ORIGINAL ARTICLE

Evaluation of the neuroprotective potential of Trans-cinnamaldehyde in female Wistar rat model of insulin resistance

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Abstract

Background: Chronic hyperglycemia in type 2 diabetes is associated with altered cognitive function. Trans-cinnamaldehyde (TCA) is one of the active components in cinnamon. It has been reported to have many pharmacological activities such as anti-bacterial and anti-inflammatory properties. *Aim and Objectives:* This present work evaluates the neuroprotective effects of TCA in the hippocampus of insulin-resistant rats. *Material and Methods:* Twenty female adult Wistar rats were fed with high fat diet for 8 weeks and then injected with a low dose of streptozotocin (30 mg/kg body weight intraperitoneally). Sixty mg/kg of TCA was orally administered for 4 weeks once daily. Y-maze and Morris water maze tests were employed to test for learning and memory. Fasting blood glucose, serum insulin, hippocampal Tumor Necrosis Factor-α (TNF-α), Nuclear Factor κB (NF-κB) were assayed. *Results:* The high fat diet/streptozotocin-induced insulin resistant-rats, characterized by hyperglycemia and hyperinsulinemia performed poorly in the Y-maze and Morris water maze (38.17 ± 1.3 s) when compared with the controls (26.67 ± 1.4 s), suggesting the impairment of learning and memory with corresponding increase in NF-κB and TNF-α in the hippocampus. *Conclusion:* Treatment with TCA significantly reversed diabetes-induced impairment of learning and memory. TCA as a prospective novel therapy in insulin-resistant subjects with dementia could be further explored.

Keywords: Hippocampus, Trans-cinnamaldehyde, Insulin Resistance, Neuro-inflammation

Introduction

Metabolic Syndrome (MeS) is the clustering of glucose intolerance, hypertension, dyslipidemia and central obesity with insulin resistance [1]. Insulin Resistance (IR) is a state of reduced responsiveness of target tissue(s) to normal circulating levels of insulin and it is the central feature of Type 2 Diabetes Mellitus (T2DM) [2]. It leads to impairment in insulin signaling which results in imbalance between glucose uptake and production [2]. Evidence suggests that insulin play important roles in learning and memory [3], thus rendering defect in insulin signaling as a risk factor for the development of cognitive dysfunction and dementia. Studies have demonstrated a strong connection between IR and increased risk in the development of Alzheimer's Disease (AD) [4].

Previous studies in human and animal models have shown that consuming High Fat Diet (HFD) is not only associated with weight gain and MeS but also with impaired hippocampal-dependent memory including reversal learning and memory processes [5]. It has been recently demonstrated that cognitive decline is more pronounced in AD patients with MeS compared with those without MeS.

This suggests that IR is strongly correlated with AD before brain pathological changes [6]. Diabetes has been implicated as a risk factor for AD [7].

Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products. Studies report that cinnamon extract has antioxidant [8], antidiabetic, and anti-inflammatory [9] properties. The most important constituents of cinnamon are cinnamaldehyde and Trans-cinnamaldehyde (TCA), which are present in the essential oil, thus contributing to the fragrance and to the various biological activities observed with cinnamon [10]. TCA possesses antimicrobial activity, antioxidant, cholesterol-lowering, antineoplastic, antibacterial and antifungal properties [11]. Experimental evidences indicate that TCA provides neuro-protective effect for 6-hydroxydopamine-induced dopaminergic injury, inhibits microglial activation and improves neuronal survival against neuro-inflammation in BV2 microglial cells with lipo-polysaccharide stimulation. It has also been found to improve pathological changes in AD and Parkinson disease [12]. However, there are no studies that examine the protective effect of TCA on cognitive impairment caused by IR. Thus, we investigated the neuroprotective effect of TCA in the hippocampus of insulin-resistant rats.

Material and Methods

Animal acquisition, care and grouping

All protocols and treatment procedures were done according to the Institutional Animal Care and Use Committee (IACUC) guidelines and as approved by the Faculty of Basic Medical Sciences Ethics Review Committee, University of Ilorin, Nigeria with approval number UERC/ASN/2018/1157. Twenty (20) adult female Wistar rats were purchased from Ladoke Akintola University, Ogbomosho, and were kept in the animal house of the faculty of Basic Medical Sciences, University of Ilorin. The rats were fed daily with rat pellets from Ogo-Oluwa feed and flour mill limited, Sango, Ilorin. The rats had access to water *ad libitum*. Proper ventilation was maintained by use of well-spaced and gauzed cages and a hygienic environment was ensured. Animals were allowed to acclimatize for fourteen days. At the end of 14 days acclimatization, the rats were randomly assigned into 4 groups; control, IR control, TCA, IR + TCA (n=5 in all groups).

Induction of IR

To induce IR, animals were fed with HFD for eight weeks and 30 mg/kg of Streptozotocin (STZ) (Sigma-Aldrich Inc., St. Louis, MO, USA Lot #MKCD4749) intraperitoneally as previously described [13-14].

TCA treatment

60 mg/kg of TCA (Sigma-Aldrich Inc., St. Louis, MO, USA Lot #MKCD4749) was administered for four weeks orally [15].

Fasting blood glucose

Blood was withdrawn from the tail vein of the animals, and the blood glucose level was checked using a digital glucometer (Accu-Check, Roche, Belgium). Animals with fasting blood glucose concentrations not less than 200 mg/mol were included in the study.

Y-maze test

Short-term spatial memory was assessed in rats using Y- maze apparatus. The Y-maze apparatus, made of wood, is shaped like a Y, with three identical arms with an angle of 120° between each pair of arms. Each of the arm is 40 cm long, 30 cm high, and 15 cm wide, the arms come together in a central area to form an equilateral triangle that is 15 cm at its longest axis. Each animal was set out at the end of one arm and was then allowed to move freely inside the maze. When the base of the animal's tail was completely placed in the arm, each arm entrance was recorded visually [16].

Morris water maze

Thirty (30) days after TCA treatment, the spatial learning and memory of rats were evaluated using Morris water maze. A circular pool of 150 cm in diameter and 60 cm high was filled with water (25 ± 2° C) to a height of 40 cm. The pool was divided into four equal quadrants; North, South, East, and West. A transparent escape platform (10 cm in diameter) was hidden 2 cm below the surface of the water at a fixed location in one of the quadrants to ensure being invisible to the rats but high enough for the rats to stand on it. Rats were trained once in a day for three days. Following the training, milk was added to the water to render it opaque, rats were gently put into the water and were given 60 s to freely search for the platform. Rats that found the platform in allotted time were allowed to stay on the platform for another 15s, while those who failed to detect the platform in 60 s were guided to the destination and also allowed to stay for 15 s. The time required for reaching the platform (escape latency), was recorded and measured by a video tracking system

Biochemical assays

Concentrations of Tumor Necrotic Factor-alpha (TNF-α), Nuclear Factor kappa B (NFk-B) and insulin in the hippocampus were determined using rat ELISA kits (Diaclone, London, UK or eBioscience, USA), according to the manufacturer's protocol.

Collection and preservation of brain samples

The following day after the behavioural studies, the wistar rats were sacrificed. Hippocampus were excised and weighted. The hippocampus was then kept in ice before being transferred into the freezer at -20°C in a Phosphate Buffer Saline (PBS) that was 4 times the volume of the brain before homogenization.

Statistical analysis

All quantitative data were analyzed using GraphPad® (version 6) and SPSS (version 20) software. Blood glucose, insulin, TNF-α and NFk-B outcomes were plotted in ANOVA followed with Tukey's multiple comparisons test. Significance was set at *$p<0.05$. The results were represented in bar charts with error bars to show the mean and standard error of mean respectively.

Results

HFD/STZ increased fasting blood glucose level while TCA treatment reversed the hyperglycemic condition

Administration of HFD/STZ significantly increased the blood glucose level of the treated groups when compared to the control (Figure 1). However, four weeks treatment of insulin resistant rats with TCA significantly reduced their blood glucose level when compared with the untreated group (Figure 2).

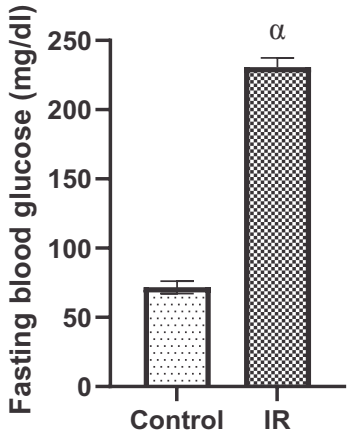

Figure 1: Fasting blood glucose after HFD and STZ administration: (Control n = 10, IR n=10).
α= significantly different from normal control. Values are expressed as mean ± SEM

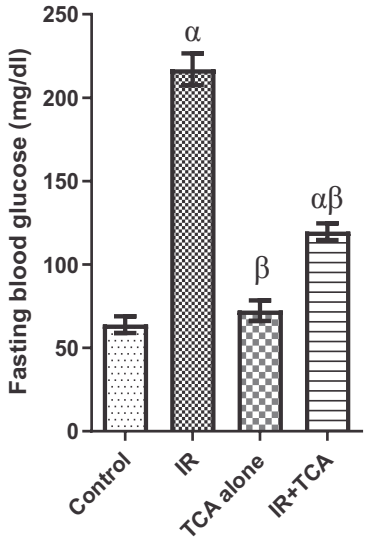

Figure 2: Blood glucose after TCA intervention: (n=5 per group).
α= significantly different from control; β= significantly different from IR. Values are expressed as mean ± SEM

TCA treatment improved memory

The effect of IR and TCA on spatial learning and memory of animals was assessed using Morris water maze. Three trials were performed for learning for three days. At the end of the learning test, the memory test was performed. The insulin resistant rats found it difficult to locate the escape platform. Treatment of insulin resistant rats with 60 mg/kg TCA for 4 weeks improved the cognitive deficit. Animals that were treated with TCA without IR located the escape platform faster than the control (Figure 3). In addition, the effect of TCA was assessed on short term memory of insulin resistant rats using Y-maze test in which insulin resistant rats had reduced short term memory while TCA enhanced short term memory in insulin resistant rats. Significant differences were not found between the control and the TCA group (Figure 4).

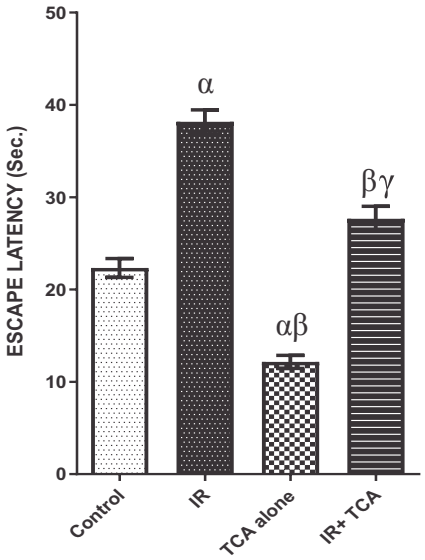

Figure 3: Bar chart showing the spatial working memory
α= significantly different from control; β= significantly different from insulin resistant; γ = significant different from TCA. Values are expressed as mean ± SEM

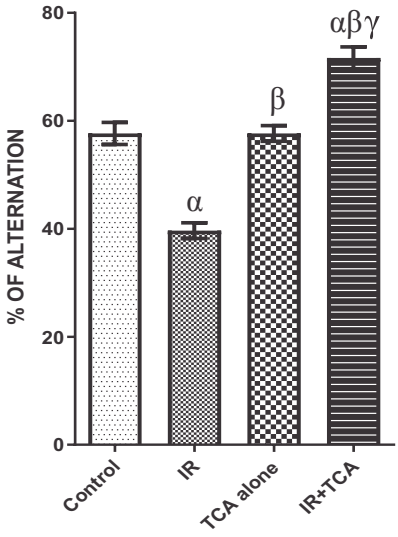

Figure 4: Bar chart showing the percentage of alternation using Y-maze test performance (n=5 per group).
α= significantly different from control; β= significantly different from insulin resistant; γ = significant different from TCA. Values are expressed as mean ± SEM

TCA suppressed neuroinflammation

The effect of TCA was assessed on inflammatory markers in the hippocampal tissues. The activity of TNF-α and NFk-B were high in insulin resistant rats. Treatment with TCA reduced the activity of TNF-α to the control level while that of NFk-B was suppressed (Figures 5 and 6).

TCA ameliorated hyper insulinemic condition

The treatment of insulin resistant rats with TCA served as therapeutic intervention as it significantly ameliorated hyper insulinemic condition in diabetic animals (Figure 7).

Treatment with TCA significantly reduced HOMA-IR

Induction of IR significantly increased blood glucose and insulin levels as well as HOMA-IR in insulin resistant group compared to the control and TCA treated groups; groups treated with TCA showed a significant decrease in blood glucose (Figure 8), insulin and HOMA-IR when compared to insulin resistant control group.

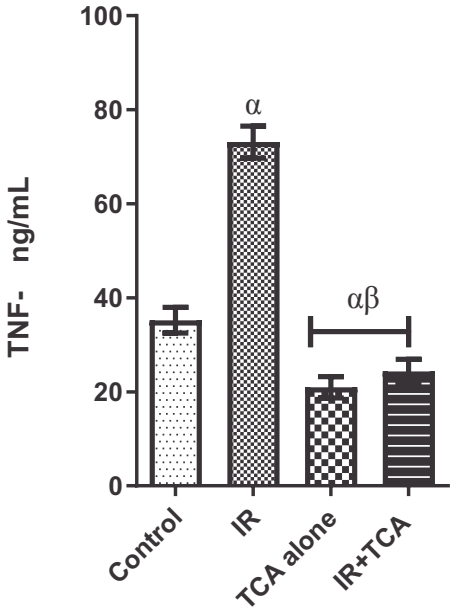

Figure 5: Hippocampal level of TNF-α (n=5 per group).
α= significantly different from control

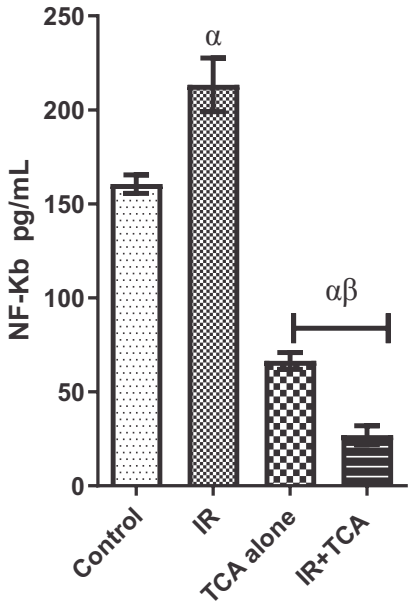

Figure 6: Hippocampal level of NF-Kb.
β= significantly different from diabetic. Values are expressed as mean ± SEM

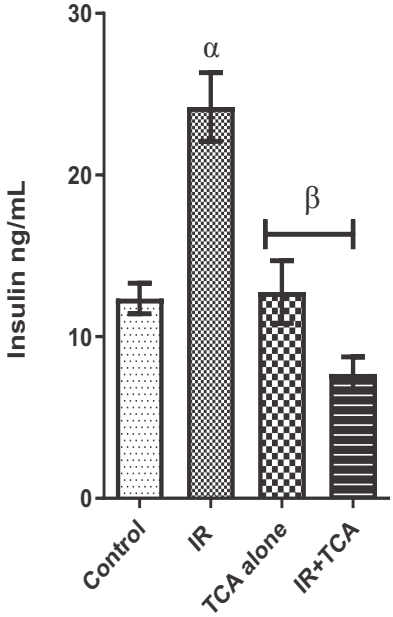

Figure 7: Serum insulin level (n=5 per group).
α= significantly different from control; β= significantly different from insulin resistant group. Values are expressed as mean ± SEM

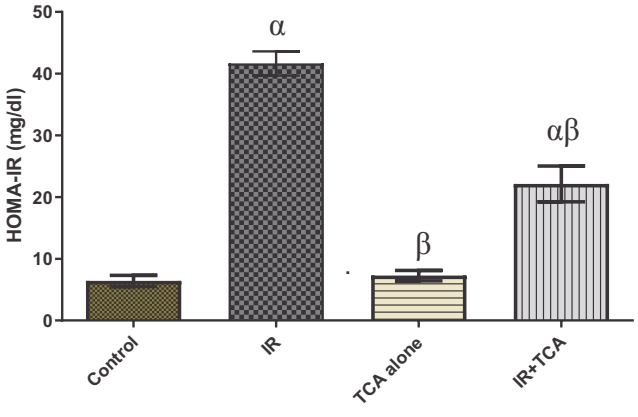

Figure 8: HOMA-IR (n=5 per group).
α= significantly different from control; β= significantly different from insulin resistant group. Values are expressed as mean ± SEM

Discussion

In this study, an insulin resistant model of cognitive decline in animals was used to assess neuroprotective function of TCA. We found that TCA reduced blood glucose level, improved memory, suppressed neuroinflammation and ameliorated hyperinsulinemia.

To determine the neuroprotective effect of TCA, we first induced an insulin resistant state in the rat by feeding the animals with HFD and inducing STZ. HFD feeding combined with low dose STZ was prescribed for the creation of animal models of type II diabetes with IR [17]. Moreover, in this present work, adult female Wistar rats were used because T2DM tend to occur later in life and more among women, contrary to vast majority of HFD/STZ Wistar rats findings that used young and male rats [18].

In this research, administration of HFD/STZ significantly increased the fasting blood glucose among the HFD/STZ treated groups (Figure 1). Previous studies had reported increased fasting blood glucose in type II diabetes models [19]. Hyperglycemia has been implicated in the etiology of mild to moderate cognitive dysfunction, decline in learning and memory including AD [19]. However, treatment with 60 mg/kg TCA significantly reduced the blood glucose level (Figure 2). Researchers have previously reported that dietary intake of cinnamon rich food regulates weight changes, lipid abnormalities, adipose tissue hormones and blood glucose in type II diabetic rats [20]. The active compound in cinnamon that elicits hypoglycemic activity is a contentious issue, however, this study has clearly shown that the active ingredient in cinnamon with hypoglycemic activity is TCA and the hypoglycemic activities of TCA was not apparent in normal rats that received TCA which corroborates previous findings [21].

Evaluating behavioural parameters in neurodegeneration in therapeutic targets represents an important means of measuring the effectiveness of treatment. Therefore, in this study we used Morris water maze and Y-maze to study the effectiveness of TCA treatment on cognitive function. In Morris water maze, we observed that there was a significant increase in escape latency among insulin resistant rats when compared to the control and TCA group. Increase in escape latency in insulin resistant group indicated a poorer learning performance due to HFD/STZ administration. Decline in hippocampal based memory has been reported in patients with T2DM and cognitive impairment in STZ-induced DM had been reported [22]. Performance in the Morris water maze correlates with the function of the hippocampus. In addition, our findings indicate that treatment with TCA was able to reverse learning and memory impairment induced by IR by reducing the escape latency in TCA treated group (Figure 3).

A significant decrease in the percentage alternation in rats treated with HFD/STZ in Y-maze test (Figure 4) was also noted, however treatment with TCA significantly increased the percentage alternation when compared to the control and TCA treated groups. These findings agree with previous reports that deficit in the hippocampal based memory performance occurs in diabetic individuals and those with predominant western diet rich in saturated fat and refined sugar have accelerated cognitive decline with aging and AD, thereby affecting cognitive functions that are

hippocampal dependent including reversal learning and memory processes [5].

Cytokines play an essential role in the coordination of immune responses in the body. Cytokine dysregulation is a key event in neuroinflammation, demyelination and neurodegeneration in the central nervous system. Activation of microglia can occur as a result of pathological states within the nervous system which may mediate glia cell injury through the production of proinflammatory cytokines. Inflammation is a common characteristic feature of many chronic diseases such as AD and DM [23].

This study shows that administration of HFD/STZ induces neuroinflammation in the hippocampus of Wistar rats evidenced by significant increase in the expression of NF-kB and TNF-α (figures 5 and 6) which agrees with some studies that reported inflammation as a key pathogenic factor in the etiology of T2DM, IR and AD [24]. NF-kB signaling pathway plays a critical role in maintaining synaptic plasticity and balance between learning and memory. Impairment in the pathways associated with NF-kB signaling causes alteration in neuronal function [25]. Activation of NF-kB has the ability to sustain microglia activation and subsequently cell death leading to neurodegenerative changes of AD-type [25].

However, TCA treatment reduced the expression of TNF-α and NF-kB showing that TCA has the ability to suppress neuroinflammation. This confirms the anti-inflammatory activity of TCA and correlates with previous findings [26]. Previous study has shown that TCA can inhibit the activity of NF-kB hence, it was not surprising to see that TCA reduced the expression of NF-kB in this present work. The anti-inflammatory activities of TCA observed in this study may be a result of its ability to inhibit inflammatory mediators and NF-kB [26].

Hyperinsulinemia, hyperglycemia and IR are all hallmark features in T2DM. The potential mechanisms of altered glucose-mediated impairment of learning and memory may be directly related to hyperinsulinemia [27]. Hyperinsulinemia and IR result in accumulation of advanced glycosylated end products which can aggravate oxidative stress of the central nervous system thereby contributing to cognitive disorders [28].

Moreover, hyperinsulinemia promotes tau phosphorylation, the main component of neurofibrillary tangles [28]. Previous studies [29] revealed that HFD was associated with IR, however TCA treatment resulted in improved hippocampal insulin (Figure 7), which supports the antihyperinsulinemic properties of cinnamon (of which TCA is an active component) earlier reported [30].

Conclusion

This study demonstrates that treatment with TCA reverses diabetes-induced cognitive deficits, normalizes blood glucose and suppresses neuroinflammation in rat models of insulin-resistance. The prospect of TCA as a novel therapy in insulin-resistant subjects with dementia could be further explored.

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