76	116.8±.56	85.33±.80	79.67±.68*`
VII	SMB 11.25mg/ Kg+GLC600µg/kg	74.17±2.87	117.00±2.113	107.67±.68	95.83±.60	72.50±.76*

The triglyceride level lowering effect of drug was observed by comparing the initial and final level of triglyceride i.e. before and after treatment at day 3rd and day 27th by applying paired sample t test. From the above table it is visible that the experimental drug SMB is significantly lowering triglyceride level to normal from day 3rd and this effect is comparable to standard drug glibenclamide.

DISCUSSION

The effect of *Swarna makshika bhasma* and standard drug Glibenclamide was observed on various biochemical parameters like blood glucose, total cholesterol and triglyceride. Total experiment was carried out in 27 days. Streptozotocin was administered on day one. On day third blood glucose level was estimated and mild diabetic (blood sugar > 200 mg/dL) rats were started with treatment. Treatment was continued and the biochemical parameters were estimated on day 10, 17 and 27.

Moderate diabetes was produced by intraperitoneal administration of streptozotocin (STZ, 45mg/kg stat) to adult rats of either sex. The effect of graded dose of SMB solution (5.85mg/kg, 11.25mg/kg and 22.5mg/kg) was studied on Blood sugar level, total cholesterol and triglyceride level. SMB was administered once daily from 4th day of STZ administration. Glibenclamide (GLC) was given in the dose of 600µg/kg orally. GLC showed decrease in blood sugar level in diabetic rats. The experimental drug SMB showed marked decrease in blood sugar level from 7th day onwards.
It can be observed from the Fig. 1 that all the three graded doses of *Swarna Makshika Bhasma* is significantly lowering elevated blood sugar level. Sugar level remains high in diabetic control rats where no treatment was given. It is also visible from the graph that the two doses high (22.5mg/kg) and medium (11.25mg/kg) are comparable in lowering BSL but Low dose (5.85mg/Kg) has more BSL lowering effect.

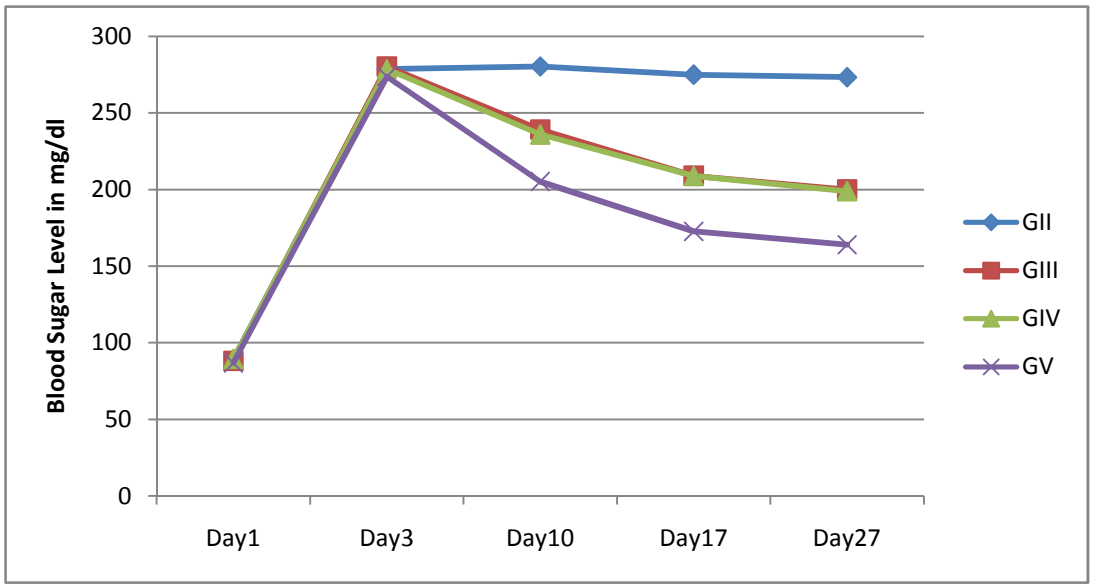

Figure 1: Showing pattern of Blood Sugar Level of Diabetic control and three graded dose treated rats.

The blood sugar level on day3 and day 27 can be observed from the figure 2. All the graded doses of *Swarna Makshika Bhasma* is significantly lowering blood sugar level. But it is visible that standard drug GLC has more BSL lowering effect. The study may be prolonged to see that if the drug is lowering the sugar to normal level. There is sharp decrease in BSL in the initial 7 days but it is lowering slowly afterwards.

The group comparison done on day 27 (i.e. final day of the treatment), shows similar BSL lowering effect of drug in group III (22.5 mg/Kg) and IV (11.25 mg/Kg). But drug in group V (5.85mg/Kg) have more BSL lowering effect in comparison to group III and IV and the difference is significant. Also BSL lowering effect of V is lesser in comparison to VI (GLC) and VII (SMB + GLC) the difference is significant.

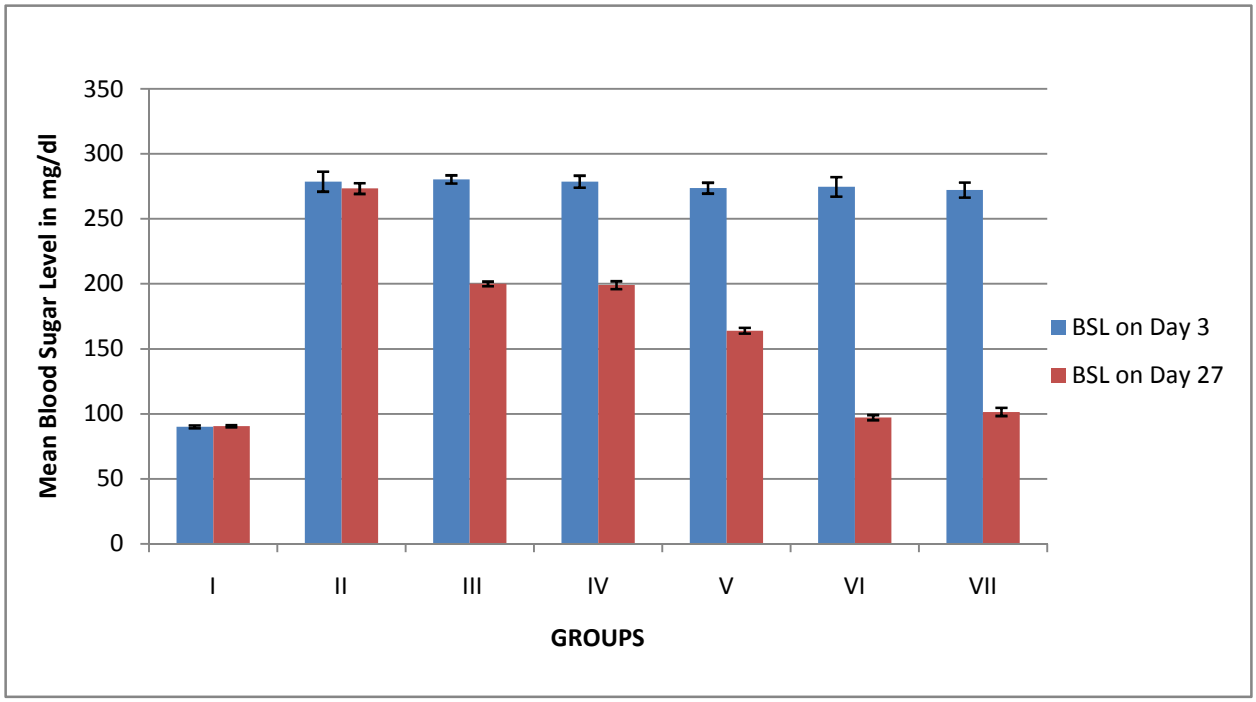

Figure 2: Showing Mean Blood Sugar Level of Seven groups on Day 3 and Day 27

Diabetic rats show significant increase in Total cholesterol (TC) and Triglyceride (TG) level. This is probably due to the fact that diabetes mellitus is accompanied with conversion in plasma lipids and lipoprotein profile. The TC and TG lowering effect of experimental drug SMB was observed in experimental animals. The effect of SMB was comparable with GLC, a known antidiabetic agent.

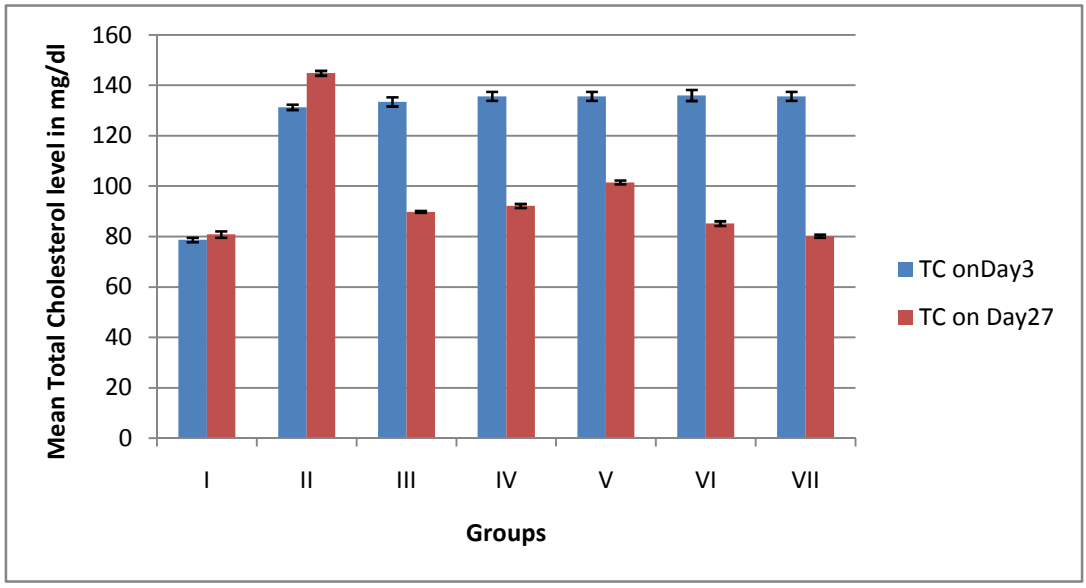

Figure 3: Showing Total Cholesterol Level of Seven groups on Day 3 and Day 27

The total cholesterol (TC) lowering effect of drug was observed by comparing the initial and final level of cholesterol i.e. before and after treatment at day 3rd and day 27th. From the above figure (Fig.3) it can be interpreted that experimental drug in all its graded doses is lowering total cholesterol level. And the TC lowering effect is significant and comparable to standard drug Glibenclamide. The effect of three graded doses is similar and their difference is non significant and thus it shows that there is nothing to do with higher dose of drug on its effect and drug is doing same work on its lower dose. But the effect of standard drug GLC and SMB +GLC has high TC lowering effect compared to SMB.

Triglyceride lowering effect in experimental animal was observed by comparing the TG level on Day 3rd when diabetes got produced and Day 27th up to which treatment was given. The result is shown in Fig 4. The three graded doses of SMB have similar TG lowering effect and their difference of mean is non significant. GLC and GLC+ SMB have more TG lowering effect than three doses of SMB. SMB (11.25mg/Kg) and SMB (5.85mg/kg) have comparable TG lowering effect with GLC

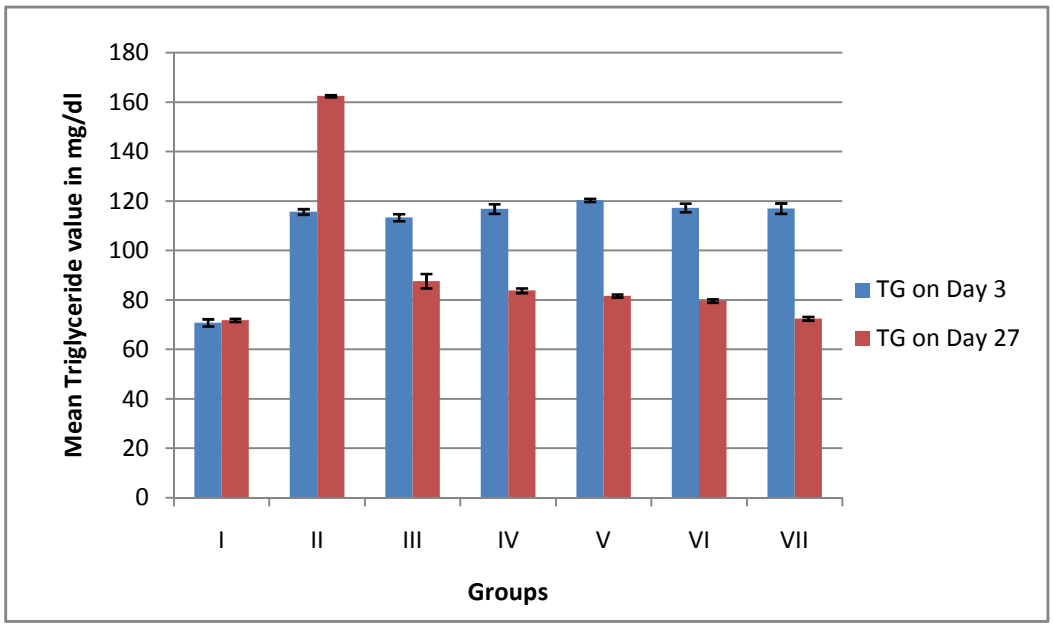

Figure 4: Showing Triglyceride Level of Seven groups on Day 3 and Day 27

Major components of *Swarna Makshika bhasma* are **Fe, Cu, Ca, Al, K, Mg** etc. Therefore, it is important to discuss here the role of these elements in human physiology and diseased condition. One of the major constituents of *Swarna Makshika bhasma* is Cu. Copper has an essential role in the biochemistry of living organisms. It is required for:

- normal infant development
- red and white blood cell maturation
- iron transport
- bone strength
- **cholesterol metabolism**
- myocardial contractility
- **glucose metabolism**
- brain development
- immune function
- protection against oxidative stress

Absorbed copper is bound primarily by albumin and transported to the liver. Copper is mainly incorporated into ceruloplasmin (ferroxidase I) in the liver. Ceruloplasmin is necessary for the absorption of iron as well as the mobilization of iron from the liver. In addition, a portion of copper is also incorporated in bile. A third portion of copper is incorporated into intracellular enzymes, such as superoxide dismutase and cytochrome oxidase.

Signs/symptoms of a copper deficiency are anemia, leukopenia, neutropenia, and osteoporosis. Other symptoms of **copper deficiency** include general weakness, impaired respiration, skin sores, decreased immune function, **elevated LDL cholesterol and reduced HDL cholesterol**. Some possible manifestations of copper deficiency are: arthritis, arterial disease, loss of pigmentation, myocardial disease, and neurological effects.A mild copper deficiency due to marginal copper intake over a long period of time can also occur. In addition to possible signs and symptoms of copper deficiency listed above, abnormal glucose tolerance may be seen.

The work by Dr. L.M. Klevay in 1973 at the U.S. Department of Agriculture, Human Nutrition Research Center pointed to a relationship between copper and cholesterol. In subsequent work, published in 1975, Dr. Klevay theorized that a metabolic imbalance between zinc and copper -- with more emphasis on **copper deficiency** than zinc excess - is a **major contributing factor in coronary heart disease**.

Subsequent work by other investigators has shown that copper complexes also can have a valuable role in the minimization of damage to the aorta and heart muscle as oxygenated blood reperfuses into tissues following myocardial infarction. This action is based on the anti-inflammatory action of copper complexes.

It has been speculated that the reason that the heart attack rate in France is lower than in the rest of Europe is because of the significant consumption by the French of red wine, which has a higher copper content than white wine because it is prepared with the skin of the grape intact.

Copper's role in the immune system has recently been supported by observations that individuals suffering from Menke's disease (an inherited disease in which there is defective copper absorption and metabolism) generally die of immune system-related phenomena and other infections. Further, animals **deficient in copper** have been shown to have **increased susceptibility to bacterial pathogens** such as salmonella and listeria. This kind of evidence has led researchers to suggest that copper compounds not only can cure various conditions, but can aid in the prevention of disease.

Potassium is an important mineral to the body and plays roles at both the cellular and electrical level. In fact, it is also considered an electrolyte because it carries a tiny electrical charge. Potassium is found in red blood cells, muscles and bones. Potassium and sodium work together to regulate the water and acid-base balance in the blood and tissues. It also works by creating a sodium-potassium pump that helps generate muscle contractions, including regulating heartbeat, according to Periodic Paralysis News Desk.

Potassium also causes a reaction in the blood vessels, according to research published in the "American Journal of Physiology Regulatory, Integrative and Comparative Physiology." Research led by F.J. Haddy determined that infusions of potassium would cause an increase in blood flow that resulted from the dilation of the arteries and relaxation of smooth muscles. The research found that dietary supplementation with potassium could lower blood pressure. This appeared to reduce the need for anti-hypertensive medications in individuals who were "salt sensitive" hypertensive. Although further research is required, the researchers theorize that potassium supplementation could help reduce other complications, such as stroke.[2]

Dietary supplementation of potassium can lower blood pressure in normal and some hypertensive patients.[3]

CONCLUSION

Swarna Makshika Bhasma is effective as an ant diabetic drug as per protocols of experimental studies of this project.

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