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PRECLINICAL EVALUATION OF A POLYHERBAL FORMULATION 'DM-99' IN STREPTOZOTOCIN INDUCED DIABETIC RATS/MICE.

Gupta K^{1*}, Chakraborty M²

Department of Pharmacology

^{1,2}Shree Devi College of Pharmacy, Mangalore- 574142.

ABSTRACT

Synthetic antidiabetic drugs have various side effects. Hence there is a search in alternate fields like herbal. DM-99 is a polyherbal formulation containing various ingredients which have shown antidiabetic property. Present study was conducted to investigate its synergistic action. Here streptozotocin induced diabetic animal model was used. Both acute study in rat and chronic study in mice was carried out. DM-99 significantly reduced blood glucose levels, increased insulin and liver glycogen levels. Significant improvement in antioxidant levels were observed. Improvement in cholesterol and triglyceraldehyde parameters were also observed. From all these parameters antidiabetic potential of DM-99 is concluded. Also acute oral toxicity study was performed.

Correspondence to Author



Kunal Gupta

Department of Pharmacology
Shree Devi College of Pharmacy
Airport Road, Kenjar,
Mangalore- 574142.

Email

kunal_gupta1985@hotmail.com

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INTRODUCTION

As per the WHO, diabetes mellitus is a heterogeneous metabolic disorder characterized by common feature of chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism. It is one of the leading cause of morbidity and mortality worldwide ^[1].

Various synthetic antidiabetic drugs are available in the market but have side effects. Insulin therapy causes hypoglycaemia and lipodystrophy at injection site. Main side effect of sulfonylurea is weight gain and loss of 44% efficacy in patients within 6 years. Biguanides causes gastrointestinal disturbances. Alpha glucosidase inhibitor causes elevation of transaminases as side effect. Thiazolidine diones causes oedema and weight gain ^[2,3].

Hence due to these drawbacks other strategies are needed. Hence there is a continuing search of new antidiabetic drugs ^[3,4].

WHO has recommended the evaluation of the effectiveness of medicinal plants in condition where the conventional allopathic treatment of diabetes is not adequate ^[5].

DM-99 is a polyherbal formulation where its antidiabetic action was shown by its *Momardica charantia* ^[6], *Azadirachta indica* ^[7], *Curcuma longa* ^[8,9], *Tinospora cordifolia* ^[10], *Eugenia jambolana* ^[11,12], *Gymnema sylvestre* ^[13], *Ficus glomerata* ^[14], *Acacia catechu* ^[15], *Phyllanthus emblica* ^[16], *Cinnamomum tamala* ^[17], purified asphaltum ^[18], *Trigonella foenum* ^[13] ingredients. The present study was conducted to find out their synergistic efficacy in diabetes.

MATERIALS AND METHOD

ANIMALS

Male wistar rats and male swiss albino mice were used respectively in acute and chronic diabetic studies. They were used after having obtained clearance from Institutional Animal Ethics Committee and CPCSEA guidelines were followed. Animals were fed with standard chow diet, water ad libitum, temperature 24-28°C, 12 hours day and night cycle. They were kept in hygienic polypropylene cages.

DRUGS AND CHEMICALS

Various drugs used were DM-99 (Goodcare Pharma, India) streptozotocin (Sigma Aldrich Co. USA), glibenclamide (Daonil, Sanofi Aventis), blood glucose kit (Erba, India), triglyceraldehyde kit (Erba, India), insulin kit (BI Insulin, IRMA). All other chemicals used were of analytical grade.

ACUTE ORAL TOXICITY TEST

The acute oral toxicity study was done according to OECD 423 guidelines ^[19]. 300mg/kg and 2000mg/kg were selected as lower and higher dose respectively.

ACUTE DIABETIC STUDY

Diabetes was induced in overnight fasted male wistar rats by single intraperitoneal injection of streptozotocin, 50 mg/kg dissolved in 0.1M cold citrate buffer pH 4.5 ^[20]. Then 48 hours after streptozotocin injection blood samples were drawn by retro orbital route. Blood glucose levels were determined to confirm diabetes ^[21]. The diabetic rats were again distributed evenly based on blood glucose into 4 groups. The groups were:

Group 1 – Normal treated

Group 2 – Diseased control (only streptozotocin)

Group 3 – Streptozotocin and DM-99 (300mg/kg dissolved in water p.o)

Group 4 – Streptozotocin and DM-99 (2000mg/kg dissolved in water p.o)

Group 5 – Streptozotocin and standard (glibenclamide 10mg/kg p.o)

After the respective drug administration blood samples were withdrawn from retro orbital route at 0, 60, 120, 180 mins ^[22]. Measure of blood samples were done by using blood glucose kit based on glucose oxide-peroxidase method.

CHRONIC DIABETIC STUDY

In chronic diabetic study sub diabetogenic dose of streptozotocin (40mg/kg) was dissolved in 0.1M cold citrate buffer and injected intraperitoneally to mice for 5 consecutive days ^[23]. Groupings were same as that of the acute study. Treatment period consisted of 28 days. Blood glucose were measured at 0, 7, 14, 21, 28th day. On 28th day the animals were sacrificed. Liver was

dissected out and homogenized in ice cold tris hydrochloride buffer. The prepared homogenates were centrifuged and used for the determination of antioxidant parameters like superoxide dismutase ^[24], catalase ^[25], glutathione ^[26], malondialdehyde ^[27]. Serum was isolated for estimation of insulin (kit procedure), liver glycogen ^[28], blood glucose and triglyceraldehyde. VLDL was accordingly calculated by Friedewald equation ^[29].

RESULT

STATISTICAL ANALYSIS

TABLE 1: COMPARISON OF BLOOD GLUCOSE

Group	0 min	60 min	120 min	180 min
1	85.66±2.65	90.17±3.28	91.83±3.02	93.17±3.114
2	207.1±4.48*	208.3±5.81*	215.9±4.82*	218.8±5.66*
3	206.7±3.98	201.7±4.00	195.0±5.07**	186.7±4.50***
4	208.2±4.81	198.9±4.72	183.6±5.66***	165.8±4.21***
5	208.0±4.89	194.8±6.10	172.7±4.91***	160.2±5.70***

Values are expressed as mean ± sem. Values are in mg/dl.

*Values are significant (P<0.01) when compared with control.

**Values are significant (P<0.05) when compared with group 2.

***Values are significant (P<0.01) when compared with group 2.

CHRONIC DIABETIC STUDY

Both doses of DM-99 significantly reduced blood glucose levels from day 7 when compared to diseased control group. The effect was pronounced on 28th day when the treatment groups of DM-99 were compared with diseased control group. See table 2. Streptozotocin diabetic rats showed decreased superoxide dismutase, catalase, glutathione levels whereas increased malondialdehyde levels. On treatment all the parameters increased with a decrease in

TABLE 2: COMPARISON OF BLOOD GLUCOSE

Group	0 th day	7 th day	14 th day	21 st day	28 th day
1	95.25±2.86	98.71±2.552	98.83±1.56*	97.5±2.85*	97.75±2.29*
2	98.0±3.67	144.1±3.90	209.0±7.61	295.3±6.93	371.0±11.03
3	99.75±2.41	133.3±2.63*	172.3±3.80*	222.8±5.99*	266.0±12.5*
4	98.4±2.33	126.4±2.67*	163.4±4.10*	200.8±4.72*	236.7±5.69*
5	98.0±2.22	116.7±1.68*	141.7±2.77*	168.4±3.04*	184.3±4.17*

Statistical analysis were done by using graph pad prism 4 software (Graph Pad, USA). ANOVA followed by Dunnetts multiple comparison test was applied. Data is presented as mean ± sem. Confidence level was taken as 95%.

ACUTE DIABETIC STUDY

The lower and higher doses of DM-99 significantly reduced blood glucose levels when compared to diseased control group from 120th minute. By this we can tell about the marked effect of the drug. See table 1.

malondialdehyde levels significantly in treatment groups. See table 3. Increase in triglyceraldehyde and VLDL level was observed in disease control group. DM-99 significantly reduced the value after treatment. See table 4. In normal condition there is an increased content of glycogen in liver. In diabetes glycogen content decreases. After treatment period DM-99 significantly increased its content in concerned groups. See table 5. Improve in insulin levels was shown by both lower and higher doses of DM-99. See table 6.

Values are expressed as mean \pm sem.

*Values are significant ($P < 0.01$) when compared with group 2.

TABLE 3: COMPARISON OF ANTIOXIDANT PARAMETERS

Group	SOD (unit/ml)	CAT(g protein/ml)	GSH(μ g/g)	MDA(/g)
1	70.73 \pm 4.15*	0.1 \pm 0.00*	115.0 \pm 2.63*	18.65 \pm 1.20*
2	27.04 \pm 6.96	0.9 \pm 0.00	28.35 \pm 1.44	98.33 \pm 1.20
3	41.40 \pm 2.34	1.5 \pm 0.00*	52.65 \pm 2.60*	61.0 \pm 3.20*
4	53.35 \pm 1.00*	1.6 \pm 0.00*	63.0 \pm 2.63*	47.0 \pm 2.09*
5	62.56 \pm 1.91*	1.8 \pm 0.00*	80.0 \pm 1.51*	34.0 \pm 2.08*

Values are expressed as mean \pm sem.

*Values are significant ($P < 0.01$) when compared with group 2.

TABLE 4: COMPARISON OF LIPID PROFILE

Group	TG (mg/dl)	VLDL (mg/dl)
1	83.0 \pm 3.05*	15.6 \pm 0.61*
2	174.4 \pm 2.34	36.69 \pm 0.46
3	132.0 \pm 2.65*	25.6 \pm 0.52*
4	119.6 \pm 1.85*	24.73 \pm 0.37*
5	105.2 \pm 2.73*	20.07 \pm 0.54*

Values are expressed as mean \pm sem.

*Values are significant ($P < 0.01$) when compared with group 2.

TABLE 5: COMPARISON OF LIVER GLYCOGEN

Group	LIVER GLYCOGEN (mg/ml)
1	0.40 \pm 0.0089*
2	0.10 \pm 0.0058
3	0.21 \pm 0.0067*
4	0.29 \pm 0.0088*
5	0.37 \pm 0.0057*

Values are expressed as mean \pm sem.

*Values are significant ($P < 0.01$) when compared with group 2.

TABLE 6: COMPARISON OF INSULIN

Group	INSULIN (μ IU/ml)
1	0.88 \pm 0.05*
2	0.34 \pm 0.05
3	0.45 \pm 0.05
4	0.66 \pm 0.05
5	0.77 \pm 0.05*

Values are expressed as mean \pm sem.

*Values are significant ($P < 0.01$) when compared with group 2.

DISCUSSION

As per the WHO diabetes mellitus is a heterogeneous metabolic disorder characterized by common feature of chronic hyperglycaemia with

disturbance of carbohydrate, fat and protein metabolism. Insulin is produced by the beta cells of the pancreas. This insulin is responsible for the proper metabolism of carbohydrate, fat, protein. Diabetes mellitus is of two types. One is insulin dependent and other one is non insulin dependent. In insulin dependent there is complete destruction of beta cells whereas in non insulin dependent there is masking of its receptors. Hence insulin fail to show its actions. This leads to hyperglycaemia which in turn causes many complications.

Various animal models are used which will show similar clinical conditions as of humans in diabetes ^[30]. One such is streptozotocin induced diabetes mellitus. It cause destruction of beta cells which lead to diabetes ^[31]. Hyperglycaemia produces reactive oxygen species which result in tissue injury. This is because of reduced antioxidant production ^[32]. Under normal conditions insulin activates enzyme lipoprotein lipase which hydrolyses triglyceride. But in diabetes as this does not happen it lead to hyperlipidaemia ^[33]. Decrease in liver glycogen occur because of destruction of glycogen synthase system. This occurs due to lack of insulin ^[34].

DM-99 causes improvement in diabetes by correcting all these derangements. It helps in secretion of insulin by beta cells which cause increase in antioxidants. This neutralizes the free radicals. It also improves blood glucose levels. Also increase in enzymes lipoprotein lipase and glycogen synthase occurs due to increase in insulin levels which will cause an improvement in lipid parameters and liver glycogen.

CONCLUSION

The polyherbal formulation, DM-99 failed to show any toxic effects in study. Also due to its wide mechanism of action it improved the diabetic condition by improving blood glucose levels, various antioxidant levels, insulin, liver glycogen, triglyceraldehyde and very low density lipoprotein parameters. Hence this drug looks promising in the treatment of diabetes.

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