Effect of Curcumin on Spatial Working Memory and Oxidative Stress Biomarkers in Alloxan-induced Diabetic Swiss Albino Mice

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Authors' contributions

Article Information

Original Research Article

ABSTRACT

The study was undertaken to evaluate the effect of curcumin on blood glucose level and neurobehavioral response in Alloxan-induced diabetic Swiss Albino mice. The animals were divided into five groups of four each (n=4). Group I served as control and received distilled water, group II, III, IV and V were diabetic and received olive oil 1 mL/kg, glibenclamide 1 mg/kg, curcumin 50 mg/kg and curcumin 100 mg/kg respectively. Diabetes was induced by injection of alloxan 150 mg/kg intraperitoneally. All administrations were done *via* oral gavage for duration of 21 days. Oxidative stress biomarkers (catalase, superoxide dismutase and glutathione peroxidase) and lipid peroxidation (MDA) were assayed using standard assay kits and cognitive impairment

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was determined using spontaneous alternation in the Y-maze. The results of the Y- maze significantly increase the spontaneous alteration percentage in spatial working memory at the dose of 100 mg/kg curcumin when compared to diabetic control. As regards to the oxidative stress biomarkers administration of 50 and 100 mg/kg b.w curcumin significantly increase the level of catalase as compared to diabetic control. Furthermore, there was a significantly increase in the level of superoxide dismutase at the dose of 100 mg/kg as compared to the control. However, there was a significant decrease in the malondialdehyde (MDA) level as compared with the control. Also when compared with the standard drug glibenclamide with the two doses of circumin, there was a significant decrease in the MDA level. This study demonstrated that curcumin at a dose of 100 mg/kg significantly ($p < 0.05$) attenuated diabetes-induced cognitive impairment in the Y-maze. It may be concluded that oral administration of cucurmin for 21 days increases spontaneous alteration in spatial working memory, and has protective effects against oxidative stress biomarkers in alloxan induces diabetes.

1. INTRODUCTION

Cognitive impairments in the diabetic population are emerging problems that warrant immediate research attention. Evidences from neurocognitive tests suggest that cognitive dysfunction should be listed along with retinopathy, neuropathy, nephropathy and cardiovascular complications as one of the complications of diabetes [1,2]. Diabetes mellitus affected more than 415 million people in 2015 and this is projected to double by the year 2040. Nigeria has a prevalence of 0.8% to 11% involving both rural and urban dwellers with about 2% reported in Zaria [3,4]. The management of diabetes is enormous burden on individuals and government. The mechanisms underlying the development of cognitive dysfunction in diabetes have not been fully elucidated. Many hypotheses have been suggested based on the pathophysiological mechanisms through which diabetes might affect the initiation and progression of the pathology of dementia [5].

It is well established that oxidative stress is implicated in both the onset and progression of diabetes and its complications. It has been shown that cognitive deficit can cause hyperglycemia in diabetic rat, which is associated with an increase in ROS levels and reduction of antioxidant levels [6,7]. In addition, increased ROS generation has been shown to activate various cellular signaling pathways, such as the polyol pathway, protein kinase C activation, and an increase of glucose shunting *via* the hexosamine pathway. All of which are related to neuronal injury and cerebral damage. It was shown that administration of antioxidants could reverse the cognitive dysfunction in the diabetic rats [6,7].

Curcumin, commonly called diferuloyl methane, is a hydrophobic polyphenol derived from the *Curcuma longa*. Turmeric has been used traditionally for many ailments because of its wide spectrum of pharmacological activities. Curcumin has been identified as the active principle of turmeric; chemically, it is a bis-a, b-unsaturated b-diketone that exhibits keto-enol tautomerism. Scientific research spanning over more than four decades has confirmed the diverse pharmacological effects of curcumin and established its ability to act as a chemo preventive agent as well as a potential therapeutic agent against several chronic diseases [8].

Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, and anti-carcinogenic activities. It also has hepatoprotective and nephroprotective activities, suppresses thrombosis, protects against myocardial infarction, and has hypoglycemic and anti-rheumatic properties [9]. Moreover, curcumin has been shown in various animal models and human studies to be extremely safe even at very high doses. In spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent [9,10,11]. The aim of the study was to determine the effect of curcumin on spatial working memory and oxidative stress biomarkers in alloxan-induced diabetic Swiss albino mice.

2. MATERIALS AND METHODS

2.1 Chemicals and Drugs

All chemicals and drugs were of analytical grade. Curcumin was purchased from Arkure Health Center (Haryana, India) (96% pure). Alloxan was purchased from Sigma chemical Company

St. Louis U.S.A. A digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany) was used for the determination of the blood glucose levels of the animals.

2.2 Experimental Animals

A total of twenty 20 Swiss albino mice of equal number of both sexes weighing 20 – 30 grams were used for the study. The animals were housed in plastic cages under standard laboratory conditions with free access to food and water. Animals were allowed for two weeks to acclimatization to the laboratory environment before the commencement of the study.

2.3 Induction of Experimental Diabetes Mellitus

The animals were fasted for 12-16 h with free access to water prior to the induction of diabetes. Diabetes was induced by single intraperitoneal injection of Alloxan monohydrate (Sigma St. Louis, U.S.A.) at a dose of 150 mg/kg b w dissolved in 0.9% cold normal saline [12]. The mice were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemic [13]. The blood samples were obtained from the tail. A digital glucometer was used to measure the blood glucose levels using glucose oxidase principle [14] using the digital glucometer. Hyperglycemia was defined by fasting blood glucose level > 200 mg/dl [15].

2.4 Experimental Design

The diabetic animals were randomly divided into four groups of four mice each and a normoglycemic group of four mice to serve as normal control. Curcumin was dissolved in 1 mL/kg olive oil. All substances were given by oral gavage for duration of 21 days as follows.

Group I: Normoglycemic control, received distilled water
Group II: Diabetic control, received olive oil 1 mL/kg.
Group III: Diabetic, received glibenclamide (glib) 1 mg/kg
Group IV: Diabetic, received curcumin (cur) 50 mg/kg [16]
Group V: Diabetic, received curcumin (cur) 100 mg/kg [16]

2.5 Determination of Spatial Working Memory Using Y-maze

Spatial working memory was assessed using spontaneous alternation version in Y-maze. In this version each mice was placed in the Y-maze for 5-6 min and the number of arms entered as well as the sequence of entries were recorded and a score was calculated to determine alternation rate. An alternation is defined as entry into all three arms consecutively. Y-maze function is sensitive to damage in areas concerned with learning and memory functions such as the hippocampus, and is also disrupted by drugs that cause memory loss [17].

2.6 Estimation of Oxidative Stress Biomarkers

2.6.1 Catalase assay

The serum catalase (CAT) activity was determined using mice catalase ELISA (GenAsia, GA-E3956RT) kit in accordance to the manufacturer's manual. Principle: The kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay mice CAT activity.

2.6.2 Determination of superoxide dismutase (U/mg protein)

The superoxide dismutase (SOD) activity was determined using mice superoxide dismutase ELISA (GenAsia, GA-E3956RT) kit in accordance to the manufacturer's manual. Principle: The kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay mice SOD.

2.6.3 Determination of glutathione peroxidase U/mg protein

The glutathione peroxidase (GPx) was determined using mice glutathione peroxidase (GenAsia, GA-E3957RT) ELISA kit in accordance to the manufacturer's manual. Principle: The kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay mice GPx.

2.6.4 Malondialdehyde level

The serum malondialdehyde (MDA) level was determined using mice malondialdehyde (GenAsia, GA-E0164RT) ELISA kit in accordance to the manufacturer's manual. Principle: The kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay mice MDA level.

2.7 Statistical Analysis

Data obtained were expressed as mean ± SEM. The data were statistically analyzed using ANOVA followed by Fischer's least significant difference (LSD) and Tukey's post hoc analysis to compare the level of significance using Statistical Package for Social Sciences (SPSS) version 22. The value of $p < 0.05$ was taken as significant.

3. RESULTS

3.1 Effect of Alloxan- induced Diabetes on Blood Glucose Levels

Administration of alloxan 150 mg/kg significantly increases the blood glucose levels after 72 hours of treatment as showed in Table 1.

Table 1. Showing the Effect of Alloxan on Blood glucose levels after 72 hours of Administration

Groups	Blood glucose level (mg/dl)
Normal control	96.25 ± 6.79
Diabetic control	240.75 ± 5.85
Glibenclamide 1 mg/kg	272.75 ± 14.53
Curcumin 50 mg/kg	267.50 ± 11.53
Cur cumin 100 mg/kg	294.25 ± 31.15

3.2 Percentage Alternation of Curcumin Treated Diabetic Swiss Albino Mice

The effects of curcumin (50 mg/kg and 100 mg/kg) on spatial working memory of alloxan-induced in diabetic Swiss albino mice showed in Fig. 1. Curcumin at the dose of 100 mg/kg showed significant ($p < 0.05$) increase in the percentage spontaneous alternation when compared to the diabetic control with values of 74.39 ± 8.06% compared to 47.50 ± 13.65% respectively.

3.3 Antioxidant Activity of Curcumin Treated Diabetic Swiss Albino Mice

The effects of curcumin (50 mg/kg and 100 mg/kg) on serum catalase, SOD and GPx assays of alloxan-induced diabetic Swiss albino mice was show in Fig. 2. The doses of 50 mg/kg and 100 mg/kg of curcumin showed significant ($p < 0.05$) increase in the serum catalase (78.92 ± 3.94 IU/L and 85.05 ± 3.23) and SOD (8.94 ± 1.16 U/mg protein and 12.84 ± 0.84 U/mg protein level after 21 days of administration compared to diabetic control (62.27 ± 7.07 U/mg protein and 5.75 ± 0.96 U/mg protein respectively).

3.4 Lipid Peroxidation of Curcumin Treated Diabetic Swiss Albino Mice

The results of the effects of curcumin (50 mg/kg and 100 mg/kg) on serum MDA level of alloxan-induced diabetic Swiss albino mice as shown in Fig. 3. Curcumin at dose of 100 mg/kg showed significant ($p < 0.05$) decrease in the serum MDA level after 21 days of administration, when compared to diabetic control with values of 7.44 ± 1.62 compared to 13.27 ± 1.19 respectively.
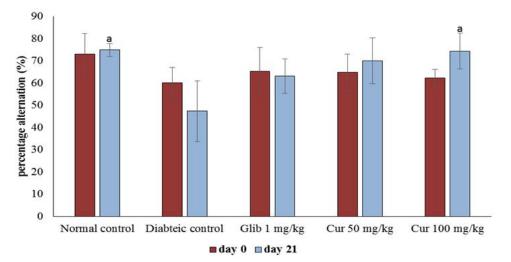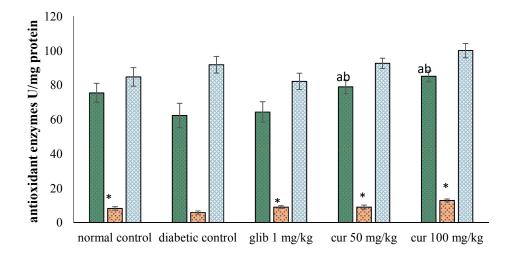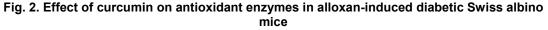

Fig. 1. Effect of curcumin on spatial working memory
Values with error bars having superscripts letter (a) are significantly ($p < 0.05$) different from diabetic control

Fig. 2. Effect of curcumin on antioxidant enzymes in alloxan-induced diabetic Swiss albino mice
Values with error bars having different superscripts letters are significantly (p < 0.05); a, = compared with diabetic control, b = compared with glib*
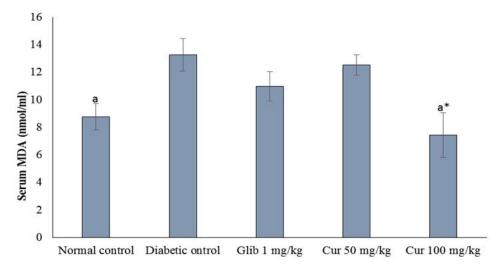

Fig. 3. Effect of curcumin on serum malondialdehyde (MDA) level in alloxan-induced diabetic Swiss albino mice
*Values with error bars having superscripts letters are significant (p < 0.05); a = Compared with diabetic control, * = Compared with glib*

4. DISCUSSION

Hyperglycemia is one of the leading cause of neurotoxicity and cognitive impairment through the increased generation of reactive oxygen species (ROS), activation of polyol pathway and advanced glycation end products and glucose shunting into hexosamine pathway which lead to end organ damage and neuronal death [18,19]. Diabetic mellitus is a metabolic disease associated with impaired glucose metabolism which alters intermediary metabolism of lipids and proteins adversely [20]. Alloxan, a beta cytotoxin, destroys pancreatic β-cells of islets of Langerhans resulting in a decrease in endogenous insulin secretion and paves ways for the decreased utilization of glucose by body tissues leading to elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides [21].

Turmeric has been used traditionally for many ailments because of its wide spectrum of pharmacological activities.

It is evident that hyperglycemia is associated with memory impairment as observed in all the groups that were diabetic (day 0) in this study. This was further confirmed by the result obtained in the diabetic control group which showed impairment in the spatial working memory after 21 days. Suggesting that the effect of the hyperglycemia, reactive oxygen species formation might be responsible for the further impairment in spatial working memory in diabetes. Hyperglycemia, ROS and inflammation have been implicated in the pathogenesis of cognitive impairment in diabetes [22]. The results obtained in the high dose (100 mg/kg) of curcumin-treated group showed a significant ($p < 0.05$) increase in percentage spontaneous alternation in Y maze test when compared to the diabetic control group. This indicated that curcumin at high dose could ameliorate the spatial working memory impairment induced by hyperglycemia. The group that received standard anti-diabetic drug did not show significant change in spatial working memory compared to the diabetic control. Also the low dose of curcumin (50 mg/kg) showed improvement which was not significant, compared to the diabetic control. Comparing between day zero (pre-treatment) and day twenty one (post-treatment), there was significant increase in the percentage spontaneous alternation at 100 mg/kg dose of curcumin. This indicated that twenty one days administration of curcumin ameliorated the spatial working memory impairment induced by diabetes. The effect might be as a result of the ability of curcumin to reverse the oxidative stress induced by alloxan. Hyperglycemia and reactive oxygen species are the leading causes of dementia and cognitive deficits [22].

The result showed a significant ($p < 0.05$) increase in antioxidant enzymes catalase and superoxide dismutase (CAT and SOD) concentration in the curcumin-treated group, when compared to diabetic control. However there was an increase in the level of GPx but it is not significant when compared to control. The increase in antioxidant enzyme activities in the curcumin-treated groups may be responsible for the decrease in ROS and subsequent MDA level.

The MDA is a reactive aldehyde the major electrophilic species known to elicit stress of toxic nature in cells and know to form covalent protein adducts. Administration of 50 mg/kg cucurmin.

Significantly decrease the level of MDA when compared to control. However, 100 mg/kg curcumin decrease the level of the MDA but it is not significant when compared to control. However when compared with glibenclamide and the two doses of curcumin, there was a significant decrease in the MDA level. The rise in serum MDA indicated in diabetic group that any oxidative stress incurred sufficiently could cause free radical mediated peroxidation of lipid component in cell membrane, thus MDA is a good indicator for evaluating oxidative stress in degenerative disease such as diabetes mellitus. SOD catalysis anions which are important reactive oxygen species in cells and involved in cell membrane damage. The elevation of GPx and SOD activities may be endogenous compensatory mechanism for prolonged over production of free radical and oxidative stress. This indicated that curcumin at the dose of 50 and 100 mg/kg possessed antioxidant effects by elevating the levels of the enzymes. The antioxidant enzymes play a crucial role in the cellular defense against ROS [23]. The SOD offers the first line of defense against ROS by scavenging and catalyzing dismutation of superoxide, produced by cellular metabolism, into hydrogen peroxide (H_2O_2) and oxygen (O_2) [24,25]. CAT and GPx are involved in the reduction of H_2O_2 into H_2O and O_2 [26]. The observed increase in SOD and CAT in curcumin-treated groups indicated that curcumin was able to scavenge free radical by sparring the endogenous antioxidant. This result disagree with the finding of Al Rubaei *at al.* (2014)[27] who reported decrease in antioxidant activity in curcumin- treated rats *in vivo* whereas the results of the present study agree with that of Tokac et al. [28] who reported an increase in the antioxidant activity in curcumin-treated groups.

Lipid peroxidation result obtained in the curcumin-treated at the dose of 100 mg/kg showed a significant ($P < 0.05$) decrease when compared to the diabetic control group. The decrease in serum MDA level observed in the present study suggested that the lipid peroxidation was decreased by daily administration of curcumin. The decrease in lipid peroxidation indicated that curcumin has antioxidant activity. This result agree with the finding of Tokac et al. [28] who reported a decrease in MDA level in curcumin-treated rats at lower and that of Nayereh, [29] who reported a

decrease in MDA level in curcumin-treated patients of type II diabetic patients. The dose of 100 mg/kg of curcumin showed more activity compared to the 50 mg/kg.

5. CONCLUSION

In conclusion, oral administration of curcumin improves short term memory and has significant a protective effects against oxidative stress biomarkers in alloxan induces diabetes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standard laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20242

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