Garcinia kola prevents scopolamine-induced memory impairment in mice: Role of oxidative and nitrosative stress

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ABSTRACT

Background: The high and increasing incidence of Alzheimer Disease (AD) worldwide is a major global concern. Oxidative stress has been considered to be linked to the aetiology of many diseases, including neurodegenerative diseases (NDDs) such as AD. *Garcinia kola* Heckel (Clusiaceae) has important antioxidant functions that can help protect against AD.

Objective: This study sought to investigate the protective effect of ethanol seed extract of *Garcinia kola* (GCK) on scopolamine-induced memory impairment in rodents.

Methods: GCK (25, 50 and 100 mg/kg, p. o.) or tacrine (5 mg/kg, i. p.) was administered for 3 consecutive days. Scopolamine (3 mg/kg, i. p.) was given, 1 h after last-treatment on day 3, followed by estimation of memory functions using the Y-maze and elevated plus maze (EPM) tasks in mice as well as Morris water maze test (MWM) in rats. The biochemical markers of oxidative stress were determined in the prefrontal cortex, hippocampus and striatum after the MWM test on day 8.

Results: Scopolamine induced significant decrease in spontaneous alternation behaviour in Y-maze and increased transfer latency in EPM in mice which was prevented by pretreatment of mice with GCK. In the MWM experiment, GCK protected rats against spatial learning deficit induced by scopolamine, evidenced in session dependent decrease in escape latency. Furthermore, scopolamine induced increased oxidative and nitrosative stress status in the prefrontal cortex, striatum and hippocampus as compared with vehicle-treated control was ameliorated with GCK administration which was similar to the effect of tacrine.

Conclusion: ***G. kola*** prevented scopolamine-induced cognitive impairment through attenuation of oxidative/nitrosative stress status. Thus, could be a potential phytotherapeutic agent in the treatment of dementia.

Keywords: Antioxidant system; hippocampus; Morris water maze; scopolamine; Y-maze

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La *Garcinia kola* empêche l'affaiblissement de la mémoire induite par la scopolamine chez la souris: rôle du stress oxydatif et nitrosant

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RÉSUMÉ

Contexte: L'incidence élevée et croissante de la maladie d'Alzheimer (MA) dans le monde est une préoccupation majeure à l'échelle mondiale. Le stress oxydatif a été considéré comme étant lié à l'étiologie de plusieurs maladies, y compris les maladies neuro-dégénératives (MND) telles que la MA. *Garcinia kola* Heckel (Clusiaceae) a des fonctions anti-oxydantes importantes qui peuvent aider à protéger contre la MA.

Objectif: Cette étude a cherché à étudier l'effet protecteur de l'extrait de graine d'éthanol de *Garcinia kola* (GCK) sur l'altération de la mémoire induite par la scopolamine chez les rongeurs.

Méthodes: On a administré du GCK (25, 50 et 100 mg/kg, p. o.) ou de la tacrine (5 mg/kg, *i. p.)* pendant 3 jours consécutifs. La scopolamine (3 mg/kg, *i. p.*) a été administrée, 1 h après le dernier traitement le jour 3, suivie de l'estimation des fonctions de mémoire à l'aide des tâches du labyrinthe Y et du labyrinthe surélevé (EPM) chez la souris ainsi que du test de labyrinthe de l'eau de Morris (MWM) chez le rat. Les jalons biochimiques du stress oxydatif ont été déterminés dans le cortex préfrontal, l'hippocampe et le striatum après le test MWM le jour 8.

Résultats: La scopolamine a induit une diminution significative du comportement d'alternance spontanée dans le labyrinthe Y et une augmentation de la latence de transfert dans EPM chez la souris, ce qui a été empêché par prétraitement de souris avec GCK. Dans l'expérience de MWM, les rats protégés par GCK contre le déficit d'apprentissage spatial induit par la scopolamine, mis en évidence dans la diminution dépendante de la session de latence d'échappement. En outre, la scopolamine a induit une élévation du niveau de stress oxydatif et nitrosant dans le cortex préfrontal, le striatum et l'hippocampe comparativement au témoin traité au véhicule a été améliorée avec l'administration de GCK qui était similaire à l'effet de la tacrine.

Conclusion: *G. kola* a permis d'éviter l'affaiblissement cognitif induit par la scopolamine par atténuation du niveau de stress oxydatif/nitrosant. Ainsi, cela pourrait être un agent phyto-thérapeutique potentiel dans le traitement de la démence.

Mots-clés: Système antioxydant; Hippocampe; labyrinthe eau de Morris; La scopolamine; labyrinthe Y-

INTRODUCTION

Alzheimer's disease (AD) is the most common ageassociated neurodegenerative disease characterized by progressive memory impairment and deterioration of other cognitive functions.[1] Neuropathological hallmarks of AD include senile plaques and

neurofibrillary tangles in neocortical and limbic brain lesions, which are coupled with neuronal loss or dysfunction.[2] Another important phenomenon in AD, is the dysfunction of the cholinergic system by amyloid plaques in several brain areas such as the basal forebrain, cortical regions, and the hippocampus. The loss of cholinergic neurons in the brain is closely associated with the reduction of acetylcholine synthesis.[3] Previous studies have shown that the disruption in cholinergic neurotransmission is one of the earliest neuropathological changes in preclinical AD and may be associated with abnormal beta-amyloid (Aβ) accumulation.[4] Therefore, disruption of cholinergic neurotransmission with scopolamine may unmask otherwise undetectable cognitive deficits in preclinical AD. Thus, the use of a cholinergic neurotransmission enhancer may be an effective way of identifying novel agents for the prevention or treatment of dementia of Alzheimer's type.

In Southwest, Nigeria, Garcinia kola seed is chewed for its anti-aging and memory enhancing activities.[5,6] *Garcinia kola Heckel (Clusiaceae) also known as bitter kola (English), "orogbo'' (Yoruba-Southwestern, Nigeria), "cida goro'' (Hausa-Northern, Nigeria), "Ugugolu'' (Igbo-Eastern, Nigera),* and Garushinia Kora (Japanese) is a medium-sized tree of about 12-15 m high and a girth of 1.80 m grown in the costal rainforest in West and Central Africa. The tree is highly valued for its edible nuts.[7] The seeds as a bitter astringent taste when chewed resembling that of raw coffee bean followed by a slight sweetness, thus consumed as a stimulant.[8] The seeds are used in traditional African Medicine for the treatment of several ailments including; liver disorders, gonorrhoea, bronchitis, laryngitis, diarrhoea, diabetes, aphrodisiac and neurological disorders.[9,10] Moreover, hepatoprotective and aphrodisiac effects of *G. kola* has been reported.[11,12] The present study was carried out to investigate effect of *G. kola* seed extract on memory impairment induced by scopolamine in mice and rats. In addition, examine the effect of the extract on scopolamine-induced oxidative and nitrosative stress biomarkers in discrete brain regions of rats.

MATERIALS AND METHODS Laboratory animals

Male Sprague-Dawley rats (150 - 170 g) and Swiss albino mice (18-22 g) used in this study were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos, Lagos, Nigeria. The animals were kept in well ventilated environment, housed in standard cages and were fed on standard pellets (Livestock Feeds, Lagos, Nigeria) and tap water *ad libitum*. The animals were acclimatized for 7 days before the commencement of the experiment. The experimental procedures adopted in this study were in compliance with the ethical standards of the Research Grant and Animal Experimentation Committee of the College of Medicine, University of Lagos, Nigeria and in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (2011).

Plant materials

Garcinia kola seeds were purchased from Mushin herbal market, Lagos state, Nigeria. Botanical identification and authentication was done by Mr. T.K. Odewo (a forestry expert) in the Department of Botany, University of Lagos, Akoka, Lagos State, Nigeria. A voucher specimen with herbarium number LUH 6138 was deposited in the herbarium of same Department for reference purpose.

Preparation of extract

One hundred and twenty grams of peeled seeds were sliced and air dried at room temperature. The air dried seeds were pulverized with a Warring commercial blender. The powdered seed was soaked in 1.4 L of ethanol for 72 h at room temperature with

intermittent agitation (12 hourly; manually). After extraction, the sample was filtered and the filterate evaporated to dryness under reduced pressure by Heidolph rotary evaporator to yield 22 g (18.33% w/w; brownish extract). The extract was suspended in 2% carboxymethylcellulose in corn oil as vehicle.

Drugs and chemicals

Ethanol, tacrine, glacial acetic acid, Folin-Ciocalteu reagent, scopolamine hydrobromide, thiobarbituric acid, sodium chloride, sodium hydroxide, potassium ferricyanide, trichloroacetic acid, napthylethylenediamine dihydrochloride, ferric chloride, DPPH solution, dithio-bis-nitrobenzoic acid (DTNB), and bovine serum albumin (Sigma Aldrich, St. Louis MO, USA), normal saline (Unique Pharmaceutical Ltd, Lagos, Nigeria).

Qualitative and quantitative phytochemical analysis

Preliminary qualitative phytochemical analysis

The Preliminary qualitative phytochemical screening of GCK was carried out using the method of Edeoga *et al.*[13]

Determination of total phenolic content

Total phenolic content of GCK was determined using Folin-Ciocalteu reagent as described by Turkmen *et al*[14]. with slight modifications. Briefly, 0.1 ml of GCK (4 mg/ml) or standard solution at different concentrations, was mixed with 0.75 ml of FolinCiocalteu's phenol reagent (10-fold diluted with water). The mixture was kept at room temperature for 5 min and 0.75 ml of 6% sodium carbonate was added. After 90 min of reaction, its absorbance was read at 725 nm. The standard calibration (0-25 µg/ml) curve was plotted using gallic acid. The total phenolics were expressed as mg gallic acid equivalent/gram dry weight. Negative control was prepared by adding 0.1 ml of ethanol instead of GCK.

***In vitro* evaluation of antioxidant activities**

Nitric oxide radical (NO) scavenging assay

Nitrite generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci *et al.*[15] Briefly, the reaction mixture (5 ml) containing SNP (5 mM) in phosphate buffered saline (*p*H 7.3), with or without the various concentrations GCK (10- 100 µg/ml), was incubated at 25°C for 180 min in front of a visible polychromatic light source (25W tungsten lamp). The $NO^{\cdot}$ radical thus generated interacted with oxygen to produce the nitrite ion which was assayed at 30 min intervals by mixing 1 ml of incubation mixture with an equal amount of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediaminedihydrochloride (NAD). The absorbance of the chromophore (purple azo dye) formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with NAD was measured at 546 nm. The nitrite generated in the presence or absence of the GCK was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out at least three times and the data presented as an average of three independent determinations. Gallic acid was used as the positive control. The percentage scavenging of $NO^{\cdot}$ by GCK and gallic acid (standard antioxidant) was calculated according to the equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

The free radical scavenging potential of GCK were determined according to the procedure of Awah et al.[16] with some minor modifications. Briefly, an aliquot of 50 µL of GCK of various concentrations (10- 100 µg/ml) were mixed with 950 µL of ethanolic solution of DPPH (3.4 mg/ 100 ml). The reaction mixture was incubated at 37°C for 1 h in the dark. The free radical scavenging potential of GCK was expressed as the disappearance of the initial purple colour. The absorbance of the reaction mixture was recorded at 517 nm using UV-Visible spectrophotometer (Agilent 8453, Germany). Gallic acid was used as the positive control. The percentage inhibition of DPPH free radical was calculated according to this formula:

The effective concentration needed to scavenge DPPH free radical by 50% (IC_{50}) was calculated by regression analysis of the dose response curve plotted between percentage inhibition versus concentration of the test samples and the standard.

Ferric ion reducing power assay

The reducing capacity of GCK may serve as a good indicator of its potential antioxidant property. The reducing power capacity of GCK was investigated using the method of Oyaizu.[17] Various concentration of GCK or standard solution (1.0 ml) were mixed 2.5 ml of potassium buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [K3Fe(CN)6] (1% w/v). After 30 min of incubation at 50°C, 2.5 ml of 10% trichloroacetic acid solution was added to each test tube and the mixture was centrifuged at 3,000 rpm for 10 min. Then, 2.5 ml of the supernatant solution was withdrawn from the tube and mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride solution (0.1%, w/v) and the absorbance was measured at 700 nm and gallic acid was used as standard.

Acute toxicity test

The acute toxicity effect of GCK was determined using the fixed dose protocol of the Organization of Economic Co-operation and Development (OECD) guidelines for testing of chemicals, TG_{420}[18] (2001) for oral administration. 11 female mice were given GCK (250 mg/kg, *p*.o., n =1; 2000 mg/kg, *p*.o., n =5; and 4000 mg/kg, *p*.o., n = 5). Behavioural signs of toxicity and mortality were observed following extract administration; during the first 30 minutes, then the second, fourth, sixth hour and once daily for 14 days for delayed toxicity or mortality.

Behavioural test Y-maze test

The test relies on the innate tendency of mice to explore a novel environment to assess spatial recognition. Male Swiss albino mice (18-22 g) were randomly divided into 6 groups (n = 6) and treated as follows for 3 days: Group1-vehicle (10 ml/kg; *p.o*.; normal control), Group 2- vehicle (10 ml/kg, *p.o.;* amnesic model), Group 3- tacrine (5 mg/kg; *i.p*.), Group 4-6: GCK (25, 50 and 100 mg/kg; *p.o.,* respectively). Fifteen minutes after the last treatment on day 3, scopolamine (3 mg/kg, i.p.)[19] was administered to mice in Group 2-6. Five minutes postscopolamine injection, each mouse was placed at the centre of the Y-maze facing the south arm 'B' and allowed to explore the maze freely for a period of 5 min. The number and the sequence of arm entries were observed. An arm entry was scored when all four paws were in the arm. Alternation behaviour was defined as consecutive entries into all three arms (i.e., ABC, CAB, or BCA but not BAB).[20] The percentage of spontaneous alternation was measured as an index of working memory by calculating the ratio of the actual number of alternations to the possible number (defined as the total number of arm entries minus two) multiplied by 100, i.e.,

% Alternation = [(number of alternations)× 100 (total number of arm entries x 2)]

The total number of arm entries was measured as an index of locomotor activity.

Elevated plus maze test (EPM)

The plus maze consists of two open and two closed arms (30 × 5 × 25 cm each) elevated to a height of 38.5 cm. Male Swiss albino mice (18-22 g) were randomly divided into 6 groups (n = 6) and treated as follows for 3 days: Group1-vehicle (10 ml/kg; *p.o*.; normal control), Group 2- vehicle (10 ml/kg, *p.o*., amnesic model), Group 3- tacrine (5 mg/kg; *i.p*.), Group 4-6: GCK (25, 50 and 100 mg/kg; *p.o.,* respectively). Fifteen minutes after the last treatment on day 3, scopolamine (3 mg/kg, *i.p*.)[19] was administered to mice in Group 2-6. Five minutes postscopolamine injection, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by the mouse to move into any one of the closed arms with all its four legs. After each test, the maze was carefully cleaned up with 10% ethanol solution. The cut off time was 90 s. TL was recorded on the first day. Memory retention was examined 24 h after the first day trial. Significant reduction in TL value of retention indicated improvement in memory.[21]

Morris water maze test

The acquisition and retention of a spatial navigation task was examined using a Morris water maze (MWM). Male Sprague Dawley rats (150-170 g) were randomly divided into 6 groups (n = 6) and treated as follows for 3 consecutive days: Group1-vehicle (10 ml/kg; *p.o*.; normal control), Group 2- vehicle (10 ml/kg, *p.o*., amnesic model), Group 3- tacrine (5 mg/kg; *i.p*.), Group 4-6: GCK (25, 50 and 100 mg/kg; *p.o.,* respectively). Fifteen minutes after the last treatment on day 3, scopolamine (3 mg/kg, i.p.)[19] was administered to rats in Group 2-6.

The MWM consists of a circular water tank (120 cm diameter and 50 cm height) filled with water (26±2 °C) to a depth of 30 cm located in a darkened test room, Four equally spaced points around the edge of the pool were designed as N (North), E (East), S (South) and W (West). A black coloured round platform height of 28 cm, and diameter of 10 cm was placed 2 cm below the surface of water in a constant position in the middle of the NE quadrant in the pool. The time taken for the rat to escape from the water onto the platform was measured. The position of platform was kept unaltered throughout the trials. Five minutes post-scopolamine injection on day 3, the animal was gently released into the pool from the SW quadrant in all the trials (day 1 of training). The animal was given 60 s (cut-off time) to find the hidden platform and allowed to stay on it for 10 s. The time taken for the rat to locate the escape platform was recorded using a stop watch. In the event that the animal was unable to locate the hidden platform within 60 s, it was gently guided to it and was allowed to stay on it for 10 s. Each animal was subjected to a daily session of 3 trials per day for 5 days consecutively (i.e. days 3 to 7). Escape latency time

(ELT) to locate the hidden platform in water maze was noted as an index of learning.[19] [The choice of rats for the Morris water maze paradigm was due to the fact that, it will easier to isolate the hippocampus and striatum for biochemical assays from rats compared to mice due to their brain size].

Brain tissue preparation

The rats were decapitated, 45 min after the MWM task on day 7 under chloral hydrate (300 mg/kg, i.p.) anaesthesia. The skull was cut open and the brain was exposed from its dorsal side. The whole brain was quickly removed and the prefrontal cortex (PFC), striatum (STR) and hippocampus (HIP) were isolated on ice bar plate. The isolated brain areas were homogenized with an Ultra-Turrax T25 (USA) homogenizer at a speed of 9500 rpm in 0.03 M sodium phosphate buffer, *p*H-7.4. The homogenate was used to measure MDA, GSH, SOD and nitrite.

Determination of lipid peroxidation

Malondialdehyde (MDA), which is an indicator of lipid peroxidation, was spectrophotometrically measured by using the thiobarbituric acid assay as previously described by Ohkawa *et al.*[22] Two hundred microlitre of the supernatant was added and briefly mixed with 1 mL of 50% trichloroacetic acid in 0.1 M HCl and 1 mL of 26 mM thiobarbituric acid. After vortex mixing, samples were maintained at 95°C for 20 min. Afterwards, samples were centrifuged at 960 × *g* for 10 min and supernatants were read at 532 nm. A calibration curve was constructed using MDA as standard and the results were expressed as U/mg protein.

Determination of reduced glutathione (GSH) level

GSH was determined by its reaction with 5, 5′-dithiobis (2-nitrobenzoic acid) (Ellman's reagent) to yield a yellow chromophore which was measured spectrophotometrically.[23] To measure the GSH level, the tissue homogenate (in 0.1 M phosphate buffer pH 7.4) was taken. The brain homogenate was mixed with an equal amount of 10% trichloroacetic acid (TCA) to precipitate the proteins. The mixture was kept for 5 min prior to centrifugation (Remi cold centrifuge) at 2000 *g* for 10 min at 4 °C. The supernatant (100 µl) of processed tissue sample mixed with 0.5 ml of Ellman's reagent (5, 5′-dithio *bis* -2-nitrobenzoic acid) (0.1 mM) (DTNB)) prepared in 2 ml of phosphate buffer (pH 8.4) and 0.4 ml of double-distilled water and the reaction mixture was shaken vigorously on vortex. The absorbance was read at 412 nm within 15 min.

Determination of catalase activity

Catalase (CAT, EC 1.11.1.6) activity was assayed following the method of Sinha[24]. The reaction mixture consisted of 150 µL phosphate buffer (0.01 M, pH 7.0), 100 µL supernatant. Reaction was started by adding 250 µL H_2O_2 0.16 M, incubated at 37°C for 1 min and reaction was stopped by addition of 1.0 mL of dichromate: acetic acid reagent. The tubes were immediately kept in a boiling water bath for 15 min and the green colour developed during the reaction was read at 570 nm on a spectrophotometer. Control tubes, devoid of enzyme, were also processed in parallel. Results were expressed as U/mg protein.

Determination of superoxide dismutase (SOD) activity The activity of superoxide dismutase (SOD, EC 1.15.1.1) was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). Each 1.5 mL reaction mixture contained 100 mM

TRIS/HCl (pH 7.8), 75 mM NBT, 2 µM riboflavin, 6 mM EDTA, and 200 µL of supernatant. Monitoring the increase in absorbance at 560 nm followed the production of blue formazan. One unit of SOD is defined as the quantity required to inhibit the rate of NBT reduction by 50% as previously described by Winterbourn et al.[25] The enzyme activity is expressed as units/mg protein.

Determination of nitrite level

Nitrite was estimated in the rats brain using the Greiss reagent and served as an indicator of nitric oxide production. One hundred microliter of Greiss reagent

acid and 0.1% napthaylamine diamine dihydrochloric

acid in water) was added to 100µl of supernatant and

[26] measured at 542nm. Nitrite

concentration was calculated using a standard curve for sodium nitrite.

Protein estimation

GCK. Protein was measured in all brain samples by the method of Lowry *et al*.[27] Bovine serum albumin (BSA) (1

mg/ml) was used as standard and measured in the

range of 0.01–0.10 mg/ml.

Statistical analysis Results obtained are expressed as mean ± SEM (n=6). Level of significance was analysed using one or two way ANOVA (whichever is applicable) followed by Tukey *post hoc* multiple comparison test using Graphpad prism version 6 (Graphpad prism Inc, CA, USA).

Results

Acute toxicity test increased progressively (Table 1). Oral administration of GCK up to 4000 mg/kg neither induced toxic behaviours nor mortality throughout the period of 14 days observation.

Effect of GCK on scopolamine-induced memory deficit in the Y-maze test

Scopolamine (3 mg/kg) markedly impaired spontaneous alternation behavior (Fig. 1). GCK

Preliminary phytochemical analysis and total phenolic contents

absorbance was

The preliminary phytochemical analysis revealed the presence of saponins, alkaloid, flavonoid, tannin,

terpenoids, and steroid. Quantitative estimation of GCK, showed that the total phenolic content is

36.58±0.06 mg GAE/g of dry

Effect of GCK on free radical generation *in vitro*

The results of the *in vitro* antioxidant assay showed the

ability of GCK to scavenge free radicals induced by DPPH, nitric oxide and ferric ion as shown in Table 1. The

results show that the hydrogen donating ability of GCK was lower than that of gallic acid. Similarly, the NO scavenging ability of GCK was similar to that of gallic acid. Moreover, the ferric ion reducing power assay measures the electron donating capacity of an antioxidant. An increased absorbance is indicative of higher reducing power. The results showed that the reducing power of GCK and the standard, gallic acid increased progressively (Table 1). Oral administration of GCK up to 4000 mg/kg neither induced toxic behaviours nor mortality throughout the period of 14 days observation.

Table 1: The *in vitro* inhibitory concentration of *G. kola* and gallic acid against free radical generation

Parameters	GCK IC50 µg/ml	Gallic acid IC50 µg/ml
DPPH	40.27	18.96
Nitrite	18.76	34.57
FRAP	114.68	88.55

IC_{50}- median inhibitory concentration

DPPH - 1, 1-Diphenyl-2-picrylhydrazyl

FRAP- ferric ion reducing capacity assay

(1:1 solution of 1% sulphanilamide in 5% phosphoric

significantly [$F(5, 30) = 15.27$, $P < 0.001$] attenuated the impairment of spontaneous alternation behavior

induced by scopolamine. However, scopolamine produced no significant effect on the total number of arm entries when compared with vehicle-treated control. Also, GCK [*F* (5, 30) = 1.535, *P=0.2087*] had no significant effect on the total number of arm entries (Fig. 2).

Effect of GCK on scopolamine-induced memory deficit in the EPM test

In the EPM, no significant difference was found in the mean transfer latency in acquisition trial. One way ANOVA revealed no significant effect of GCK treatment [F(5,24)=2.481,P=0.0602] during acquisition trial in the

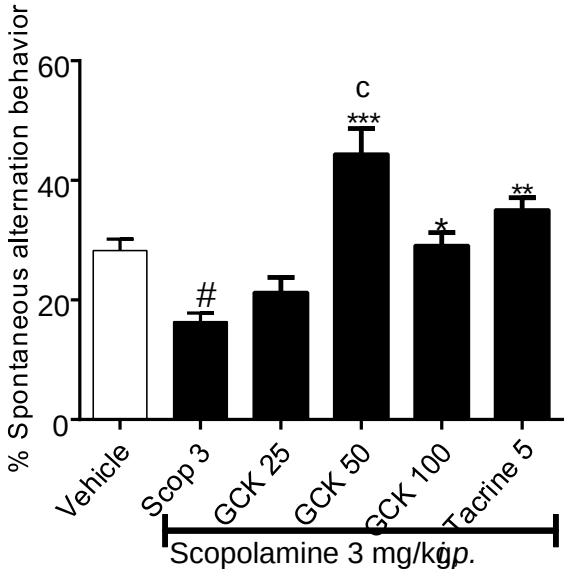

Figure 1: Effect of GCK on spontaneous alternation behaviour in scopolamine-induced memory deficit test in Y-maze test in mice. Values are expressed as mean±SEM (n =6), #p<0.05 versus vehicle only treated; *p<0.05, **p<0.01, ***p<0.001 versus vehicle + scopolamine treated; cp<0.001 versus GCK 25 mg/kg treated. Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison test.

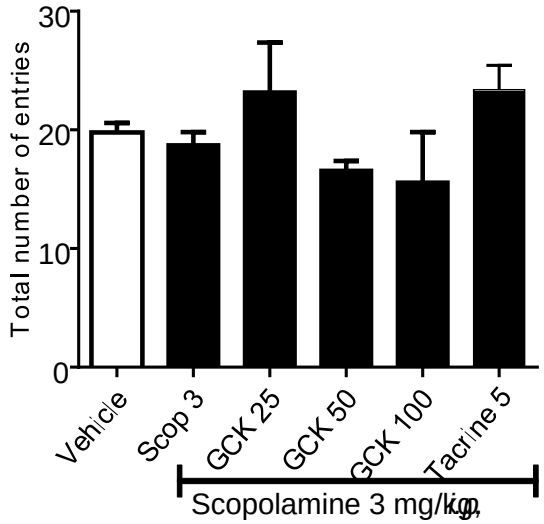

Figure 2: Effect of GCK on total number of arm entries in scopolamine-induced memory deficit test in Y-maze task. Values are expressed as mean±SEM (n =6). Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison test.

EPM test in mice. A significant difference was found in the mean retention trial TL between the groups; [F(5,48)=6.195, P< 0.01]. 3 days pre-treatment of mice with GCK reversed the memory impairment induced by scopolamine. Post hoc analysis showed that

scopolamine (3 mg/kg) significantly increased transfer latency when compared with vehicle-treated control during retention trial on day 2 indicating amnesia. GCK (50 mg/kg) treatment produced significant (P<0.01) reduction in transfer latency in retention trial when compared with acquisition trial (Fig. 3).

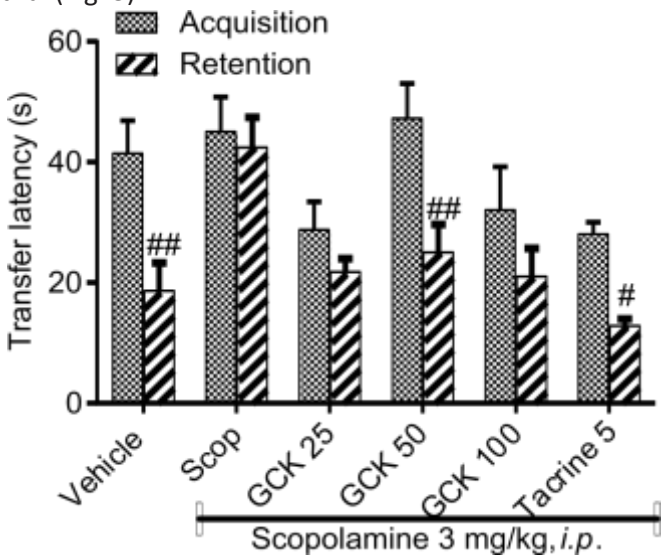

Figure 3: Effect of GCK on transfer latency in scopolamine-induced memory deficit in EPM test in mice. Values are expressed as mean±SEM (n =6). #P<0.05, ##P<0.01 versus acquisition trial. Statistical level of significance analysis by one way (acquisition trial) or two way (retention trial) ANOVA followed by Tukey *post hoc* multiple comparison test.

Effect of GCK on spatial learning in Morris water maze test

Vehicle only treated rats quickly acquired the spatial task as observed by a gradual and session-dependent decrease in escape latency time (ELT) [F (4, 20) =57.02, p<0.001]. Intraperitoneal administration of scopolamine induced spatial memory impairment as indicated by no significant change [F (4, 20) =2.52, P=0.0665] in ELT from 2nd to 5th sessions when compared with the first session. However, pre-treatment of rats with tacrine before scopolamine injection produced a session dependent and significant decrease [F (4, 20) =17.28, P<0.001] in ELT from the 2nd to 5th session when compared with the first session. Similarly, oral administration of GCK prevented the spatial-learning deficit induced by scopolamine in rats. GCK (25 or 100 mg/kg) significantly decreased mean ELT from third session [F(4,20)=13.69, P<0.01],
[F(4,20)=9.17,P<0.001], respectively, when compared to first session, while GCK 50 mg/kg reduced the mean ELT significantly from the second to the fifth session [F (4, 20)=9.98,P<0.001]. The decrease in ELT produced by GCK was comparatively similar.to that of tacrine-treated groups (Fig.4).

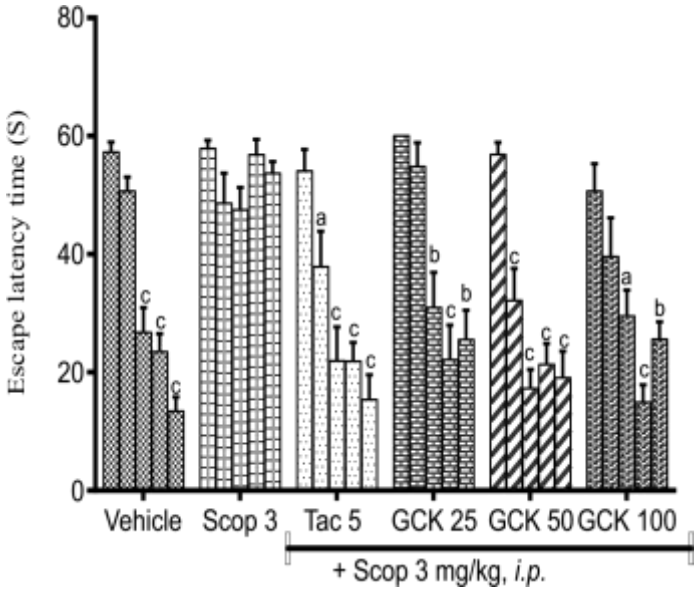

Figure 4: Effect of GCK on spatial learning task following scopolamine-induced memory impairment in Morris water maze test in rats. Values are expressed as mean ELT (sec)±S.E.M (n = 6). [a]p<0.05, [b]p<0.01, p<0.001 versus session 1. [c] Statistical level of significance analysis by two way ANOVA followed by Tukey *post hoc* multiple comparison test.

Effect of GCK on malondialdehyde level

The effect of GCK on the levels of MDA in the prefrontal cortex, striatum and hippocampus of rats after the MWM, was examined to evaluate its effect on scopolamine-induced lipid peroxidation. Scopolamine administration induced significant increase in the levels of MDA in discrete brain regions as compared with vehicle-treated control group. However, pretreatment of rats with GCK (25, 50 and 100 mg/kg) or tacrine (5 mg/kg) significantly (P<0.001) reduced the MDA levels in the prefrontal cortex, striatum and hippocampus. Two way ANOVA revealed significant effect of GCK or tacrine treatment ($F(5,72)=43.61$, $P<0.001$), scopolamine pretreatment ($F(2,72)=4.18$, $P=0.019$) and pretreatment × treatment interaction ($F(10,72)=7.915$, $P<0.001$) (Fig. 5).

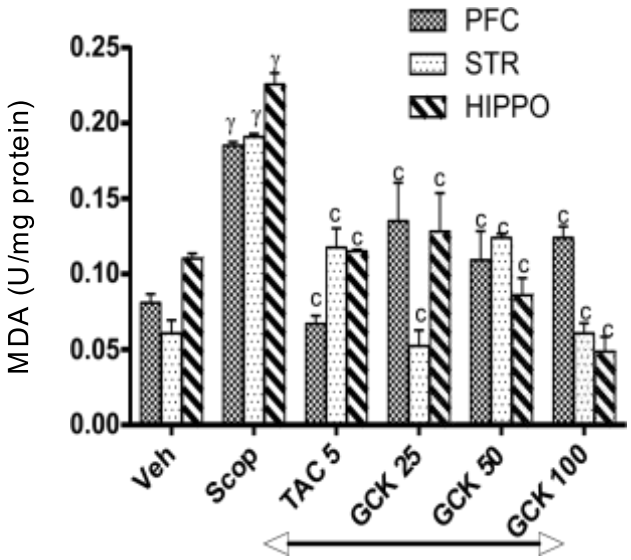

Figure 5: The effect of GCK on malondialdehyde activity within the prefrontal cortex, striatum and hippocampus of scopolamine-induced amnesic rats. Values are expressed as mean±SEM (n=6). $^{\gamma}P<0.001$ versus vehicle-treated, control; $^{c}P<0.001$ versus vehicle + scopolamine treated. Statistical level of significance analysis by two way ANOVA followed by Tukey *post hoc* multiple comparison test.

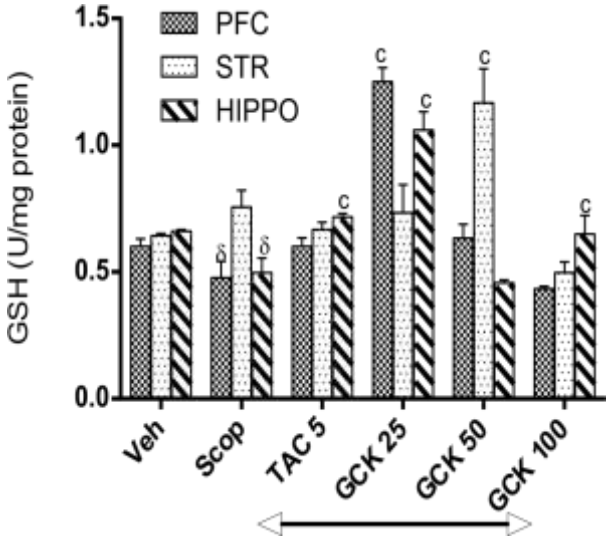

Figure 6: The effect of GCK on glutathione activity within the prefrontal cortex, striatum and hippocampus of scopolamine-induced amnesic rats. Values are expressed as mean±SEM (n=6). $^{\delta}P<0.001$ versus vehicle-treated, control, $^{c}P<0.001$ versus vehicle + scopolamine treated. Statistical level of significance analysis by two way ANOVA

Effect of GCK on glutathione activity

Administration of scopolamine resulted in a significant decrease in reduced glutathione level ($F(2,72)=0.4744$, $P=0.6242$) in the prefrontal cortex, striatum and hippocampus when compared with vehicle-treated control. The reduction in GSH level induced by scopolamine was reversed by pretreatment of rats with GCK or tacrine in the prefrontal cortex, striatum and hippocampus. Moreover, two ANOVA revealed the main effect of GCK (25, 50 and 100 mg/kg) treatment ($F(5,72)=54.43$, $P<0.001$) and pretreatment × treatment interaction ($F(10,72)=14.84$, $P<0.01$) (Fig. 6). followed by Tukey *post hoc* multiple comparison test.

Effect of GCK on superoxide dismutase level

Administration of scopolamine induced significant (F(2,72)=98.53, P<0.001) reduction in superoxide dismutase in the prefrontal cortex, striatum and hippocampus when compared with vehicle-treated control. However, 3 days pretreatment of rats with GCK or tacrine prevented the decrease in superoxide dismutase level in the prefrontal cortex, striatum and hippocampus. Two way ANOVA revealed significant t r e a t m e n t e f f e c t o f G C K o r t a c r i n e (F(5,72)=126.60,P<0.001) and pretreatment × pretreatment × treatment interaction (F(10,72)=18.32, P<0.01) (Fig. 8).

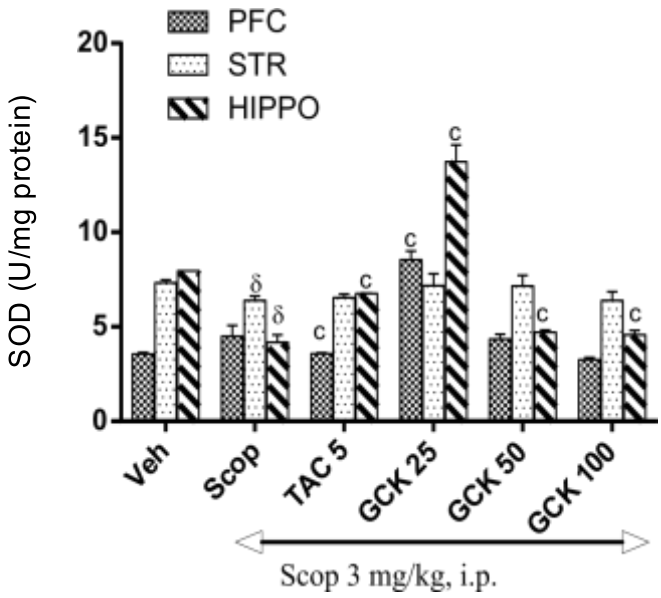

Figure 7: The effect of GCK on superoxide dismutase activity within the prefrontal cortex, striatum and hippocampus of scopolamine-induced amnesic rats. Values are expressed as mean±SEM (n=6). $^{\delta}$P<0.001 versus vehicle-treated, control; ^{c}P<0.001 versus vehicle + scopolamine treated. Statistical level of significance analysis by two way ANOVA
treatment interaction (F(10,72)=22.38, P<0.01) (Fig. 7). followed by Tukey *post hoc* multiple comparison test.

Effect of GCK on nitrite generation

Scopolamine administration induced a significant increase in the level of nitric oxide in the prefrontal cortex, striatum and hippocampus of rats
(F(2,72)=59.30, P<0.001) when compared with vehicletreated control. However, 3 days pretreatment of rats with GCK or tacrine prevented (P<0.001) the scopolamine induced increase in nitrite generation in the prefrontal cortex, striatum and hippocampus. Two way ANOVA revealed significant treatment effect of GCK or tacrine (F(5,72)=53.15,P<0.001) and

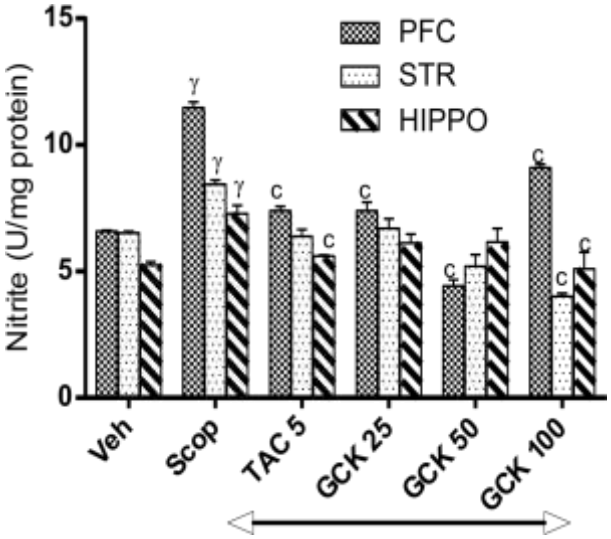

Figure 8: The effect of GCK on nitrite level within the prefrontal cortex, striatum and hippocampus of scopolamineinduced amnesic rats. Values are expressed as mean±SEM (n=6). P<0.001 versus vehicle-treated, control; P<0.001 γ c versus vehicle + scopolamine treated. Statistical level of significance analysis by two way ANOVA followed by Tukey
post hoc multiple comparison test.

DISCUSSION

Findings obtained from this study showed that GCK possesses memory enhancing effect evidenced in its ability to prevent scopolamine-induced working memory and spatial learning deficits in rodents as well as its ability to scavenge oxidative and nitrosative stresses in discrete brain regions. Scopolamine, a muscarinic acetylcholine receptor antagonist, is used as the gold standard for inducing cognitive deficits in man and animals.[3,28] The scopolamine model is still used extensively for preclinical testing of new substances designed to treat cognitive impairment.[29,30] It is well known that cholinergic neuronal systems play an important role in the cognitive deficits associated with AD, ageing and neurodegenerative diseases.[3] The spontaneous alternation behaviour in the Y- maze, as an index of short-term memory is a highly useful method to screen compounds against amnesic rodent model.[20,31]. The GCK-induced increase in alternation behavior did not affect the number of arm entries thus, ruling out psychostimulant activity. The EPM test on the other hand was originally developed to estimate anxiety in rodents.[32] However, it was modified to evaluate spatial learning and memory in rodents. The parameters measured are not the same: the number of entries into the open and closed arms, the time spent in the open arms for anxiety, but transfer latency (TL), which reflects the time the mice took to move from the open arm to either of the enclosed arms for the memory processes.[33,34] In the present study, scopolamine induced spatial learning deficit which was ameliorated by GCK treatment. The decrease in transfer latency on 2nd day (i.e., 24 h after the first trial) when compared with acquisition trial indicated improvement of memory.[34,35]

Morris water maze test evaluates spatial memory and detects changes in the central cholinergic system.[36] In this study, scopolamine administration increased the escape latency indicating spatial learning and long term memory deficits at a dose of 3 mg/kg, this dose has been reported to have no effect on swimming ability and appears to be dissociated from drug-induced hyperactivity.[36] The vehicle-treated control group rapidly acquire spatial learning in the MWM task. Similarly, pretreatment of rats with *G. kola* significantly shortened escape latency time from day 3. The session dependent decrease in escape latency produced by *G. kola* represents long-term memory.[37] These results suggest that the extract improves long-term memory in amnesic rat model induced by scopolamine treatment. Oxidative and nitrosative stress occur in biological systems due to the dysregulation of the redox balance, caused by a

deficiency of antioxidants and/or the overproduction of free radicals.[38] It has been proposed that the progressive increase in ROS and consequent oxidative damage play the major role in neurodegenerative disorders.[39] Ciobica et al[39] showed that the levels of SOD and GPX decrease in rats by scopolamine and the level of malondialdehyde increase in same rats, compared with saline-treated rats. Several research works validate the harmful influence of scopolamine on cortex and hippocampus-dependent learning and memory.[40-42] Jeong *et al.*[30] showed that memory impairment in the scopolamine-induced animal model is associated with increased oxidative and nitrosative stress within the brain. This improvement was mainly shown by the increased levels of GSH, SOD, and catalase as well as the decreased intracellular reactive oxygen species, MDA and nitrite concentrations in the aforementioned central areas. Previous studies identified some active compounds in *G. kola* which include Garcinia biflavonoid (GB) 1, GB2, kolaviron, kolaflavone and kolaflavanone.[43] The phytochemical screening of *G. kola* showed that it contains high quantities of alkaloids, and moderate quantities of saponins, tannins, flavonoids, cyanogenic glycosides, sterols and phenols. In addition, these results indicated that the different constituents of *G. kola* could be potent free radical scavenger, as they scavenged the DPPH radical, hydroxyl radical and NO radical *in vitro* system. *G. kola* reacted with DPPH radical, and converted it to 1, 1-diphenyl-2- picrylhydrazine, and the degree of discoloration (indicated by decrease in absorption) indicated the scavenging activity.[44] The strong scavenging capacity of the extract on DPPH was possibly due to the hydrogen donating ability of the polyphenolic compounds. Nitric oxide is considered as relatively less reactive, its metabolic product such as peroxynitrite, formed after reacting with oxygen, is extremely reactive and can induce toxic reactions, including thiol group oxidation, protein tyrosine nitration, lipid peroxidation, and DNA modification.[45] The nitrite scavenging ability of extracts further expands the role of this plant as a potent antioxidant. The reducing power capacity of the extract may provide a significant indication about the potential antioxidant capacity of the seeds. However, further study is ongoing to investigate possible amelioration of scopolamine-induced cognitive impairment by kolaviron in rodents.

CONCLUSION

Findings from this study demonstrated memory enhancing properties of *Garcinia kola* through enhancement of antioxidant systems in the prefrontal cortex, striatum and hippocampus. In addition, *Garcinia kola* might offer a useful therapeutic choice in either the prevention or the treatment of dementia.

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