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Neuro-protective effect of Carvedilol, an adrenergic antagonist against scopolamine-induced cognitive impairment in mice

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ABSTRACT

The use of β- adrenoceptor blocking agents (β -blockers) in the clinical treatment of cardiovascular disorders and glaucoma are associated with enhancedvigilance, attention, reward, learning and memory. The present study was designed to explore the possible role of Carvedilol, an adrenergic antagonist in ameliorating scopolamine-induced neurotoxicity in rats. Mice were divided into control and treatment groups. Control mice for each test received 10 ml normal saline/kg while the treatment groups (n = 6) received Carvedilol (2.5, 5 and 10 mg/kg orally) and1 mg scopolamine/kg intraperitoneally). One hour after sildenafil and thirty minutes after scopolamine administration orally and intraperitoneally respectively, the animals were assessed for 5 minutes on elevated plus maze, Y-maze and open-field. The parametersmeasured on the EPM were memory acquisition and memory retention latencies with and without scopolamine while spontaneous alternation behaviour was measured in Y-maze. The effect of Carvedilol on locomotion was assessed in mice using open field.Carvedilol significantly ($p<0.001$) shortened memory acquisition and retrieval latencies in mice with scopolamine–induced cognitive deficit. Carvedilol produced significant ($p<0.0001$) increase in spontaneous alternation behaviour in both memory intact and memory deficit models. Carvedilol however, had no effect on locomotor activity of mice. The results suggest that Carvedilol enhanced memory acquisition and retrieval in cognitive deficit and cognitive intactmice.It also improved short term memory as indicated by increase in spontaneous alternation behaviour in mice.Carvedilol may therefore be useful in management of dementing disorders such as Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is a degenerative disease of the braincharacterized by the impairment of memory, disturbances in reasoning, planning, languageand perception (Parle *et al.,* 2004; Rahamat *et al.,* 2012). Amyloid β-peptide (Aβ) has been identified as a possible source of oxidative stress in AD because it can acquire a free-radical state that contributes to its toxic effects(Perry *et al.,* 2002;Parle *et al.,* 2004;Kumar *et al.,* 1994). Aβ- induced cytotoxicity is caused by intracellular accumulation of reactive oxygen species, which leads to lipid peroxidation and cell death (Kumar *et al.,* 1994). Although the precise mechanisms by which Aβ induces neurotoxicity is still unknown, modulation of Aβ insult has been speculated to be an important preventive and neuro protective approach to control the onset of AD (Kim *et al.,* 2001). Use of antioxidants has been recognized as an effective method in minimizing pathological and toxic effects associated with Aβ-induced oxidative stress (Rahamat*et al.,* 2012).

Therefore several agents with antioxidant properties are being evaluated for possible therapeutic application in management of AD. Such agents include both natural products of plants origin and synthetic compounds.*Ginkgo biloba* L.(Oken *et al.,* 1995)), *Huperziaserrata* (Thunb. Ex Murray) Trevis.(Skolnick, 1997) and salvianolic acid B (Durairajan *et al.,* 2008) are being extensively investigated as natural therapeutic agents for the treatment of AD patients. Other agents with antioxidant effects being investigated include vitamin C, vitamin E, β-carotene, propranolol and Carvedilol.

Carvedilol a non-selective β-adrenoceptor blocker acts as antioxidant as well as throughβ -adrenoceptor blocking, vasodilatation, inhibition of apoptosis (Abreu *et al.,* 2000), anti-inflammatory (Yaota *et al.,* 2002), mitochondrial protective (Abreu *et al.,* 20001), non-competitive inhibition of NMDA receptor and calcium channel blocking (Lysco*et al.,* 1992).

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Carvedilol has also been shown to exert neuroprotectiveeffects in several models of transient focal stroke, and a cardioprotective effect in several models of cardiovascular ischemia and reperfusion (Savitz et al, 2000). These effects are related to its antioxidant and free radical scavenger properties. The antioxidant activity of Carvedilolhas been attributed to its carbazole moiety and it is approximately 10-fold more potent as an antioxidant than vitamin E(Kumar and Dogras, 2009, Lysco*et al.*, 1998).Thus, the present study was designed to investigate the neuroprotective effects of Carvedilol against scopolamine-induced behavioral alterations in mice.

MATERIAL AND METHODS

Chemicals and drugs

Carvedilol(Sigma chemical Co., USA),Scopolamine hydrochloride(Sigma chemical Co., USA).

Animals

Male albino mice (18 - 20 g) obtained from the Animal Facility Centre ofNational Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria were used in the study. The mice were fed standard laboratory diet, given water ad libitum and maintained under laboratory conditions of temperature (22 ± 1°C), relative humidity(14±1%) and 12 h light and 12 h dark cycle. All experiments were performed between 0700hr and 1100hr daily in accordance to the "Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

Ethical Approval

Approval was given by the Department of Pharmacology and Toxicology (NIPRD) Ethical Committee on Animal Experimentation for the care and use of laboratory animals.

Assessment of effect of carvedilol on normal mice using Elevated Plus Maze

This experiment was carried out to assess cognitive performance of mice after oral treatment with Carvedilol using elevated plus maze paradigm. The elevated plus maze consists of two opposite open arms (50 x 10 cm), crossed with two closed arms of same dimensions with 10 cm high walls. The arms are connected with Central Square (10 x10 cm). Acquisition of memory was assessed on day 1 before pre-treatment with Carvedilol. Mice were placed individually at one end of an open arm facing away from the central square. The time taken to move from open arm and enter into the closed arms was recorded as initial transfer latency (ITL). If a mouse did not enter an enclosed arm within 90 s, it was gently pushed into the enclosed arms and the transfer latency (TL) was assigned 90 s. Retention of memory was assessed by placing a mouse at one end of the open arm facing away from the central square. The time taken by the mouse to enter into the enclosed arm was noted as the retention latency after 24 h of ITL (Verna et al, 1992). Mice were grouped into 4 groups of 6 mice each and treated orally as follow: Group 1: 5 ml normal saline/kg body weight; Group II: 10 mg Carvedilol/kg body weight; Group III: 20.0 mg Carvedilol/kg body weight; Group IV: 40 mg Carvedilol/kg body weight.

Assessment of effect of Carvedilol on Scopolamine-induced amnesia in mice on EPM

This experiment was carried out to assess the ability of Carvedilol to reverse scopolamine-induced cognitive deficit in mice on elevated plus maze (EPM). This consists of two opposite open arms (50 x 10 cm), crossed with two closed arms of same dimensions with 10 cm high walls. The arms are connected with Central Square (10 x10 cm). Acquisition of memory was assessed on day 1 before treatment with Carvedilol. Mice were placed individually at one end of an open arm facing away from the central square.

The time taken to move from open arm and enter into the closed arms was recorded as initial transfer latency (ITL). If a mouse did not enter an enclosed arm within 90 s, it was gently pushed into the enclosed arms and the transfer latency (TL) was assigned 90 s. Retention of memory was assessed by placing a mouse at one end of the open arm facing away from the central square. The time taken by the mouse to enter into the enclosed arm was noted as the retention latency after 24 h of ITL (Verna et al, 1992). Mice were grouped into 4 groups of 6 mice each and treated orally as follow: Group 1: 5 ml normal saline/kg body weight; Group II: 10 mg Carvedilol/kg body weight; Group III: 20.0 mg Carvedilol/kg body weight; Group IV: 40 mg Carvedilol/kg body weight. Thirty minutes before retention latency assessment all the experimental groups were given 1 mg scopolamine/kg body weight intraperitoneally.

Effect of carvedilol in mice on Y-maze

This study was carried out to evaluate effect of Carvedilol on spatial working memory in mice. Spontaneous alternation behaviour in mice is a valid measure of spatial working memory. The Y-maze apparatus is suitable for assessment of short term memory, general locomotor activity and stereotypic behaviour (Tijani *et al.*, 2012, Akanmu *et al.*, 2011, sarter *et al.*, 1988). The Y-maze is a three –arm horizontal maze (40 cm long and 5 cm wide with walls 10 cm high) in which the three arms are symmetrically separated at 120^0. Mice were initially placed within one arm (A), and the arm entry sequence (e.g ABCCAB, where letters indicate arm codes) and the number of arm entries were recorded manually for each mouse over a 6 min period. The maze arms were cleaned with 70% ethanol between tasks to remove residual odours.

Alternation was determined from successive entries into the three arms on overlapping triplet sets in which three different arms are entered. An actual alternation was defined as entries into all three arms consecutively (i.e. ABC, CAB or BCA but not BAB). An entry was defined as placing all four paws within the boundaries of the arm. One hour before this test, mice were allotted to groups of five mice each and treated with graded doses

of Carvedilol (10, 20.0 and 40 mg/kg, oral) and 30 min later memory impairment was induced by administration of scopolamine (1 mg/kg, intraperitoneally) while the control group received 5 ml normal saline/kg. The percentage alternation for each mouse was determined as the ratio of actual to possible alternations (defined as the total number of arm entries minus 2), multiplied by 100 as shown by the following equation: % Alternation =

[(Number of alternations) / (Total arm entries-2)] x 100 (Kim *et al.*, 2007; Heo et al, 2009)

Effect of carvedilol in mice on open field apparatus

This experiment was designed to evaluate the effect of Carvedilol on locomotor activity. Twenty mice were grouped into 4 groups of 5 mice each and treated orally as follow: Group 1:5 ml normal saline/kg, Group II: 10mg Carvedilol/kg body weight; Group III: 20.0 mg Carvedilol/kg body weight ; Group IV: 40 mg Carvedilol/kg body weight. This study was carried out on day 1 (before) and 24 h after administrationofCarvedilol. Each mouse was observed for a period of 5 min in an open field apparatus. The open field apparatus is made of square (45 cm) transparent glass box with a wooden floor.

The wooden floor was divided into 9 equally sized squares of 15 cm x 15cm each. The number of square box crossed by each mouse was expressed as counts per 5 min. The frequency of square box crossings was taken as the index of locomotor activity.

Statistical Analysis

All data were expressed as the mean ±standard error of mean (SEM). Statistical analysiswas carried out using one-way analysis of variance (ANOVA). Any significant differencebetween means was assessed by student's t-test at 95% level of significance.

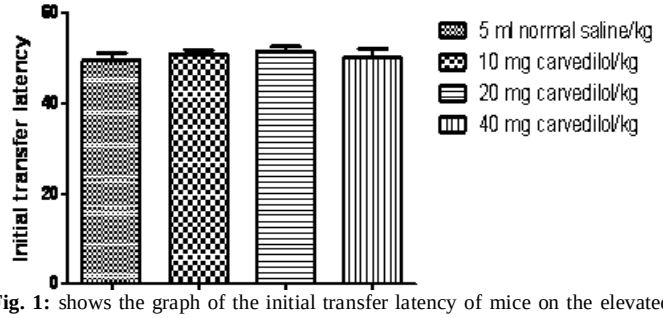

Fig. 1: shows the graph of the initial transfer latency of mice on the elevated plus maze.

RESULTS

Effect of Carvedilol on EPM

There was no significant ($F_{3,\ 16}$=0.34, p=0.4637) difference in the initial escape latency of rats in all the treatment groups (fig. 1). However, Carvedilol significantly (F 3, 16= 3.5,p<0.001,) and dose- dependently shortened the retention latency on the elevated plus maze (Fig 2).

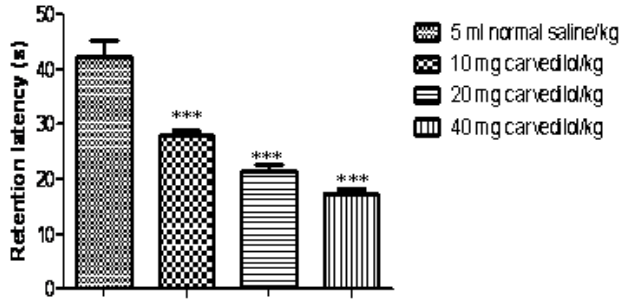

Fig. 2: shows the graph of retention latency of mice treated with graded doses of carvedilol.

Effect of Carvedilol on cognitive deficit –induced by scopolamine

There was no significant ($F_{3, 16}$=1.4, p=0.2670) difference in the initial escape latency of rats in all the treatment groups (fig. 3).Carvedilol significantly ($F_{3,\ 16}$= 151.3,p<0.0001,) and dose-dependently shortened the retention latency on the elevated plus maze (Fig 4) when compared to control.

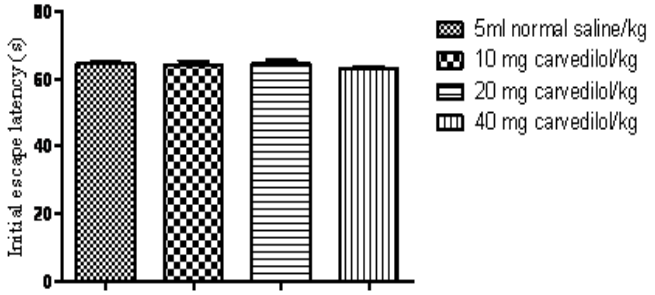

Fig. 3: shows the graph of the initial transfer latency of mice on the elevated plus maze.

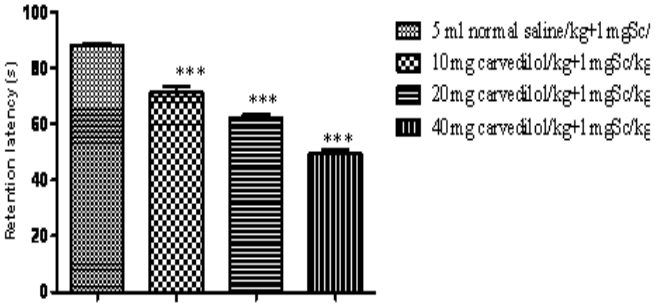

Fig. 4: shows the graph of effect of graded doses of carvedilol on retention latency of mice with cognitive deficit.

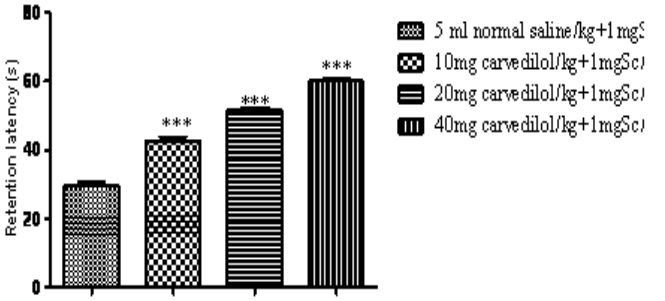

Fig. 5: shows the effect of graded doses of carvedilol on spontaneous us altemation be haviour of mice with cognitive deficit. *** significantlydiffernt from the control at p<0.0001, n=5

Effect of Carvedilol on spatial memory on Y-maze

Carvedilol significantly ($F_{3,16}$ = 180.8, p<0.0001) increased the spontaneous alternation behaviour in mice with cognitive deficit –induced by scopolamine when compared to the control (fig. 5)

Effect of Carvedilol on locomotor activity of mice on open field

Carvedilol had no effect ($F_{3, 16}$= 0.66, p= 0.5889) on total locomotor effect of mice on open field apparatus (fig. 6)

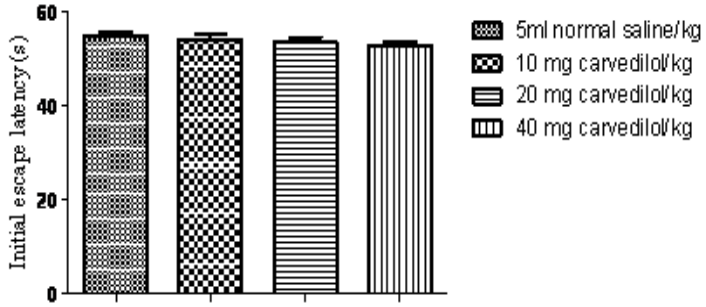

Fig. 6: shows the effect of graded doses of carvedilol on locomotor activity of mice of mice on the elevated plus maze.

DISCUSSION

The results of this study showed that Carvedilolwas effective in ameliorating scopolamine-induced memory deficit in mice on Elevated PlusMaze (EPM) and Y-maze. Scopolamine is a muscarinic cholinergic receptor antagonist and, when used at a low dose as in the present study, is well known to cause memory impairments in rodents similar to those found in age-related senile central nervous system dysfunction (Ebert and Kirch, 1998, Tijani et al, 2012). In the present study, Carvedilolimproved the memory retention of mice as reflected by diminished transfer latency values on elevated plus maze. The transfer latency is an index used for assessment of memory retention potential. The first trial was to expose the animals to a novel arena while the second assessment in the open arm of the Elevated Plus Maze queries ease of recognition and retrieval of acquired memory.Carvedilol was also able to reverse the effect of scopolamine on transfer latency ofcognitive deficient mice on the elevated plus maze. This reversal of scopolamine –induced cognitive deficit by Carvedilol may suggest modulatory effect of the adrenergic blocker on cholinergic system.Systemic administration Scopolamine induces central cholinergic blockade, produced a reversible and well described impairment in both (i) maintainingattention and (ii) processing of information and the acquisition of new knowledge in rodents and in humans (Souza *et al.,* 2010; Becker, 1991).

Alzheimer's disease (AD) is a brain disorder characterized by a progressive cognitive decline, leading to dementia, due to degeneration of the cholinergic nervous system (Cummingsand Kaufer, 1996). Animal and human studies suggest that disruption of the cholinergic nervous system is a major factor in the early state of Alzheimer's disease (Jann, 2000, Kang et al, 2005). The ameliorative effect of Carvedilol on learning and memory was investigated in the Y-maze test in order to confirm its effect on spatial short term memory. The choice of Y-maze for the study was based on the work of Kim *et al.,* (2006) which showed that spontaneous alternation behaviour is an indicator of spatial memory. Scopolamine produced significant reduction reduction in Spontaneous alteration behaviour (SAB), whereas Carvedilol attenuated scopolamine-induced reduction in spontaneous alternation behaviour. This observation suggests that Carvedilol is capable of reversing scopolamine-induced reduction in short term memory. In spite of evidence that scopolamine produces motor disturbances, alteration in locomotor activity was not found in Carvediloltreated groups in this study. The absence of motor disturbance in this study was revealed by the absence of significant difference the number of arm entries of the experimental groups in the Y-maze test. Sarter *et al.,* (1988) has proposed that absence of difference in the total arm entries by mice in Y-maze after drug treatment indicates that treatment produced no alteration in general locomotor activity in the Y-maze test. The results of effect of Carvedilol on locomotor activity on open field further supports the assertion that Carvedilol alteration of spontaneous alternation behaviour in Y-maze is centrally mediated and not associated with adverse effect of Carvedilol on locomotor activity. Carvedilol has been reported in the literature as acts as a NO modulatory agent in vascular endothelial cells and in cell-free systems (Yoshioka et al, 2000). It has also been shown to inhibit superoxide ion release from activated neutrophils (Maèièkova et al, 2005).Carvedilol also has been shown to preserve the endogenous antioxidant system (i.e., vitamin E andreduced glutathione), which is normally consumedwhen tissues or cells are exposed to oxidative stress (Feuerstein et al, 1998). This preservation of endogenous antioxidants may explain why the Carvedilol treatment was able to reverse scopolamine induced cognitive deficit in mice. Carvedilol has also been reported to protect the expression of many inflammatory mediators and cytokine- like TNF-and IL-1, which are mainly responsible for causing oxidative damage. Cholinergic neurons are positive markers for the evolution of memory and related disorders affecting acetylcholine and resulting in decreased activity of acetylcholinesterase and choline acetyl transferase(Germano and Kinsella, 2005). The efficacy of Carvedilol might be attributable to its β-blocking effect (adrenergic blocker), but its reversal of scopolamine-induced cognitive deficit in mice suggests presence of an additional effect on cholinergic systemswhich may be an additional therapeutic benefit for AD.

CONFLICT OF INTEREST

There is no conflict of interest associated with the authors of this paper.

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