PROTECTIVE EFFECT OF COMMIPHORA MUKUL GUM RESIN ON BRAIN IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The present study was undertaken to investigate the hypolipidemic activity of ethanolic extract of Commiphora mukul gum resin (EtCMGR) on the brain of streptozotocin (STZ) induced diabetic Wistar rats.

Methods: Thirty two rats, included for the study, were divided into four groups: control (C), control treated with EtCMGR (C+CM), diabetic (D) and diabetic treated with EtCMGR (D+CM). Diabetes was induced by single intraperitoneal injection of STZ (55 mg/kg b.w.).

Results: Diabetic rats showed significant reduction in the levels of total lipids, phospholipids, cholesterol, glycolipids and protein level and significant decrease in the activity of acetylcholinesterase while the levels of triglycerides, acetylcholine and the activities of glutamate pyruvate transaminases (GPT) and glutamate oxaloacetate transaminases (GOT) increased significantly when compared to control group. Oral administration of EtCMGR (suspended in 5% Tween-80 in distilled water prior to use) daily at a concentration of 200 mg/kg b.w. to group-D+CM rats for 60 days reversed the above changes significantly.

Conclusion: These results suggest that EtCMGR exhibits hypolipidemic effect in the STZ-induced diabetic rats.

Keywords: Acetylcholinesterase, Commiphora mukul, Hypolipidemic activity, Streptozotocin.
protective effect on brain lipids and activities of transaminases and acetyl cholinesterase in STZ induced diabetic rats.

MATERIALS AND METHODS

Chemicals

STZ was obtained from the Sigma (St. Louis, MO, USA) and 2,4-dinitrophenyl hydrazine (DNPH) was procured from SD-Fine Chemical, India. All other chemicals and solvents of analytical grade were procured from Sisco Research Laboratories Ltd., Mumbai, India.

Collection of plant material

EtCMGR (brown, dry powder, and cream colour) was purchased from Chemioids (manufacturers and exporters of herbal extracts) Vijayawada, Andhra Pradesh, India. Herb to product ratio was 8:1 and the extract was suspended in 5% Tween-80 in distilled water prior to use.

Induction of diabetes

Two-three week-old male Wistar rats of body weight 125-150 g procured from Sri Raghavendra Enterprises (Bangalore, India), were acclimatized for 7 days to our animal house, and maintained at standard conditions of temperature and relative humidity, with a 12 h light/dark cycle. Water and commercial rat feed were provided ad libitum. The current work was carried out with a prior permission from our institutional animal ethical committee (Regd. no. 470/01/a/CPCSEA, dt. 24th August 2001). Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared STZ solution (55 mg/kg b.w., in ice cold 0.1 M citrate buffer, pH 4.5 in a volume of 0.1 ml per rat). After 72 h of STZ administration, the plasma glucose level of each rat was determined using Liquid Gold Diagnostic kit [35]. The dose of lipid extract sample was subjected to evaporation and 1 ml of 10 N H2SO4 was added and digested for 1 h in a boiling water bath. Then 20 µl of H2O2 was added and the solution was boiled until the liquid becomes colourless. The tubes were cooled and phosphorus was estimated by Fiske Subbarow method [37]. Two hundred µl of lipid extract sample was subjected to evaporation and 1 ml of 10 N H2SO4 was added and digested for 1 h in a boiling water bath. Then 20 µl of H2O2 was added and the solution was boiled until the liquid becomes colourless. The tubes were cooled and phosphorus was estimated by Fiske Subbarow method [37].

Experimental design

In the present experiment, a total of 32 rats (16 diabetic rats; 16 normal rats) were used. The rats were divided into four groups of 8 each: control (C); control rats treated with EtCMGR (C+CM); diabetic (D); and diabetic rats treated with EtCMGR (D+CM). The dose of EtCMGR in the current study is based on the earlier reports on the effect of EtCMGR, a dose less than 200 mg/kg b.w. was not expected to be effective in rats [32].

Animal sacrifice and organ collection

After the experimental period of 60 days, the animals from each experimental group were starved for 12 h and sacrificed by cervical dislocation and immediately the whole brain was dissected out and washed with ice-cold saline and used for analysis.

Extraction of lipids from brain tissue

Tissue (250 mg) homogenate was prepared in Folch reagent (2:1 chloroform-methanol) using Potter-Elvehjem homogenizer and centrifuged at 3000 rpm. Five ml of the supernatant was mixed with 3 ml of distilled water and again centrifuged at 3000 rpm and resulting organic phase was used for total lipids, triglycerides, cholesterol, phospholipids and glycolipids analysis by the method of Foch et al. [33]. Total lipids were estimated by gravimetric method.

Estimation of triglycerides

Triglycerides (TG) were estimated by GPO-PAP enzymatic method using Liquid Gold Diagnostic kit according to the method of Fossati et al. [34]. Sixty µl of the lipid extract, taken in an eppendorf tube, was allowed to evaporate in an incubator. To this 1.0 ml of the triglyceride reagent was added, mixed and incubated at 37 °C for 10 min. Triglyceride standard (200 µg/ml) and water blank were also treated in a similar manner. After incubation, absorbance was read at 505 nm and values are expressed as mg/g tissue.

Estimation of cholesterol

Total cholesterol was estimated by CHOD-PAP enzymatic method using Liquid Gold Diagnostic kit [35]. Sixty µl of lipid extract, taken in an eppendorf tube, was allowed to evaporate in an incubator. To this 1.0 ml of the cholesterol reagent was added, mixed and incubated at 37 °C for 10 min. Cholesterol standard (200 mg%) and water blank were also treated in a similar manner. After incubation, absorbance was read at 510 nm and values are expressed as mg/g tissue.

Estimation of phospholipids

Phospholipids (PL) were estimated by the method of Connerty et al. [36]. Phospholipids were digested with H2SO4 and the liberated inorganic phosphate was estimated by the method of Fiske and Subbarow method [37]. Two hundred µl of lipid extract sample was subjected to evaporation and 1 ml of 10 N H2SO4 was added and digested for 1 h in a boiling water bath. Then 20 µl of H2O2 was added and the solution was boiled until the liquid becomes colourless. The tubes were cooled and phosphorus was estimated by Fiske Subbarow method. To the above digest 1.0 ml of molybdate II, 0.4 ml of ANS (1-amino-2-naphthol-4-sulfonic acid) reagents were added and the volume was made up to 10 ml with distilled water. After an incubation period of 15 min, the blue colour formed was read at 660 nm. The phospholipid content is calculated by multiplication of phosphate value by 25. The results are expressed as mg/g tissue.

Estimation of glycolipids

Glycolipids (GL) were estimated based on the method of Roughan and Batt [38]. Two hundrend µl of brain homogenate was subjected to evaporation and 2 ml of 2 N sulphuric acid was added and digested for 2 h in a boiling water bath. After hydrolysis, 4 ml of chloroform was added and centrifuged. The aqueous layer was separated and 50 µl of 80% phenol was added followed by 4 ml of concentrated H2SO4. The orange colour was measured at 480 nm. A series of galactose standards (20-200 µg) were treated in similar manner. Glycolipid concentration was estimated by multiplying galactose content with 4.45. The values are expressed as mg/g tissue.

Preparation of brain homogenate for activities of enzymes (protein and acetyl choline and activities of acetylcholinesterase and transaminases)

Ten per cent brain homogenate was prepared in 0.15 M potassium chloride by using Potter-Elvehjem homogenizer at 0 °C and centrifuged at 12,000 rpm for 45 min at 0-4 °C. The supernatant thus obtained was distributed into eppendorf tubes, labelled and stored at-20 °C and used for enzyme assays.

Determination of protein content

The protein content of tissue homogenates was determined by the Lowry protein assay using bovine serum albumin as the standard [39].

Estimation of acetyl choline

Acetyl choline (Ach) content was estimated by the method of Metcalf et al. [40] as described by Augustinson [41]. In this method the acetyl group reacts with alkaline hydroxylamine to form acetyl hydroxamate, which then reacts with ferric chloride in acidic medium to form a brown coloured complex which was measured spectrophotometrically at 540 nm against reagent blank. The acetyl choline content is expressed as µmoles of Ach/g of tissue.

Estimation of Acetylcholinesterase activity

The activity of Acetylcholinesterase (AchE) was estimated by the method of Ellman et al. [42]. Thio-choline, released from acetyl thio-choline by the action of enzyme, reacts with--SH group of DTNB [5, 5'-Dithio-Bis (2-Nitrobenzoic Acid)] reducing it to thiol, which has maximum absorbance at 412 nm. The activity was calculated by using molar extinction coefficient of SH group of DTNB as 14.3x10^5 and expressed as µmoles of Ach hydrolysed/min/mg protein.
Estimation of transaminases activities ([glutamate pyruvate transaminases (GPT) and glutamate oxaloacetate transaminases (GOT)]

Pyruvate gives a brown colored compound with 2, 4-Dinitrophenyl hydrazine (DNPH) which is measured chromatically at 520 nm [43]. The enzyme activities are expressed as µg of pyruvate liberated/min/mg protein.

Statistical analysis

The results were expressed as mean±SEM for eight rats in each group. Data were analysed for significant difference using Duncan’s multiple range test (P<0.05) [44].

RESULTS

Effect of EtCMGR on brain lipid profiles

Table 1 represents data on total lipids constitute phospholipids, glycolipids, cholesterol, triglycerides and also the brain lipids profile of groups-C, C+CM, D and D+CM rats at the end of experimental period. STZ induced diabetic rats showed significant decrease in phospholipids, glycolipids, and cholesterol (29%, 72.5% and 25%) and significant increase intriglycerides (33%) when compared with group -C.

Effect of EtCMGR on brain lipid profiles for 60 days treatment for 60 days in group -D+CM resulted in significant increase in triglycerides (22%) in group-D+CM rats when compared to group-D. But this significant increase in phospholipids, glycolipids, and cholesterol could not reach the control values whereas triglycerides levels were normalized. Thus phospholipids, glycolipids, and cholesterol levels (20%, 41% and 13%) in group-D+CM rats were still significantly lower (25%, 40% and 14%) when compared to group-C whereas EtCMGR treatment for 60 days to group-C+CM rats showed slight increase in glycolipids (54 %) and decrease in cholesterol and phospholipids (10% and 5%) and no significant change in triglycerides levels when compared to group-C.

Effect of EtCMGR on acetylcholine and acetylcholine esterase

Table 2 illustrates the concentration of Ach and the activity of AchE in the brain of four experimental groups. Diabetic group showed significant decrease in the activity of AchE (8%) and increase in Ach (29%) content when compared with group-C. EtCMGR treatment for 60 days resulted in significant increase in the activity of AchE (40%) and significant decrease in Ach (48%) content in group-D+CM rats when compared to group-D. At 60 days, group-C+CM rats showed significantly higher activity of AchE (68%) and slight decrease in Ach (36%) content when compared to group-C. Thus, the present study indicates the protective effect of EtCMGR treatment in maintaining the normal level of neurotransmitter i.e., Ach even under STZ induced diabetic state.

Table 1: Effect of EtCMGR administration on brain lipid profile in diabetic rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Total lipids (mg/g tissue)</th>
<th>Phospholipids (mg/g tissue)</th>
<th>Triglycerides (mg/g tissue)</th>
<th>Cholesterol (mg/g tissue)</th>
<th>Glycolipids (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C</td>
<td>330.65±14.70</td>
<td>64.80±1.42</td>
<td>8.44±0.22</td>
<td>32.52±0.97</td>
<td>3.49±0.22</td>
</tr>
<tr>
<td>Group C+CM</td>
<td>300.21±6.27</td>
<td>61.76±1.29</td>
<td>7.6±0.32</td>
<td>29.21±3.36</td>
<td>4.39±0.27</td>
</tr>
<tr>
<td>Group D</td>
<td>135.16±15.76</td>
<td>46.21±0.64</td>
<td>11.28±0.24</td>
<td>24.42±1.39</td>
<td>0.96±0.11</td>
</tr>
<tr>
<td>Group D+CM</td>
<td>220.02±0.53</td>
<td>51.69±1.53</td>
<td>8.8±0.44</td>
<td>28.3±0.80</td>
<td>2.06±0.22</td>
</tr>
</tbody>
</table>

C: control, C+CM: control rats treated with EtCMGR, D: diabetic, D+CM: diabetic rats treated with EtCMGR. Values are expressed as mean±SEM (n=8 animals). Means with different superscripts within the column are significantly different at P<0.05 (Duncan’s multiple range test).

Table 2: Effect of EtCMGR administration on brain acetylcholine content and activity of acetylcholinesterase in diabetic rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Acetylcholine (µmoles of Ach/g tissue)</th>
<th>Acetylcholinesterase (µmoles of Ach hydrolysed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C</td>
<td>0.96±0.019</td>
<td>3.59±0.17</td>
</tr>
<tr>
<td>Group C+CM</td>
<td>0.49±0.026</td>
<td>2.28±0.11</td>
</tr>
<tr>
<td>Group D</td>
<td>0.274±0.009</td>
<td>5.39±0.18</td>
</tr>
<tr>
<td>Group D+CM</td>
<td>0.382±0.023</td>
<td>2.80±0.18</td>
</tr>
</tbody>
</table>

C: control, C+CM: control rats treated with EtCMGR, D: diabetic, D+CM: diabetic rats treated with EtCMGR. Values are expressed as mean±SEM (n=8 animals). Means with different superscripts within the column are significantly different at P<0.05 (Duncan’s multiple range test).

Effect of EtCMGR on transaminases

Data presented in table 3 indicate total protein content and activities of transaminases (GOT and GPT) measured in the four experimental groups. STZ induced diabetic rats showed significant decrease in protein content (28%) with significantly increased activities of GOT (67%) and GPT (24%) in brain when compared with group-C. EtCMGR treatment for 60 days in group-D+CM rats resulted in significant increase in protein content (18%) with significantly decreased activities of GOT (27%) and GPT (15%) in brain compared to group-D. But this change in protein content and transaminases in group-D+CM rats could not reach the control values. However group-C+CM rats showed no significant variation in protein content and activities of GPT and GOT when compared to group-C.

DISCUSSION

Diabetes mellitus is a chronic disease which cannot be completely cured and may lead to development of complications if not properly...
regulated. Diabetes is primarily characterized by hyperglycemia which results from lack of insulin or a weak response of tissues to this hormone. It is associated with long-term complications affecting the eyes, kidneys, cardiovascular system and nervous system [45-47].

In the current study, the significant decrease in total lipid content of group-D rats may be due to increased catabolism of lipids and/or enhanced LPO due to oxidative stress observed under diabetic conditions. Earlier studies in our laboratory demonstrated a defective metabolism of lipid peroxides in brain tissue of diabetic animals [48]. EtCMGR treatment for 60 days showed partial recovery from diabetic induced decrease in total lipid content of group-D+CM which may be due to its protective effect against diabetic induced alterations in lipid metabolism.

In STZ induced diabetic rat brain alterations may be responsible for demyelination and nerve degeneration. The alterations in brain lipids lead to changes in physiological properties of membranes, in enzyme activities, receptors, transport, and cellular interactions and belongs to a family of hydrolases. It hydrolyses acetylcholine and is used as a marker for cholinergic neural function. Degradation of acetylcholine is necessary to depolarize nerves so that it might be available for recycling and oxidative stress under hyperglycemic conditions indicates altered protein metabolism in diabetes. Increased GPT activity may be related to increased transport of NADH from the cytosol to mitochondria [70]. Matthews et al. [71] reported that both the enzymes degrade glutamate; though only GPT was able to reduce toxic (500 µM) levels of glutamate into the physiologic (<20 µM) range. The excitotoxic effect of glutamate is believed to be the cause of several neurodegenerative processes [72] and several enzymes with the capacity to degrade glutamate have been suggested as possible neuro-protectants [71]. Thus, enhanced transaminase activities in group-D rats indicate an adaptive mechanism to decrease the glutamate level in the brain. Our reports of decrease in protein content and increase in extent of GPT and GOT activities are supported by earlier studies on diabetic rats [73]. This may be due to increased protein turnover and also increased catabolism of amino acid in the brain of diabetic rats due to enhanced protein oxidation and protein glycation observed under hyperglycemic conditions [48]. The partial rectification of decreased protein content and enhanced transaminase activities observed in group-D+CM by EtCMGR treatment which may be due to its antihyperglycemic effect with enhanced insulin secretion. Thus C. mukul treatment to diabetic rats showed improved brain energy metabolism.

CONCLUSION

In summary, EtCMGR has been shown to have, besides hypoglycemic properties, strong hypolipidemic action on diabetic hypertriglyceridemia and hypercholesterolemia as well. This could be useful for prevention or early treatment of diabetic disorders. Further studies are in progress in identifying the active components in C. mukul and their role in controlling diabetes.

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CONFLICT OF INTERESTS

Declared None

REFERENCES


