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EFFECT OF *MLEAA* TREATMENT ON THE LIPID PROFILE OF THE DIABETIC RAT BRAIN IN COMPARISON WITH METFORMIN DRUG

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ABSTRACT
The present investigation was intended to probe the response of MLEAA (methanolic leaf extract of *Artemisia absinthium*) treatment on the levels of lipid profile in experimental diabetic rats. A total of 40 male rats were divided into 5 groups, 8 in each group; Normal group (N), the Normal group treated with *Artemisia absinthium* (NA), Diabetic group (D), Diabetic group treated with *Artemisia absinthium* (DA) and Diabetic group treated with metformin (DM). STZ (Streptozotocin-55 mg/kg body weight) was used to induce diabetes mellitus through intraperitoneal injection in normal male Wistar rats by a lone shot. Two months treatment with MLEAA has produced a notable rise in the content of total lipids (TL), phospholipids (PL), glycolipids (GL) and cholesterol (C) in the diabetic group rats, but a momentary decrease in triglyceride content in DA group rats was reported when compared to D group rats like in DM group rats that has been medicated with metformin drug. DA group rats that were treated with MLEAA for 2 months has emphasized the defensive response of the *Artemisia absinthium* plant against STZ induced diabetes in Wistar albino rats in comparison to metformin, a commercial antidiabetic drug.

KEYWORDS: Total lipids (TL), phospholipids (PL), glycolipids (GL) and Metformin.

INTRODUCTION
Diabetes mellitus is a biochemical metabolic defect associated with carbohydrate, amino acid and lipid metabolic pathways. Hyperglycemia, insulin deficiency, long-term complications of different organs like brain, eyes, heart, kidney and limbs are most prominent features of diabetes.[1] Insulin dependent diabetes mellitus - Type 1 diabetes and non-insulin-dependent diabetes mellitus -Type 2 diabetes are the two different types of this disorder. In Type 1 diabetes damage of insulin producing pancreatic beta cells is due to autoimmune condition raised against the self during this disorder. Impairment of insulin secretion and peripheral insulin resistance causes Type 2 diabetes.[2,3] Several independent estimates along with World Health Organization (WHO) has reported that by 2030, more populated Asian countries like India and China's expenditure is going to be more than a trillion US dollars yearly for medication of diabetes and its complications due to huge rise in the cases of diabetes.[4,5]

Experimental diabetic rats due to STZ are good animal models to study the molecular, cellular and morphological changes in the brain induced by stress during diabetes.[6] Cellular and morphological changes in the brain are caused by oxidative stress due to hyperglycemia in diabetic rats.[7] Peroxidation of lipids and release of free radicals damage the brain resulting neurophysiological changes that leads to decreased blood flow to the brain tissues, damage of the layers of blood brain barrier and cerebral edema.[8] All such everlasting aggravated alterations lead to coronary artery disease, renal failure, blindness, foot amputation and nerve damage in diabetic condition.[9-11] Huge dependency on glucose for energy resources, more consumption of oxygen, high lipid content and scanty antioxidant system of brain makes it more susceptible to oxidative stress.

Increased free fatty acid mobility across biological layer is linked to decreased lipase activity due to deficiency of insulin hormone. STZ induced diabetic model serves as model of Type-I diabetes mellitus and hyperlipidemia.[12-14] Increased TAG (triacylglycerol) and LDL (low density lipoproteins) levels and decreased HDL (high density lipoprotein) levels are responsible for developing premature arteriosclerosis in diabetes.[15] None of the modern oral hypoglycemic drugs offers faultless glycemic controls including insulin.[16] Worldwide many medicinal plants and their extracts are used for treating diabetes. Since decades traditional Indian medication systems like Ayurveda, Homeopathy, Siddha and Unani has included several herbs and medicinal plants for the treatment of diabetes mellitus.[17]

Artemisia absinthium is indigenous to Jammu and Kashmir region in India even though it is globally

distributed naturally. Wormwood (*Artemisia absinthium*) is an aromatic shrub that has been used as herbal medicine for treating several health disorders since ancient times. Pharmacological examination of *Artemisia absinthium* has revealed its free-radical scavenging activity, anti-oxidative stress function, antioxidant activity and neurite outgrowth function in former studies.[18] Historical medicinal values of *Artemisia absinthium* have motivated us to investigate its protective action on STZ induced alterations in the lipid profile of brain tissues of diabetic rats.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals and reagents of the study were procured from Sigma Chemical Company (USA) and SISCO Research laboratory Pvt. Ltd, India.

Plant material

MLEAA (methanol leaf extract of *Artemisia absinthium*) was purchased in dried powder form from Mahaks Herbal & Aromatic Agro Products, Srinagar, and Jammu & Kashmir. Then MLEAA extract was suspended in 5% Tween-80 in distilled water until utilization.

Maintenance of Wistar rats

Male Wistar rats with average body weight of 150–200 g were acclimatized for a week in the animal house and maintained at a standard temperature of 24-28^{o}C with a 12 h light/dark schedule cycle. Rats were fed with a rodent pellet diet and water *ad libitum* under aseptic conditions. The study was conducted in the Post Graduate Department of Pharmacology Laboratory, Sree Siddaganga College of Pharmacy, Tumkur, with due permission from the Institutional Animal Ethics Committee (IAEC) with Regd. no: 123/PO/C/99/CPCSEA.

DESIGN OF THE EXPERIMENT

Experimental groups

A total of 40 rats were divided into five groups of eight in each as: Normal rats (N), Normal rats treated with MLEAA (NA), Diabetic rats (D), Diabetic rats treated with MLEAA (DA) and Diabetic rats treated with Metformin drug (DM).

Induction of diabetes

In D, DA and DM marked groups diabetes was induced after 16 hours of fasting by a single intraperitoneal injection of freshly prepared streptozotocin solution with a dosage of 55 mg /kg body weight[19, 20] in ice cold 0.05 M citrate buffer pH 4.5 at a volume of 0.1 ml per rat. 72 hours after the inoculation of STZ dose, rats with determined plasma glucose levels above 300 mg/dl were considered as diabetic and confirmed for usage in the experimentation.

Treatment with MLEAA

The dose of MLEAA (500 mg/kg body weight) in the treatment is based on the previous research reports on the *A. absinthium* extract.[21,22] NA and DA groups were treated daily with MLEAA (500 mg/kg body weight) but DM group is treated with Metformin drug (100 mg/kg body weight) orally by gastric intubation in 5% Tween-80 in distilled water per rat once a day for two months. N (Normal) and D (diabetic) rats were given distilled water instead of MLEAA. Body weight, fasting plasma glucose and levels of insulin were monitored at 15-day intervals till the end of the study. Treatment dose of the MLEAA (500 mg/kg b.w.) was fixed based on the former experimental results on the dose-dependent antihyperlipidemic effect of *A. absinthium* extract, which reported that a dose less than 200 mg/kg b.w. was not expected to be effective in rats.[21,22]

Brain tissue collection for assay of lipids

At the end of the experimentation all the rats from different groups were starved for 12 hours and sacrificed by cervical dislocation. After that the total brain tissue was dissected out and washed with saline (0.9% NaCl-ice cold) and utilized for the analysis of various lipid parameters.

BIOLOGICAL ASSAY OF LIPID PARAMETERS

Assay methods

Estimation of total lipids (TL) by gravimetric method

5ml of $CHCl_3$ (chloroform) and 5ml of CH_3OH (methanol) are added to 5grams of brain tissue and homogenized. After homogenization again 5ml of $CHCl_3$ is added to the mixture and homogenized for another 30seconds. Finally water (5ml) is added to this mixture, and the sample is again homogenized for 30 more seconds. Then the mixture is allowed to separate, the lower solvent phase is removed and filtered through a Whatman no. 1 filter paper, the filtrate is collected. This procedure is repeated once again with the addition of chloroform step. At the final step of the procedure the filtrate is allowed to separate in a graduated cylinder and the volume of the lower $CHCl_3$ layer is recorded. Pre-weighed aluminum pans (3 pans per sample) are used to evaporate 0.5 ml aliquots of the $CHCl_3$ layer overnight in a hood to estimate the total lipid (TL) content of the brain tissue by recording the weights, and then converting to percent lipids.[24]

Extraction of lipids (PL, GL, TG and cholesterol) from brain tissue

Tissue homogenate was prepared by adding Folch reagent (2:1 chloroform – methanol mixture) to brain tissue before centrifuging at 3000 rpm. Then 5 ml of supernatant was mixed with 3 ml of distilled water, centrifuged at 3000 rpm and resulting organic phase was used for the estimation of PL, GL, TG and cholesterol analysis.[23]

Phospholipids (PL)

Method of Connerty *et al* was implied for the estimation of Phospholipids (PL).[27] Through Fiske and Subbarow method released inorganic phosphate (Pi) was estimated by digesting PL with H_2SO_4.[28] About 200 µl of lipid

extract was evaporated; it is added to 1 ml of 10 N H_2SO_4 and digested for an hour in a hot water bath. Then H_2O_2 (20µl) was added to it, boiled till colorless liquid is seen and the tubes were cooled. Later phosphorus (Pi) was estimated by Fiske Subbarow method. 1.0 ml of molybdate II, 0.4 ml of ANSA (1-amino-2-napthol-4-sulfonic acid) reagents were added to the digest and the volume was made up to 10 ml with distilled H_2O. At 660 nm developed colour (blue) was recorded after15 minutes of incubation. Phospholipid (PL) content is obtained by multiplication of phosphate (Pi) value by 25. The results are expressed as mg/g tissue.

Triglycerides (TG)

Triglycerides (TG) content was assayed through GPO-PAP (Glycerol phosphate oxidase-p-aminophenazone) enzymatic method using Liquid Gold Diagnostic kit.[25] Lipid extract of the brain (60µl) in an eppendorff tube, was evaporated using an incubator. Then it is added to 1.0 ml of the TG reagent and incubated at 37˚C for 10 minutes. In the same way standard (TG 200 mg/dl) and blank (H_2O) were also treated. At 505 nm absorbance was recorded and the results are expressed as mg/g tissue after incubation for all the samples.

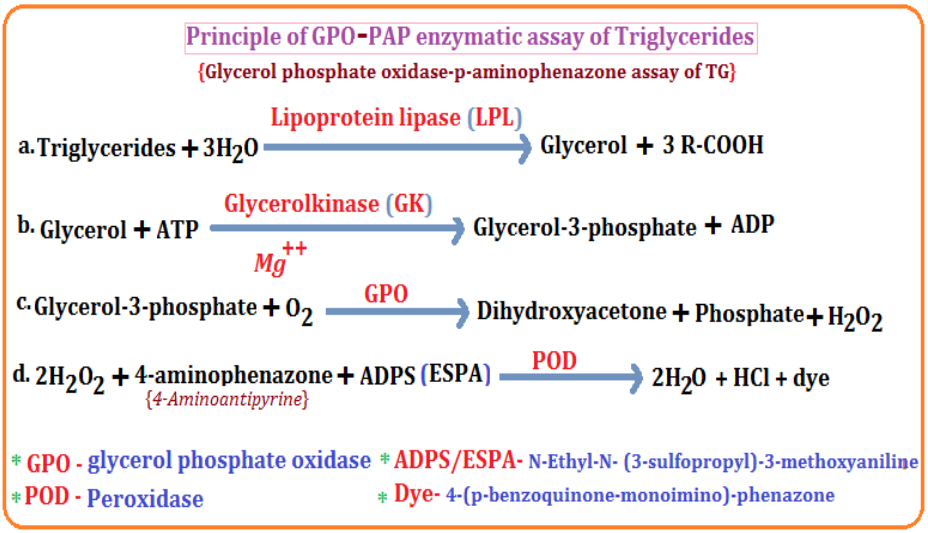

Figure 1: Principle mechanism of estimation of triglycerides.

Cholesterol (C)

Total cholesterol content was assayed through CHOD-PAP (cholesterol oxidase - phenol 4-aminoantipyrine peroxidase) enzymatic method.[26] Cholesterol reagent (1ml) was added to the lipid extract (60µl) that has been pre evaporated and the mixture is incubated at 37˚C for 10 minutes. Same treatment is implied to the blank (H_2O) and Cholesterol standard (200 mg /dl). The results are expressed as mg/g tissue upon reading the absorbance values of the incubated samples at 510 nm.

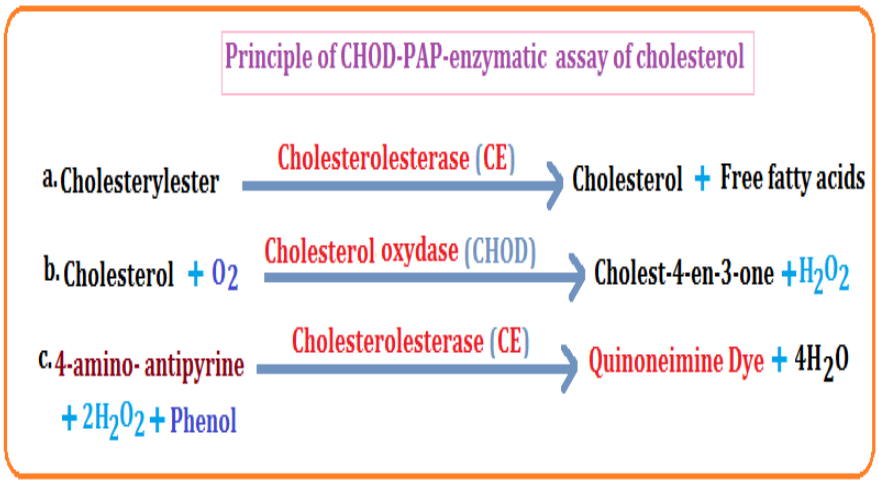

Figure 2: Principle mechanism of estimation of cholesterol.

Glycolipids (GL)

Procedure of Roughan & Batt method was used to estimate Glycolipid (GL) content in lipid extract of brain tissue.[29] Tissue homogenate (200 µl) was evaporated, added to 2 ml of 2 N sulphuric acid and digested for two hours in a water bath (boiling). After hydrolytic digestion, it is centrifuged by adding 4 ml of $CHCl_3$ (chloroform). The Supernatant aqueous layer was

collected after centrifugation and it is added with 50 µl of 80% C_6H_5OH (phenol) and 4 ml of con.H_2SO_4. The colour (orange) developed was read at 480 nm. Standards of galactose sugar (20-200µg) were also treated in the same procedure. Multiplication of galactose sugar concentration with 4.45 gives the concentration of glycolipid and the results are expressed as mg/g tissue.

Statistical data analysis

The results were displayed as mean ± S.E.M. Research data was analyzed for significant difference using Duncan's Multiple Range (DMR) test ($P < 0.05$) (Duncan, 1955).[30]

RESULTS

Effect of MLEAA on lipid profile

Total lipids (TL) comprises phospholipids (PL), glycolipids (GL), cholesterol and triglycerides (TG). Brain tissue lipid profile of normal (N), normal treated with *A.absinthium* (NA), diabetic (D), diabetic treated with *A.absinthium* (DA) and diabetic treated with metformin (DM) rat groups are displayed in table1 & figure3. Diabetic rats (D) exhibited significant decrease in TL (62%) content when compared with normal group (N). *Artemisia absinthium* treatment for 2 months improved TL (103%) content greatly in DA rats when compared to D group. But this marked hike in TL content in DA did not reach the normal values. Thus DA rats showed somewhat lesser TL (24%) content when compared to N group. DA group has also displayed lesser TL (19.57%) content when compared to DM group. NA rats displayed a minor (9%) but not marked reduction in TL content when compared to N rats. D rats displayed quite downturn in the levels of PL, GL, and cholesterol (35%, 72% and 25%) but very much boost in TG (54%) levels when compared to N rats at the end of experimentation. DA rats after systematic medication with *Artemisia absinthium* extract for 60 days exposed growth in the levels of PL, GL, and cholesterol (26%, 140% and 21%) and much decline in TG (34%) levels when compared to D rats. PL, GL and cholesterol levels of DA rats did not reach the normal values of N rats though TG levels were normalized. PL, GL and cholesterol levels of DA rats are less than the values of DM group (9.11%, 17.62%, and 7.76%) but the levels of TG are slightly more (2.31%) than the levels of TG in DM group.NA rats show cased a slight rise in GL (29%) and reduction in cholesterol and PL (4.6% and 7.8%) and slight growth (2.6%) in TG levels when compared to N rats.

LIPID PROFILE

Table 1: Lipid profile (mg/g tissue) in STZ lured diabetic rats upon MLEAA treatment.

Lipid (mg/g tissue)	N	NA	D	DA	DM
Total lipids	340.36±13.42^{d}	307.12±5.32^{c}	127.15±16.32^{a}	258.27±4.56^{b}	321.15±2.21^{d}
Phospholipids	67.42±1.45^{c}	62.11±1.32^{c}	43.32±2.12^{a}	54.72±1.15^{b}	60. 21±1.11^{d}
Triglycerides	7.84±0.12^{a}	7.63±0.22^{b}	12.14±0.31^{d}	7.96±0.54^{c}	7.78±0.13^{c}
Cholesterol	30.21±0.85^{d}	28.82±0.23^{c}	22.51±1.24^{a}	27.43±0.51^{c}	29.74±0.21^{a}
Glycolipids	3.72±0.16^{c}	4.82±0.23^{d}	1.01±0.11^{a}	2.43±0.21^{b}	2.95±0.18^{c}

Communicated results are mean ± S.E.M (n=8). Means with various superscripts inside the segment are fundamentally different at P<0.05 (Duncan's multiple range test).

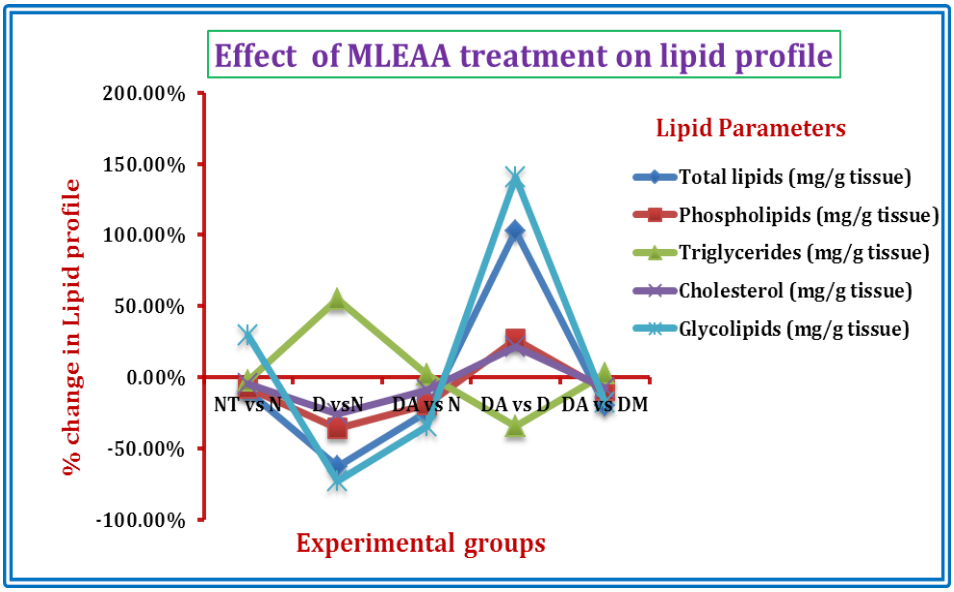

Figure 3: Change in lipid profile of STZ lured diabetic rats upon MLEAA treatment.

DISCUSSION

Determining the levels of various lipids is part of the evaluation of lipid metabolism and it plays a major role in identification of the various hyperlipoproteinemias. The decreased PL, GL and cholesterol and increased TG content of D group rats compared to N rats indicate the damage or oxidation of lipid content in bilayers of brain cells in diabetes. Changes in the STZ induced diabetic rat brain may be due to nerve decadence. Our reports of decreased PL, GL and cholesterol content in diabetic rats are in agreement with earlier studies.[31] Diabetes has been linked with an expanded peril of developing early on atherosclerosis due to increase in TG and LDL levels and decrease in HDL levels. The increased levels of TG cause certain types of liver and renal diseases also along with coronary artery disease in diabetic state. Thus dyslipidemia is a well-known characteristic feature of diabetes due to altered lipid metabolism in many tissues. MLEAA treatment of STZ lured diabetic rats has presented the proof of normolipemic, anti-hypertriglyceridemia properties of *Artemisia absinthium* in comparison with diabetic drug metformin.

CONCLUSION

It is confirmed that MLEAA (methanolic leaf extract of *Artemisia absinthium*) is having counteracting ability against hypertriglyceridemia and is helpful in normalizing the levels of other lipids like PL, GL and cholesterol by maintaining the total lipid content (TL) in its optimum range in STZ lured diabetic rats. This study has furnished enough proof for the safe use of *Artemisia absinthium* in the treatment of diabetes mellitus. Further pharmacological evaluation is required for the identification of complete anti-diabetic activity of the *Artemisia absinthium* plant.

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