ANTIDIABETIC ACTIVITY OF MADHUNASHINI (MD-19) IN ALLOXAN INDUCED DIABETES MELLITUS

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Abstract: Madhunashini (MD-19), a polyherbal formulation, has been screened for antidiabetic activity. The hypoglycemic activity was carried out in normal rats, while antidiabetic effect was analyzed in alloxan-induced diabetic rats at two doses of 250 and 500mg/kg of the polyherbal formulation. Pioglitazone (30 mg/kg) was used as the standard drug. The biochemical parameters (glucose, urea, creatinine, serum cholesterol, serum triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein, SGOT, SGPT and alkaline phosphatase were assessed in diabetic rats at both the doses. MD-19 showed its effectiveness as an antidiabetic product in reducing the elevated hyperglycemia in acute and chronic study, but it does not produce hypoglycemic effect. Treatment of diabetic rats with MD-19 restored the biochemical parameters significantly. The present study supports the use of MD-19 as an antidiabetic product.

Key words: MD-19, Alloxan, Antidiabetic

INTRODUCTION

Diabetes mellitus (DM) is common endocrine disorder affecting more than 150 million people worldwide and this number is likely to increase to 300 million by the year 2025 [1], out of which more than one fifth will be Indians. According to the International Diabetes Federation, India has been declared as the diabetes capital of the world [2]. Plants have been used as sources of drugs for treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population [3].

MD-19 is herbal drug formulation for diabetes mellitus developed by us in our laboratory. It is a combination of medicinal plants with mineral namely Momordica muricata, Tinospora cordifolia, Trigonella foenum graecum, Caesalpinia bonducella, Curcuma longa, along with Shilajit. The acute and chronic toxicity studies has been previously reported in our earlier studies [4].

Some of these are known to possess antidiabetic effect and have been used in the indigenous system of medicine to treat diabetes mellitus. In the present study, the hypoglycemic activity of MD-19 was carried out in normal rats, while antidiabetic effect was analyzed in alloxan-induced diabetic rats at two doses of 250 & 500mg/kg.

MATERIALS AND METHODS

Animals used: Male Wister rats with body weights of 180-200 g were used for the current study. The animals were fed on a standard pellet diet and water ad libitum.

Reagents and drugs: Kits of Span diagnostic Ltd., Surat were used for determination of glucose, triglyceride, total cholesterol, HDL cholesterol, SGOT, SGPT, urea and creatinine. Alloxan monohydrate was obtained from Research Lab Fine Chemical Ind. Mumbai. Pioglitazone hydrochloride was obtained from, Alembic Pharmaceuticals Ltd, Mumbai.
Glucometer strips and uristrips for measuring glucose in blood and urine of Roche Diagnostics, Germany and Delta, BC, Canada respectively were used.

**Preparation of herbal extracts and development of herbomineral combination:** Individual aqueous extracts of the air-dried herbal drugs were prepared by cold maceration process using chloroform water (2ppm in distilled water) as a solvent.

It was prepared by incorporation of aqueous extracts of Momordica muricata, Tinospora cordifolia, Trigonella foenum graecum, Caesalpinia bonducella, Curcuma longa, along with Shilajit. Individual extracts were concentrated to dry mass using rotary evaporator under controlled temperature (25-40°C). Dried extract thus prepared were mixed in equal quantity, which were further mixed with shilajit. The details of composition and concentration of MD-19 are summarized in table 1.

**Drug administration:** MD-19 extract was suspended in distilled water and administered orally through orogastric tubes at the following doses of 250 and 500 mg/kg body wt. The volume of the vehicle (2 ml) was kept constant for above two doses.

**Experimental induction of diabetes in rats [5]**
The rats were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body wt. intraperitoneally. After 2 weeks, rats with moderate diabetes having glycosuria indicated by uristips and hyperglycaemia i.e. with a blood glucose of 200-260 mg/dl. were used for the experiment.

**Experimental design**

**Activity of MD-19 on normoglycaemic animals [6]:** The hypoglycemic activity of two doses of MD-19 was evaluated separately. A total of 18 rats fasted for 18 hours were divided into three equal groups. The animals of group I served as an untreated control whereas other two groups were administered with the MD-19 extract at a single dose of 250 and 500 mg/kg, respectively. Plasma glucose was estimated using glucometer strips.

**Acute study [7]:** In this study total of 30 rats (24 diabetic surviving rats and six normal rats) were used. The test samples (MD-19 and pioglitazone hydrochloride) were administered orally by using orogastric tube. Fasting blood glucose level was examined at the beginning of experiment (i.e. at zero hour), 2, 4 and 6 hours after the administration of test samples.

**Group 1:** Normal rats. They were given with 2 ml of normal saline.

**Group 2:** Diabetic control rats were given 2 ml of normal saline.

**Group 3:** Diabetic rats. They were given aqueous solution of MD-19 extract 250 mg/kg body wt.

**Group 4:** Diabetic rats given aqueous solution of MD-19 extract 500 mg/kg body wt.

**Group 5:** Diabetic rats given pioglitazone hydrochloride orally 30 mg/kg body wt.

**Chronic study [8]:** The chronic study was done in total of 24 diabetic surviving rats. Diabetes was induced in rats 2 weeks before starting the experiment. The rats were divided into six groups after the induction of alloxan diabetes. In the experiment six rats were used in each group.

**Group 1:** Diabetic control rats given with 2 ml of normal saline;

**Group 2:** Diabetic rats given aqueous solution of MD-19 extract 250 mg/kg body wt. daily orally for 21 days.

**Group 3:** Diabetic rats given aqueous solution of MD-19 extract 500 mg/kg body wt. daily orally for 21 days.

**Group 4:** Diabetic rats given pioglitazone hydrochloride orally 30 mg/kg body wt. in aqueous solution daily for 21 days.

**Collection of blood samples:** For the purpose of estimation of serum glucose, lipid profile, and other biochemical parameters the blood samples of the fasted rats were collected from the retrobulbar venous plexus immediately with capillary tubes under ether anesthesia and with 0.1 M EDTA as anticoagulant. blood samples were allowed to clot for 30 min and serum was separated by centrifugation.

**Biochemical estimations:** Blood glucose and lipid profile were estimated by enzymatic method using reagent kit procedural guidelines and details. (Span diagnostic Ltd., Surat, India).

**Statistical analysis:** The results are expressed as mean ± SEM. Data on blood glucose level were
analyzed by one-way ANOVA followed by Tukey’s post hoc test. While data on lipid profile were analyzed by Student’s ‘t’ test. Value of P less than 5% (P<0.05) was considered statistically significant.

RESULTS

Effect of MD-19 on body weight: Diabetic rats showed significant reduction in body weight during 21 days period of study (Table 2). Alloxan caused body weight reduction, which is reversed by the extracts after the 5 to 8 days of the daily treatment as compared to the standard pioglitazone treated group.

Activity of on normoglycaemic rats: The two doses of MD-19 were administered to evaluate the hypoglycemic activity of MD-19 in normal rats. The study revealed that both the test doses did not showed any hypogleaemic activity over the entire period of study (Table 3).

Antihyperglycemic effect of the extracts (Effect of extracts on blood glucose level after single administration of extracts in hyperglycemic rats): As expected in the diabetic control there was severe hyperglycemia as compared to the normal animals. The extracts and pioglitazone treated groups showed a decrease in blood glucose levels at 2, 4 and 6 hours after administration.

Comparing with the diabetic control all the two doses of herbomineral extract significantly lowered the elevated blood glucose levels. It was observed that the standard drug pioglitazone in (30 mg/kg p.o.) lowered the blood glucose level significantly bringing it nearly back to normal (Table 4).

Effect of extracts on blood glucose level after prolonged administration of extracts in hyperglycemric rats: The blood glucose level of the diabetic control group did not show any significant change, whereas the extracts and pioglitazone treated groups showed a significant decrease when observed on 7, 14 and 21 days after the treatment. The results are tabulated in Table 5.

Effect of extracts on lipid profile in alloxan-induced diabetic rats: The effect of the extract on diabetes induced hyperlipidemia was also studied. The subsequent hyperlipidemia can be used as an index for the hyperglycemia by measuring the lipid profile (total cholesterol, triglycerides, high density lipoproteins and low density lipoproteins.)

It was observed that due to diabetes there was an increase in the total cholesterol levels as well as triglyceride levels. The HDL levels were reduced in the diabetic animals and the LDL levels were increased significantly, but there were no significant variations in the VLDL levels. Serum cholesterol and triglycerides, levels were decreased significantly by pioglitazone and with both the studied doses of the extract due to long treatment. The extract showed a significant decrease in the total cholesterol and triglyceride levels. In particular, the extract given at 500 mg/kg showed a much relevant action. It also increased the HDL level and was successful in suppressing the LDL levels as compared to the standard drug (Table 6).

Effect of MD-19 on creatinine, urea, SGOT, SGPT and alkaline phosphatase in alloxan-induced diabetic rats: Creatinine, serum urea, SGOT (ASAT), SGPT (ALAT) and alkaline phosphatase were found to be increased significantly in alloxan treated diabetic rats. All these were significantly decreased by the daily treatment in diabetic rats. The results are summarized in Table 7.

DISCUSSION

In case of alloxan induced diabetes, there is degradation of islets cells by accumulation of cytotoxic free radicals. Alloxan concentrates in the islets of Langerhans’ and in liver cells, where it is reduced to dialuric acid [9]. It is unstable in aqueous medium and oxidized back to alloxan, along with generation of oxygen, hydrogen peroxide and hydroxyl radicals by Fenton type reaction [9]. The liver contains antioxidants viz., super oxide dismutase (SOD), catalase, glutathione peroxidase and reduced glutathione, which scavange these free radicals. Contrary to this, the islets of Langerhans’ have low concentrations of these enzymes and are vulnerable to the cytotoxic effects of the free radicals. [10]. It is reported that increase in islet cell super oxide dismutase activity can prevent or decrease alloxan toxicity. Experimental diabetes, carried out by different workers [11-13] showed initial islet inflammation, followed by infiltration of activated macrophages and lymphocytes in the inflammatory focus. These cells might be the source of the cytotoxic oxygen radicals.
### Table 1: MD-19 (Composition and concentration)

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Botanical name</th>
<th>Common name</th>
<th>Family</th>
<th>Part used</th>
<th>Concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Momordica muricata</td>
<td>Karela</td>
<td>Cucurbitaceae</td>
<td>Fruit</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Tinospora cordifolia</td>
<td>Gulvel</td>
<td>Menispermaceae</td>
<td>Stem</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Trigonella foenum graecum</td>
<td>Methi</td>
<td>Leguminosae</td>
<td>Seeds</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>Caesalpinia bonducella</td>
<td>Gajaga</td>
<td>Leguminosae</td>
<td>Seeds</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>Curcuma longa</td>
<td>Haridra</td>
<td>Zingiberaceae</td>
<td>Rhizomes</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Shilajit</td>
<td>-</td>
<td>Mineral</td>
<td>-</td>
<td>50</td>
</tr>
</tbody>
</table>

### Table 2: Effect of MD-19 on body weight in alloxan-induced diabetic rats. Values are expressed as Mean ± Standard error of mean [SEM] for groups of six animals each.

<table>
<thead>
<tr>
<th>Period</th>
<th>Diabetic Control</th>
<th>Madhunashini 50 mg/kg</th>
<th>Madhunashini 500 mg/kg</th>
<th>Pioglitazone 30 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>208.83 ± 1.40</td>
<td>206.33 ± 1.909</td>
<td>207.16 ± 1.493</td>
<td>212.02 ± 1.291</td>
</tr>
<tr>
<td>7 days</td>
<td>180.92 ± 2.887</td>
<td>192.04 ± 2.049</td>
<td>202.50 ± 1.478</td>
<td>205.50 ± 3.170</td>
</tr>
<tr>
<td>14 days</td>
<td>163.83 ± 2.982</td>
<td>188.50 ± 2.405</td>
<td>202.66 ± 2.445</td>
<td>207.66 ± 3.273</td>
</tr>
<tr>
<td>21 days</td>
<td>148.33 ± 1.054</td>
<td>181.33 ± 1.447</td>
<td>202.66 ± 1.944</td>
<td>192.22 ± 1.022</td>
</tr>
</tbody>
</table>

### Table 3: Effect of MD-19 on plasma glucose levels after intragastric per oral administration to normoglycaemic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma glucose g/dl. After treatment at time (Hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>71.667 ± 1.308</td>
</tr>
<tr>
<td>MD-19 250 mg/kg</td>
<td>70.166 ± 0.792</td>
</tr>
<tr>
<td>MD-19 500 mg/kg</td>
<td>73.50 ± 0.846</td>
</tr>
</tbody>
</table>

### Table 4: Effect of MD-19 on plasma glucose levels after intragastric per oral administration to hyperglycemic rats. Values are expressed as Mean ± Standard error of mean [SEM] for groups of six animals each. *P<0.05 the diabetic control was compared with the extract and pioglitazone treated group.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma glucose g/dl. After treatment at time (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>250.16 ± 1.249</td>
</tr>
<tr>
<td>MD-19 250 mg/kg</td>
<td>259.83 ± 3.092</td>
</tr>
<tr>
<td>MD-19 500 mg/kg</td>
<td>256.50 ± 4.530</td>
</tr>
<tr>
<td>Pioglitazone 30 mg/kg</td>
<td>261.33 ± 1.764</td>
</tr>
</tbody>
</table>

### Table 5: Effect of MD-19 on plasma glucose levels after prolonged oral administration to hyperglycemic rats. Values are expressed as Mean ± Standard error of mean [SEM] for groups of six animals each. *P<0.05 the diabetic control was compared with the extract and pioglitazone treated group.

<table>
<thead>
<tr>
<th>Period</th>
<th>Plasma glucose g/dl. after treatment at time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control</td>
<td>0 day</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>264.18 ± 2.432</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>287.43 ± 1.370</td>
</tr>
<tr>
<td>Pioglitazone 30 mg/kg</td>
<td>305.78 ± 0.767</td>
</tr>
<tr>
<td>212.07 ± 2.076</td>
<td>140.39 ± 3.291*</td>
</tr>
</tbody>
</table>
MD-19 is combination of extracts of herbal species with reported anti-diabetic activity and equal proportion of shilajit. The antidiabetic activity of the formulation is due to presence of shilajit as it has free radical scavenging activity of rejuvenation of cells, thus initiates to multiply β-cells in pancreas. The property appears to be enhanced by the presence of Momordica charantia and Tinospora cordifolia, as these herbs are reported to decrease hyperglycemia [14-15]. The other herbal drugs have synergistic effect along with above herbo-minerals [16]. Long-term treatment with shilajit increases the number of β-cells of pancreas, i.e. pancreaticβ-cells action, which may result in better sensitivity of pancreatic β-cells with prompt secretion of a large quantity of insulin in response to hyperglycemia [17-18]. The preliminary phytochemical screening of the extracts showed presence of glycosides, tannins, flavanoids, alkaloids and saponins [4]. In present study a significant weight reduction was observed in control models MD-19 250 mg/kg, but MD-19 500 mg/kg dose showed insignificant fluctuation. This condition was better than the standard drug pioglitazone 30 mg/kg. Further, MD-19 did not produced hypoglycaemia when administered to control rats, however, onset of action for any drug requires sufficient time, which is not checked in present study.

It was observed that there is a gradual decrease in basal sugar level (BSL) as the time passes with higher dose of MD-19 (500 mg/kg bw). Perhaps this is due to the presence of saponins in Trigonella foenum-graecum and curcumin in Curcuma longa [19,20]. Significant decrease of BSL during prolonged treatment with the formulation, as compared with pioglitazone, proves a better treatment that improves the secretory activity of β-cells generating insulin. Saponins along with shilajit also shows significant decrease in cholesterol and triglycerides as both of them are reported to decrease in cholesterol and triglycerides as well as β-cells of pancreas, i.e. pancreatotrophic β-cells with prompt secretion of a large amount of insulin to the presence of saponins in Madhunashini and Curcuma longa [19,20].
them acts synergistically for rejuvenation of the β-cells thus improving their potential to secrete insulin.

Because of free radical scavenging activity of the secondary metabolites present in the formulation daily treatment in alloxan induced diabetic rats shows significant decrease in the levels of creatinine, serum urea, SGOT, SGPT, and alkaline phosphatase [21].

These results may be due to increased uptake of glucose by the tissues and its utilization. Many oral hypoglycaemic agents are normally cleared by kidneys and so accumulate in uremic patients thus increasing risk of hypoglycemia and toxicity. MD-19 has shown significant antihyperglycemic activity and proved to be effective in nephrotoxicity.

The increased lipid profile was brought down significantly by administration of MD-19. The reduction in serum lipid may be due to decreased synthesis or increased excretion through intestinal tract by HMG CoA reductase which is important enzyme in formation of cholesterol and enhancement of the degradation of formed cholesterol by increasing the excretion through intestinal tract. [11,15,21]. From these results, it can be concluded that the extract at the studied doses possess antihyperglycemic as well as antihyperlipidemic action against alloxan induced hyperglycemia.

REFERENCES