Effects of *Mucuna pruriens* on Free Fatty Acid Levels and Histopathological Changes in the Brains of Rats Fed a High Fructose Diet

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Significance of the Study

- In this study, a high fructose diet fed to rats increased the levels of free arachidonic acid and docosahexaenoic acid, and also caused extensive gliosis, neuronal hydropic degeneration, and edema but these effects were reversed when *Mucuna pruriens* was added to the high fructose diet. Hence, *M. pruriens* could have a therapeutic benefit by reversing pathological changes caused by altered brain fatty acid metabolism and histopathological changes due to a high fructose diet.

Keywords

Brain · Free fatty acids · Fructose · Histopathology · *Mucuna pruriens*

Abstract

Objective: To investigate free fatty acid levels and histopathological changes in the brain of rats fed a high fructose diet (HFrD) and to evaluate the effects of *Mucuna pruriens*, known to have antidiabetic activity, on these changes. **Materials and Methods:** The study comprised 28 mature female Wistar rats. The rats were divided into 4 groups, each included 7 rats. Group 1: control; group 2: fed an HFrD; group 3: fed normal rat chow and *M. pruriens*; group 4: fed an HFrD and *M. pruriens* for 6 weeks. At the end of 6 weeks, the rats were decapitated, blood and brain tissues were obtained. Serum glucose and triglyceride levels were measured. Free fatty

acid levels were measured in 1 cerebral hemisphere of each rat and histopathological changes in the other. The Mann-Whitney *U* test was used to compare quantitative continuous data between 2 independent groups, and the Kruskal-Wallis test was used to compare quantitative continuous data between more than 2 independent groups. **Results:** Arachidonic acid and docosahexaenoic acid levels were significantly higher in group 2 than in group 1 ($p < 0.05$). Free arachidonic acid and docosahexaenoic acid levels in group 4 were significantly less than in group 2 ($p < 0.05$). Histopathological examination of group 2 revealed extensive gliosis, neuronal hydropic degeneration, and edema. In group 4, gliosis was much lighter than in group 2, and edema was not observed. Neuronal structures in group 4 were similar to those in group 1. **Conclusions:** The HFrD increased the levels of free arachidonic acid and docosahexaenoic acid probably due to membrane degradation resulting from possible oxi-

dative stress and inflammation in the brain. The HFrD also caused extensive gliosis, neuronal hydropic degeneration, and edema. Hence, *M. pruriens* could have therapeutic effects on free fatty acid metabolism and local inflammatory responses in the brains of rats fed an HFrD.

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Introduction

The dietary intake of excess fructose has negative effects on many tissues and may cause various metabolic disorders [1]. It has been shown that short-term feeding of rats with a high fructose diet (HFrD) leads to metabolic changes that may cause prediabetes in humans and in the case of longer HFrD exposure may result in type II diabetes [2]. A high carbohydrate diet is associated with metabolic syndrome, central obesity, hyperlipidemia, hyperglycemia, atherosclerosis, nonalcoholic fatty liver, endothelial dysfunction, insulin resistance, and hypertension [3–5]. Problems such as hypertension, stroke, renal insufficiency, cardiovascular morbidity, and high mortality could also develop as a result of these conditions [5, 6]. In addition, several histological and clinical pathologies related to both proinflammation and increased oxidative stress in the brain have been demonstrated [7–9]. Cognitive decline, mood disorders, and behavioral abnormalities can develop due to increased cytokine production and brain lipid metabolism changes [7, 10].

Brain lipid metabolism is altered by high fructose or glucose intake [7, 11, 12]. A rat model of intracerebroventricular streptozotocin-induced brain insulin resistance showed decreased cerebral cortex concentrations of ethanolamine glycerophospholipid and phosphatidylserine, and increased concentrations of unesterified palmitate, stearate, and arachidonic acid (AA), suggesting increased phospholipase A₂-mediated membrane degradation [7, 11]. Brain phospholipid concentration was reported to be reduced in a genetic mouse model of diabetes [12]. Increased hippocampal malondialdehyde concentration (a marker of polyunsaturated fatty acid and oxidative degradation, was reported in the hippocampus of genetically obese and hypertensive rats [7]. These findings indicate that the metabolism of brain free fatty acids (FFA) could be affected by these metabolic changes.

Mucuna pruriens is a legume grown in Africa, South America, and South Asia [13, 14]. *M. pruriens* has a positive effect on enzymes involved in carbohydrate metabolism, either by increasing insulin secretion or by acting as

insulin thereby reducing blood sugar levels [13]. Anti-inflammatory, hypolipidemic, and antithrombotic effects have also been reported [14]. *M. pruriens* has antioxidant and cytotoxicity-reducing effects, and its effect on neurodegenerative diseases such as Alzheimer and Parkinson diseases has been investigated [15, 16].

The roles of *M. pruriens* in preventing metabolic changes that can occur in rats fed an HFrD, and the effects of *M. pruriens* on the brain are not well understood. In this study, changes in FFA and histopathological changes in brain tissue were investigated in rats fed an HFrD, and the effects of *M. pruriens* on these changes were analyzed.

Materials and Methods

Animals and Study Groups

This study was approved by the Institutional Animal Research Ethics Committee. The Experimental Animals Implementation and Research Center supplied 28 mature female Wistar rats (aged 8–9 weeks) with normal motor activity and weighing 200–250 g for this study. The rats were housed in standard laboratory conditions (12-h light/dark cycle; room temperature 20–22°C).

The rats were divided into 4 groups, each containing 7 rats. Group 1 (control): fed a normal rat chow diet and normal drinking water for 6 weeks; group 2 (HFrD): fed a normal rat chow diet and fructose water (50 g fructose powder per 100 mL drinking water) for 6 weeks; group 3 (*M. pruriens*): fed a normal rat chow diet and normal drinking water and given 100 mg/kg *M. pruriens* every day by gavage for 6 weeks; group 4 (HFrD + *M. pruriens*): fed a normal rat chow diet and fructose water (50 g fructose powder per 100 mL drinking water) along with 100 mg/kg *M. pruriens* every day by gavage for 6 weeks. *M. pruriens* dry extract was provided by OmniActive Health Technologies (item code: 51002; Hinjewadi, Pune, India). The extract was from *M. pruriens* dried seeds.

At the end of 6 weeks, blood samples were taken from the rats' tails just before decapitation. The samples were centrifuged at 3,000 g for 10 min, sera decanted and stored at –80°C until analysis. Serum glucose and triglyceride levels were measured with enzymatic assay kits (ADVIA Chemistry), by blood analyzer device (Autoanalyzer, Siemens). Brain tissues were taken and the whole brains were divided into 2 hemispheres. FFA levels were measured using gas chromatography (GC) using a Shimadzu GC instrument in 1 hemisphere. Histopathological examinations were performed, using light microscopy by a pathologist (I.H.O.) and a neurosurgeon (B.A.), in the other hemisphere.

Preparation of Fatty Acid Methyl Esters

Lipid extraction from tissue samples was performed using the Hara and Radin method, which uses a 3:2 volume/volume (v/v) hexane/isopropanol mixture [17]. The fatty acids in lipids were converted to methyl ester derivatives for gas chromatographic analysis. To prepare the methyl ester derivatives [18], the lipid extract in the hexane/isopropanol phase was transferred into 30-mL test tubes. Five milliliters of 2% methanol sulfuric acid was added and mixed well with a vortex. This mixture was left for methylation in the incubator at 50°C for 15 h. The tubes were re-

Table 1. Serum glucose and triglyceride levels (mg/dL)

	Group 1 (control)	Group 2 (HFrD)	Group 3 (<i>M. pruriens</i>)	Group 4 (<i>M. pruriens</i> + HFrD)	<i>p</i> values			
					G1 vs. G2	G1 vs. G3	G1 vs. G4	G2 vs. G4
Glucose	85.7±10.9	113.2±6.4	78.7±8.9	92.4±7.3	0.002	0.22	0.21	0.004
Triglyceride	90.4±24.9	175.2±34	88.8±11.3	97.5±46.5	0.001	0.43	0.32	0.003

Table 2. Percentages of free fatty acid peaking in the brain tissue

Fatty acids C:D	Group 1	Group 2	Group 3	Group 4	<i>p</i> values	
					G1 vs. G2	G2 vs. G4
14:0	0.314±0.096	0.328±0.107	0.327±0.157	0.414±0.164	>0.05	>0.05
15:0	0.316±0.059	0.241±0.078	0.320±0.108	0.328±0.163	>0.05	>0.05
16:1	0.456±0.207	0.374±0.065	0.339±0.127	0.491±0.126	>0.05	>0.05
16:0	31.414±2.046	30.754±1.192	31.032±2.777	29.783±3.399	>0.05	>0.05
17:0	0.816±0.125	0.688±0.126	1.051±0.339	1.246±0.185	>0.05	>0.05
18:2	0.391±0.121	0.227±0.055	0.389±0.077	0.291±0.096	>0.05	>0.05
18:1	17.583±1.966	17.250±1.565	17.269±1.826	17.509±2.047	>0.05	>0.05
18:0	29.878±1.481	29.321±2.959	30.164±1.744	29.693±3.748	>0.05	>0.05
20:4	6.247±0.811	9.860±0.945	6.043±0.961	7.046±4.059	0.002	0.01
20:0	1.029±0.384	0.616±0.111	1.078±0.368	1.208±0.284	>0.05	>0.05
21:0	2.926±0.702	2.440±0.373	2.827±0.399	3.081±0.419	>0.05	>0.05
22:6	6.392±1.193	8.887±1.033	5.918±1.063	6.839±1.050	0.004	0.005
22:2	0.060±0.014	0.069±0.016	0.068±0.019	0.081±0.016	>0.05	>0.05
22:1	0.237±0.080	0.182±0.035	0.235±0.045	0.263±0.030	>0.05	>0.05
22:0	0.536±0.157	0.389±0.099	0.505±0.109	0.786±0.419	>0.05	>0.05
24:0	0.376±0.297	0.285±0.109	0.226±0.054	0.304±0.138	>0.05	>0.05

C, number of carbon atoms; D, number of double bonds of the molecule.

moved from the incubator, cooled to room temperature and mixed well by adding 5 mL of 5% sodium chloride. Fatty acid methyl esters were extracted with 5 mL hexane, and the hexane phase was placed into the pipette, treated with 2% potassium bicarbonate (KHCO₃) and kept for 4 h to allow the phases to separate. The solution of the mixture that contained methyl esters was then subjected to evaporation at 45°C and under nitrogen flow dissolved with 1 mL of hexane, placed into 2-mL autosampler-covered vials, and analyzed with GC.

Gas chromatographic processes of fatty acid methyl esters were analyzed with a Shimadzu GC instrument. For this analysis, a Machery-Nagel capillary column was used. The temperature of the column was kept at 120–220°C, the injection temperature was set to 240°C; the detector temperature was kept at 280°C. Nitrogen was used as the carrier gas. The mixture belonging to the standard fatty acid methyl esters was injected, then the fatty acid methyl esters of the samples, and the retention time of each fatty acid was determined. Acetone was used for preparing injection probes. After these procedures, analysis of the fatty acid methyl esters of the samples was performed. The results were determined as the percentage amount for each fatty acid among the fatty acids.

Histopathological Examination

For histopathological investigation, brain samples were fixed in neutral-buffered 10% formalin. Fixed tissue samples were embedded in paraffin and horizontally sectioned into 4-μm slices with a microtome. After deparaffinization, sections were mounted on slides and stained with hematoxylin and eosin. The slides were assessed under a light microscope.

Statistical Analysis

The data were analyzed using SPSS (Statistical Package for Social Sciences for Windows v.22.0; SPSS Inc., Chicago, IL, USA). Means and standard deviations were used as descriptive statistical methods. The Mann-Whitney *U* test was used to compare quantitative continuous data between 2 independent groups and the Kruskal-Wallis test was used to compare quantitative continuous data between more than 2 independent groups. The Mann-Whitney *U* test was used as a complement to determine the differences after the Kruskal-Wallis *H* test. The findings were evaluated at the 95% confidence interval and at the 5% significance level. *p* < 0.05 was considered statistically significant.

Fig. 1. a-f Histopathological representations of brain tissue (supratentorial region, gray matter-white matter interface) samples of rats. Hematoxylin-eosin. $\times 20$. **a** Normal neuronal structures are shown in group 1. **b** Hydropic degeneration and edema (arrow) are seen in group 2. **c** Widespread gliosis (star) is also present in group 2. **d** Neuronal structures in group 3 are similar to group 1. **e** Slight gliosis is seen in group 4. **f** Neuronal structures in group 4 are similar to groups 1 and 3.

Results

Blood Tests

The levels of serum glucose and triglyceride are presented in Table 1. Serum glucose and triglyceride levels were significantly higher in group 2 (glucose: 113.2 ± 6.4 , triglyceride: 175.2 ± 34) compared with group 1 (glucose: 85.7 ± 10.9 , triglyceride: 90.4 ± 24.9) ($p < 0.05$). Glucose and triglyceride levels were slightly higher in group 4 (glucose: 92.4 ± 7.3 , triglyceride: 97.5 ± 46.5) than group 1, but the difference was not statistically significant ($p > 0.05$). Serum glucose and triglyceride levels were significantly less in group 4 compared with group 2 (*M. pruriens*) ($p < 0.05$).

Brain Tissue FFA Levels

The peak levels of all FFA in brain tissue by group are shown in Table 2. AA (20: 4n-6) and docosahexaenoic acid (DHA) (22: 6n-3) levels were significantly higher ($p < 0.05$) in group 2 (AA: 9.860 ± 0.945 , DHA: 8.887 ± 1.033) than group 1 (AA: 6.247 ± 0.811 , DHA: 6.392 ± 1.193). Free AA and DHA levels were significantly less in group 4 (AA: 7.046 ± 4.059 , DHA: 6.839 ± 1.050) compared with group 2 ($p < 0.05$).

Histopathology

Normal neuronal structures were observed in group 1. Extensive gliosis, neuronal hydropic degeneration and edema were seen in group 2. Normal neuronal structures, similar to the control group, were also seen in group 3. In group 4, mild gliosis was seen, but edema was not observed and the neuronal structures were similar to those of group 1. The histopathological features of the 4 groups are given in Figure 1.

Discussion

In this study, HFrD did not alter saturated or mono-unsaturated FFA levels, but higher levels of 2 specific polyunsaturated fatty acids, AA and DHA. These findings could lead to impairment of membrane integrity due to lipid peroxidation, as membrane degradation may increase the amount of free AA and DHA. Rats that were fed HFrD and received *M. pruriens* showed a significant decrease in free AA and DHA levels compared to rats fed with HFrD without *M. pruriens*. These findings indicate that *M. pruriens* might have an inhibitory effect on membrane degradation in the brain that can develop with an

HFrD. Recent studies showed a significant expression of genes necessary for fructose metabolism in the brain [19]. It was clear from these data that several regions of the brain, including the cerebellum, hippocampus, cerebral cortex, and olfactory bulb, were capable of significant fructose metabolism. Additionally, this fructose metabolism was enhanced in brains after exposure to high fructose in the diet. Dietary fructose can induce some changes in brain metabolism, which could lead to possible detrimental metabolic dysregulation in the brain of individuals with a high fructose diet, as is seen in other organs [19].

Serum glucose and triglyceride levels increased significantly due to HFrD in our experiment. A high carbohydrate diet, also called a ketogenic diet, is known to cause a variety of metabolic diseases. Insulin resistance and elevated serum triglycerides are impacted by high levels of fructose in the diet. Elevated serum triglycerides have been postulated to disrupt insulin signaling by accumulating inside the cell [8]. Examination of the effects of such a diet on neurological diseases indicates that neurodegenerative disorders (e.g., Alzheimer and Parkinson diseases) may develop due to mitochondrial and cell membrane disorders. In addition, cognitive and behavioral changes may occur due to oxidative stress and inflammatory cytokine release [7, 10, 20]. Increased oxidative stress due to high fructose uptake enhances lipid peroxidation [9]. Changes in AA and DHA metabolism, which have important roles in cell membrane integrity and fluidity, have been reported in the brain due to membrane peroxidation [7, 11, 21].

The finding of this study that rats fed an HFrD revealed extensive gliosis, neuronal hydropic degeneration, and edema in the brains could be due to possible oxidative stress and local inflammatory responses depending on inflammation. This finding could be due to HFrD and *M. pruriens* together causing gliosis that was much lighter, edema was not observed, and neuronal structures were similar to those of the control group. These indicate that *M. pruriens* could protect the brain against HFrD-related histopathological changes. However, several studies [22, 23] have shown that high blood sugar led to histopathological changes correlated with neuronal damage via oxidative stress and inflammatory cytokine release. Reactive gliosis is strongly associated with brain inflammation; increased inflammation is a key physiological feature of obesity, diabetes mellitus and/or metabolic syndrome [24]. The brain is highly sensitive to inflammation and inflammatory mediators, and metabolic dysfunction in both humans and laboratory animals has been associated

with reactive gliosis and increased brain inflammation [25, 26]. Probable effects of *M. pruriens* that might be responsible for the prevention of membrane degradation, gliosis, hydropic degeneration, and edema in the brain have been reported previously [15, 16]. *M. pruriens* can reduce blood sugar levels by increasing insulin secretion or acting like insulin. Thus, it could prevent the development of insulin resistance in tissues. Also, *M. pruriens* could strengthen membrane stabilization by reducing inflammation through hypolipidemic, antithrombotic, antioxidant, and cytotoxicity-reducing effects. Some of the potential active components or fractions of *M. pruriens* may be responsible for the different effects [13, 16]. Majekodunmi et al. [13] showed that the antidiabetic activity of *M. pruriens* seeds resides in the methanolic and ethanolic fractions of the extract. *M. pruriens* contains L-dopa, so its anti-Parkinson effects were attributed to its L-dopa content. Also *M. pruriens* seeds are rich in novel alkaloids, saponin, sterols, and minerals such as Mg^{2+} , K^+ and Fe^{3+} [13, 16]. The main limitation of this study included nonidentification of which components were essentially responsible for the findings.

Conclusions

An HFrD increased free AA and DHA levels due to membrane degradation as a result of possible oxidative stress and inflammation in the brain. In addition, an HFrD caused histopathological changes in the brain, for example, extensive gliosis, neuronal hydropic degeneration, and edema. In this study, *M. pruriens* had therapeutic effects for FFA metabolism and local inflammatory disorders in the brains of rats fed an HFrD. These findings need to be verified by additional experimental and clinical studies to further contribute to the literature on the therapeutic effects of *M. pruriens*.

Disclosure Statement

There are no conflicts of interest.

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