COCHLEAR FIBROBLASTS REPAIRMENT IN RAT MODEL OF DIABETES MELLITUS AS A RESULT OF CURCUMIN TREATMENT (PRELIMINARY STUDY)

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Abstract

Background
Previous research suggests that curcumin is able to decrease malondialdehyde (MDA) expression due to oxidative stress related to noise exposure on fibroblasts of cochlear lateral wall. The literature shows that the damage of fibroblasts on cochlear lateral wall in diabetes mellitus is happened in similar manner to the damage caused by noise exposure. Therefore, this study aims to prove that curcumin also able to reduce MDA expression on fibroblast of cochlear lateral wall of diabetes mellitus rat models.

Materials and Methods
This preliminary study conducted on 9 male Wistar strain Rattus norvegicus rats which were divided into 3 groups. Group 1 serve as the control group which received intraperitoneal injection of citrate buffer and oral CMC through nasogastric tube. Group 2 is diabetes mellitus group model which received streptozotocin injection at a dose of 60mg / kg b.w single dose, administered intraperitoneally. Group 3 received the same treatment as group 2, followed by curcumin treatment with a dose 400 mg / kg b.w / day for 8 days. All rats underwent termination and necropsy procedure on their temporal bones for immunohistochemical assay to determine the expressions of MDA.

Result
The increased expression of MDA was detected in the diabetic group (without curcumin treatment). Curcumin treatment decreased the expression of MDA significantly (p <0.005).

Conclusion
Curcumin could repair the cochlear damage related to oxidative stress due to diabetes mellitus via decreased expressions of MDA on cochlear fibroblasts in rat models of diabetes mellitus.

Keywords: Diabetes Mellitus, Cochlea, Fibroblast, Curcumin, Malondialdehyde.
Introduction

Diabetes mellitus (DM) is a metabolic disease caused by chronic degenerative absolute insulin deficiency due to destruction of pancreatic β cells (type 1 diabetes) or relative deficiency due to ineffective use of insulin (type 2 diabetes) [1]. Various studies have been conducted to investigate hearing loss in patients with DM and showed a positive correlation. Hearing loss in diabetes related to hyperactivity of oxygen free radicals and leads to oxidative stress. Oxidative stress due to increased reactive oxygen species (ROS) resulted in apoptosis in neuronal cells, which underlie neuropathic complications due to diabetes. ROS is considered as one of the main causes in the process of cochlear cell death due to hyperglycemia [2,3,4].

Lipid peroxides are disintegrated quickly and form reactive carbon compounds, among these, malondialdehyde (MDA) is an important reactive carbon compound which is used commonly as an indicator of lipid peroxidation, and has become one of widely reported analytes for the purpose of estimating oxidative stress effects on lipids [5].

The antioxidant compounds overcome the toxicity of oxidative stress generated or enhanced by a number of conditions like nutritional imbalance, xenobiotics and their metabolic products, strenuous physical activities, and hereditary disorders. The attenuation properties of antioxidants protect the key biomolecules such as DNA, proteins, and lipids by scavenging reactive oxygen species (ROS). The use of curcumin, a herbal plant product, as an antioxidant is relatively safe as its toxicity is not reported even up to 10 g/day. The curcumin displays antioxidant properties due to the presence of phenolics and methoxy groups on the phenyl ring and 1,3-diketone groups. It acts as a strong antioxidant by neutralizing free radicals and by showing metal binding characteristics. Curcumin has potential to cross blood brain barrier in mammalian systems and exerts protective effect [6].

Materials and Methods

This experimental study was conducted on Wistar strain white rats (Rattus norvegicus) with randomized posttest-only control group design. Preliminary study conducted on 9 male Wistar strain Rattus norvegicus rats which were divided into 3 groups. Group 1 serve as the control group which received intraperitoneal injection of citrate buffer and oral CMC through nasogastric tube. Group 2 is diabetes mellitus group model which received streptozotocin injection at a dose of 60mg / kg b.w single dose, administered intraperitoneally. Group 3 received the same treatment as group 2, followed by curcumin treatment with a dose 400 mg / kg b.w / day for 8 days. Streptozotocin administered intraperitoneally at a dose of 60 mg / kg B.W (single dose). Curcumin derived from Curcuma longa L. (Turmeric).
All rats underwent termination and necropsy procedure on their temporal bones for immunohistochemical assay to determine the expressions of MDA.

**Result**

The expression of MDA were found to be increased in the DM model without curcumin treatment group (group 2) compared to other groups (group 1 and 3). Curcumin decreased the expression of MDA in DM model (group 3). Immunohistochemical assay on cochlear fibroblast resul shown in Figure 1 below:

![Images](image_url)

Fig. 1 The expression of MDA in each group (40x zoom): (A) group 1; (B) group 2; (C) group 3. The white arrow indicates the expression of MDA in cochlear fibroblasts marked by the brown color.

Data in table 1 showed significant differences for the expressions of MDA (P < 0.05) in DM rat model group without curcumin treatment compared to control group. A dose of 400 mg curcumin per day for 8 days showed statistically significant decrease in the expression of MDA compared to untreated DM group (P < 0.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean difference ± Standard Error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>-5.800 ± .730</td>
<td>.000</td>
</tr>
<tr>
<td>Group 3</td>
<td>.600 ± .730</td>
<td>1.000</td>
</tr>
<tr>
<td>Group 2</td>
<td>6.400 ± .730</td>
<td>.000*</td>
</tr>
</tbody>
</table>

*indicates significant p value at <0.05
Data in table 1 showed significant differences for MDA expression (P<0.05) in all groups, except in group 1 compared to group 3. A dose of 400 mg curcumin per day for 8 days showed statistically significant decrease in MDA expression compared to untreated group (P<0.05).

**Discussion**

The expression of MDA was found to be increased significantly in the cochlear fibroblasts of diabetes model group (group 2) compared to control group (group 1) (P<0.05) in this present study. The increased expression of MDA in diabetes model group was evoked by the increased levels of hydroxyl radical (OH+) due to the spontaneous reduction of hydrogen peroxide (H₂O₂) by free transition metal ions through Fenton and Haber-Weiss reactions, resulting in increased lipid peroxidation process directly to polyunsaturated fatty acids (PUFA) contained in the cell membranes. The finding in this study is in accordance with findings of Kumari et al. (2014) [7] who found increased level of MDA in diabetics patients compared to normal subjects. Sumathi et al (2013) [8] in a cross sectional study which includes hundred diabetic patients found that diabetic subjects suffering from sensorineural hearing loss has increased levels of serum MDA compared to diabetic subject without hearing loss.

Lipid peroxidation is one of the most widely used indicators of free radical formation, a key indicator of oxidative stress. Measurement of lipid peroxidation has historically relied on the detection of thiobarbituric acid (TBA) reactive compounds such as malondialdehyde (MDA) generated from the decomposition of lipid peroxidation products [9]. Lipid peroxidation is a chain reaction initiated by the hydrogen abstraction or addition of an oxygen radical, leading to oxidative damage of PUFA. The end products of lipid peroxidation include the cytotoxic aldehydes, such as MDA and 4-hydroxyalkenal (4-HAE), that possess high reactivity to proteins and DNA, and hydrocarbon gasses such as ethane [10].

In this present study, curcumin proved to be able to decrease the expression of MDA in cochlear fibroblasts (P<0.05). Curcumin is able to reduce the accumulation of MDA due to lipid peroxidation by preventing the spontaneous reduction of hydrogen peroxide (H₂O₂) caused by free transition metal ions through Fenton and Haber-Weiss reactions, resulting in decreased lipid peroxidation process directly to PUFA contained in the cell membranes.

**Conclusion**

This present study indicates that curcumin is effective agent in the treatment of oxidative damage in fibroblasts within the cochlear animal model of diabetes mellitus.
through decreased expressions of MDA. It provides more insight into the mechanism of curcumin as an exogenous antioxidant against diabetes related oxidative stress and may serve as a scientific basis in the traditional systems of medicine for the management of diabetes related sensorineural hearing loss in the future.

References


CERTIFICATE OF APPRECIATION
PRESENTED TO:

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