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Neuroprotective Potential of Minocycline in Metabolic Syndrome-Induced Cognitive Impairment: An Experimental Study in Mice

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Abstract

Metabolic syndrome (MetS) is increasingly recognized as a risk factor for cognitive decline and neurodegenerative disorders. This study investigates the underlying mechanisms of MetS-induced cognitive impairment and evaluates the therapeutic potential of minocycline. MetS was induced in Swiss albino mice using a high-fat high-carbohydrate (HFHC) diet. Behavioral, biochemical, and histopathological assessments were conducted to evaluate cognitive function, oxidative stress, and neuroinflammation. MetS animals exhibited significant metabolic alterations, impaired memory, increased acetylcholinesterase activity, elevated inflammatory cytokines, and reduced antioxidant defenses. Minocycline treatment significantly improved cognitive performance, reduced oxidative stress, and attenuated inflammatory responses. Histological analysis confirmed neuroprotection in hippocampal regions. These findings suggest that minocycline may serve as a potential therapeutic agent for managing MetS-associated cognitive dysfunction.

Keywords: Metabolic syndrome, cognitive impairment, minocycline, oxidative stress, neuroinflammation, hippocampus

1. Introduction

Metabolic syndrome (MetS) is a multifactorial disorder characterized by obesity, insulin resistance, hypertension, and dyslipidemia. It has emerged as a major global health issue affecting a significant portion of the adult population. Beyond cardiovascular complications, MetS is now strongly linked to cognitive decline and neurodegenerative diseases.

Chronic metabolic disturbances contribute to increased oxidative stress and systemic inflammation, which can adversely affect brain function. The hippocampus, a critical region for memory and learning, is particularly susceptible to such changes. Increased levels of pro-inflammatory cytokines and oxidative stress markers have been implicated in neuronal damage and synaptic dysfunction.

Minocycline, a lipophilic tetracycline antibiotic, has shown promising neuroprotective properties due to its anti-inflammatory and antioxidant actions. It modulates microglial activation and reduces cytokine production, making it a potential candidate for treating neuroinflammatory conditions.

This study aims to explore the effects of MetS on cognitive function and to evaluate the protective role of minocycline using behavioral, biochemical, and histopathological parameters.

2. Materials and Methods

2.1 Experimental Animals

Healthy adult male Swiss albino mice weighing 18–22 g were used for the study. Animals were procured from a registered animal facility and housed in polypropylene cages under controlled environmental conditions (temperature: 25 ± 2°C, relative humidity: 50–60%, 12 h light/dark cycle). Standard pellet diet and water were provided *ad libitum*.

All experimental procedures were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)**,** and the protocol was approved by the Institutional Animal Ethics Committee (IAEC).

2.2 Induction of Metabolic Syndrome

Metabolic syndrome was experimentally induced by feeding animals a high-fat high-carbohydrate (HFHC) diet for a period of four weeks. The diet consisted of maize starch, lard, casein, vitamin-mineral mixture, and sodium chloride. Additionally, drinking water was supplemented with 20% fructose solution to enhance carbohydrate intake.

This dietary regimen led to metabolic alterations including increased body weight, hyperglycemia, dyslipidemia, and elevated blood pressure, thereby mimicking features of metabolic syndrome.

2.3 Experimental Design

Animals were randomly divided into five groups (n = 10 per group):

i. **Group I (Control):** Received standard diet and water
ii. **Group II (Scopolamine):** Received standard diet + scopolamine (0.4 mg/kg, i.p.)
iii. **Group III (MetS):** Received HFHC diet for 4 weeks
iv. **Group IV (Minocycline Control):** Received standard diet + minocycline (50 mg/kg, p.o.)
v. **Group V (MetS + Minocycline):** Received HFHC diet + minocycline (50 mg/kg, p.o.) during last 10 days

2.4 Behavioral Studies

Behavioral assessments were performed to evaluate learning and memory functions in experimental animals. All tests were conducted in a quiet room under controlled conditions to minimize stress and external disturbances.

2.4.1 Elevated Plus Maze (EPM) Test

The elevated plus maze apparatus consisted of two open arms (25 × 5 cm) and two closed arms of the same dimensions with 15 cm high walls. The arms were arranged in a plus configuration and elevated approximately 50 cm above the floor.

Each animal was placed at the end of an open arm facing away from the central platform. The time taken by the animal to move from the open arm to any of the closed arms was recorded as **transfer latency (TL)**.

a. **Initial Transfer Latency (ITL)** was recorded on the first day
b. **Retention Transfer Latency (RTL)** was recorded after 24 hours

A decrease in TL on the retention day indicates improvement in memory, whereas an increase suggests cognitive impairment.

The maze was cleaned with alcohol between trials to eliminate olfactory cues.

2.4.2 Passive Avoidance Test

The passive avoidance apparatus consisted of two compartments: a brightly illuminated chamber and a dark chamber separated by a guillotine door. The floor of the dark compartment was equipped with stainless steel grids capable of delivering mild electric shock.

During the training session, each mouse was placed in the illuminated chamber. Upon entering the dark chamber, an electric foot shock (1 mA for 5 seconds) was administered.

After 24 hours, the **step-down latency (SDL)** was recorded without delivering shock. The time taken by the animal to re-enter the dark chamber was considered as a measure of memory retention.

a. Increased SDL indicates better memory retention
b. Decreased SDL indicates cognitive impairment

A cut-off time of 300 seconds was considered for the experiment.

2.4.3 Use of Amnestic Agent

Scopolamine (0.4 mg/kg, i.p.) was used as a standard amnestic agent to induce memory impairment and validate the behavioral models.

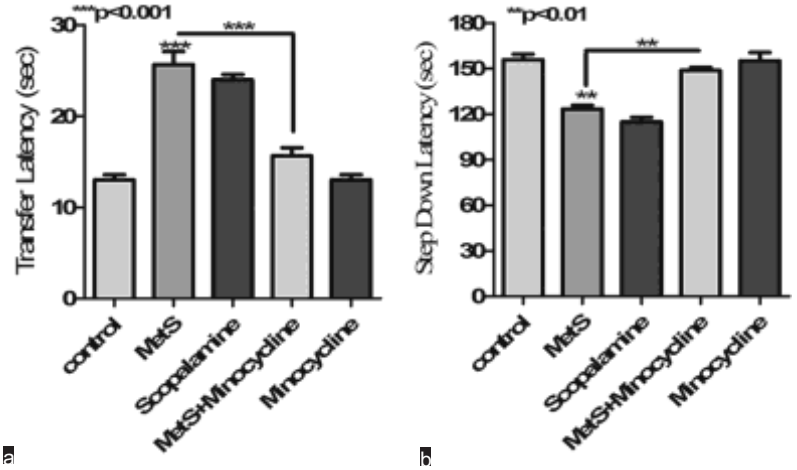

Figure 1 here: Effect on learning and memory

2.5Biochemical Parameters

a. Acetylcholinesterase (AChE) activity
b. Reduced Glutathione (GSH)
c. TNF-α and IL-1β

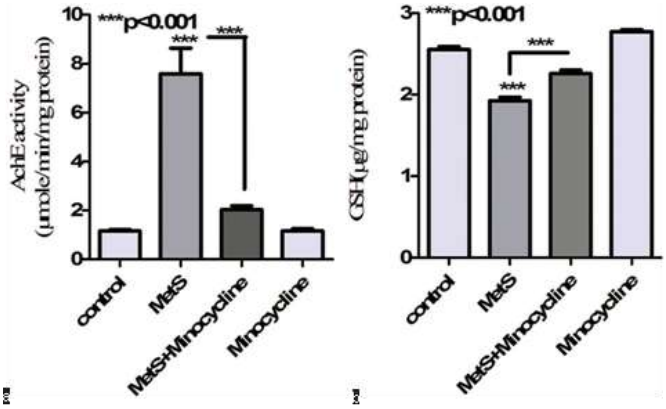

Figure 2 here: AChE and GSH levels

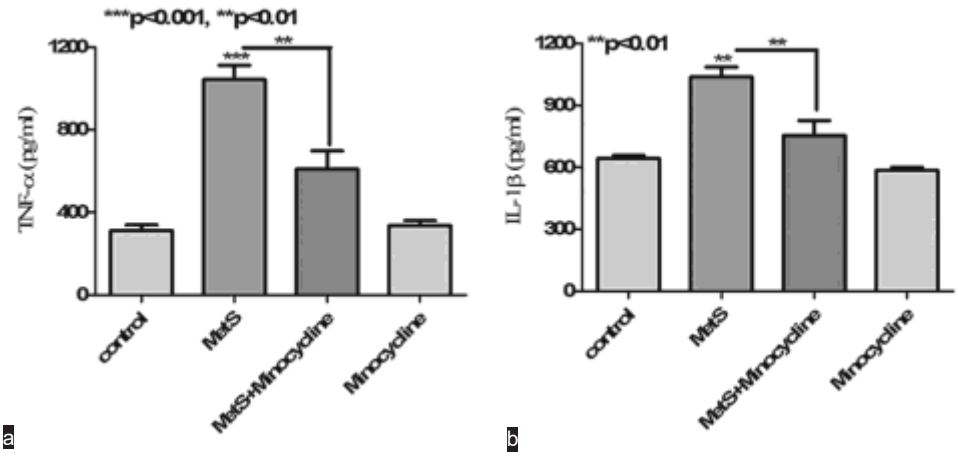

Figure 3 here: Cytokine levels

2.6 Histopathology

Hippocampal regions (CA1 & CA3) were examined.

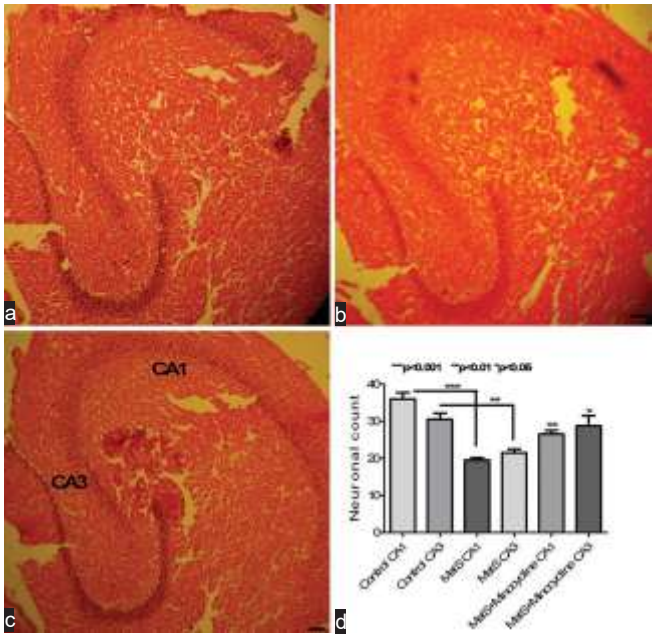

Figure 4 here: Histopathological images

2.7 Statistical Analysis

All experimental data were expressed as mean ± standard error of mean (SEM)**.** Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by multiple comparison post hoc test to evaluate intergroup differences.

The analysis was carried out using statistical software such as GraphPad Prism (Version 8.0 or later). A value of $p < 0.05$ was considered statistically significant.

Levels of significance were represented as:

i. $p < 0.05$ – statistically significant
ii. $p < 0.01$ – highly significant
iii. $p < 0.001$ – extremely significant

3. Results

3.1 Effect on Metabolic Parameters

Administration of a high-fat high-carbohydrate (HFHC) diet for four weeks resulted in significant metabolic alterations in experimental animals.

A marked increase (**$p < 0.001$**) in body weight was observed in the MetS group compared to the control group, indicating the development of obesity. Similarly, fasting blood glucose levels were significantly elevated (**$p < 0.001$**), confirming the induction of hyperglycemia.

Furthermore, the HFHC diet led to a significant increase in serum lipid profile parameters, including total cholesterol, triglycerides, and low-density lipoprotein (LDL), along with a reduction in high-density lipoprotein (HDL) **levels (**$p < 0.01$ to $p < 0.001$**).**

In addition, systolic blood pressure was significantly elevated in MetS-induced animals, further validating the successful induction of metabolic syndrome.

Treatment with minocycline in the MetS + minocycline group showed a significant reduction ($p < 0.05$–0.01) in body weight, blood glucose levels, and lipid profile parameters when compared to the MetS group. These findings suggest a protective effect of minocycline against metabolic disturbances.

Bodyweight (g)	**20±2**	**34.2±0.9****
Waistcircumference(cm)	7.1±0.8	**8.6±0.8*****
Fastingbloodglucose(mg/dl)	110±4.5	**265±3.2*****
Systolicbloodpressure(mmHg)	111.2±2.6	**137±2.4****
LDL(mg/dl)	32±0.6	**88.5±2.5*****
Triglyceride(mg/dl)	**110±2.5**	**154.3±2.8****

Table 1 here: Metabolic parameters

3.2 Effect on Cognitive Function

Cognitive performance was assessed using the Elevated Plus Maze (EPM) and Passive Avoidance test.
MetS-induced animals exhibited significant impairment in learning and memory functions as evidenced by a decrease in step-down latency (SDL) and a significant increase in transfer latency (TL) compared to the control group ($p < 0.001$). These findings indicate compromised memory retention and acquisition.
Administration of minocycline to MetS animals resulted in **a** significant improvement ($\boldsymbol{p} < 0.01$) in both SDL and TL values, suggesting enhanced cognitive performance and memory retention.
The scopolamine-treated group also showed marked cognitive deficits, validating the experimental model.

3.3 Effect on Oxidative Stress Markers

MetS induction led to a significant reduction ($\boldsymbol{p} < 0.001$) in hippocampal reduced glutathione (GSH) levels, indicating elevated oxidative stress and impaired antioxidant defense mechanisms.
Treatment with minocycline significantly restored GSH levels ($p < 0.01$) when compared to the MetS group, suggesting its potent antioxidant activity.

3.4 Effect on Neuroinflammatory Markers

MetS animals demonstrated a significant elevation ($\boldsymbol{p} < 0.001$) in pro-inflammatory cytokines, including TNF-α and IL-1β, compared to control animals.
Minocycline treatment significantly reduced the levels of these cytokines ($p < 0.01$), indicating its anti-inflammatory potential and ability to suppress neuroinflammation.

3.5 Histopathological Findings

Histological examination of hippocampal sections revealed marked neuronal degeneration and reduced neuronal density in the CA1 and CA3 regions of MetS-induced animals.
In contrast, animals treated with minocycline showed preservation of neuronal architecture**,** with reduced neuronal loss and improved cellular organization.
These findings confirm the neuroprotective effect of minocycline at the structural level.

4. Discussion

The present study demonstrates that metabolic syndrome significantly impairs cognitive function through mechanisms involving oxidative stress and neuroinflammation.
Chronic consumption of a high-fat high-carbohydrate diet resulted in metabolic disturbances, which are known to disrupt neuronal homeostasis. The observed decline in cognitive performance is consistent with increased oxidative stress, as evidenced by reduced GSH levels, and elevated inflammatory cytokines such as TNF-α and IL-1β.
Oxidative stress plays a critical role in neuronal damage by promoting lipid peroxidation, protein oxidation, and DNA damage, ultimately leading to impaired synaptic function. Simultaneously, increased levels of pro-inflammatory cytokines contribute to microglial activation and neuroinflammation, further exacerbating neuronal injury, particularly in the hippocampus.
Minocycline treatment significantly attenuated these pathological changes. Its neuroprotective effects may be attributed to:

a. Inhibition of microglial activation
b. Suppression of pro-inflammatory cytokine release
c. Enhancement of antioxidant defense mechanisms

The improvement in behavioral parameters, along with restoration of biochemical and histological alterations, indicates that minocycline effectively mitigates MetS-induced cognitive dysfunction.
These findings are in agreement with previous reports highlighting the role of inflammation and oxidative stress in neurodegenerative conditions and support the therapeutic potential of minocycline beyond its antimicrobial properties.

5. Conclusion

The present study establishes that metabolic syndrome induces significant cognitive impairment through oxidative stress and inflammatory pathways. The observed biochemical and histopathological alterations confirm neuronal damage, particularly in hippocampal regions.
Minocycline demonstrated significant neuroprotective effects by improving cognitive function, restoring antioxidant levels, reducing inflammatory cytokines, and preserving neuronal integrity.
These findings suggest that minocycline may serve as a promising therapeutic agent for the management of metabolic syndrome-associated cognitive dysfunction. However, further clinical studies are required to validate its efficacy and safety in humans.

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