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Modulating Effect of Green Tea and Vitamin C in the Management of "Alzheimer's disease: Oxidative Stress, Cell-Based Anti-Inflammatory and Genotoxic Profiles

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Abstract

Alzheimer's disease is a progressive brain disorder. Green tea (GT) and vitamin C are known for their antioxidant and anti-inflammatory properties. This study aims to investigate the potential oxidative stress, cell-based anti-inflammatory and genotoxic Profiles for synergistic effect of green tea and vitamin c against to Alzheimer's disease**.**

In vitro assessments included their several antioxidant parameters and ability to inhibit inflammatory markers such as nitric oxide (NO), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and matrix metallopeptidase 1(MPP1) in lipopolysaccharide-activated macrophages. *In vivo* approach evaluated genotoxicity and their potential neurotherapy **against** Alzheimer's disease using a rat model. Behavioral, biochemical, and histological evaluations were conducted.

Green tea methanolic extract (GTME) and vitamin C showed enhanced antioxidant and anti-inflammatory modulator activities. The neurotoxicity rat's model exhibited cognitive decline and brain injury in contrast to the control group. While treated rats with GTME and vitamin C exhibited improved cognitive function, behavior and reduced AChE levels the combination exerted a significantly more potent effect. Histological analysis confirmed improved brain tissue structure in rats receiving the combined treatment. Crucially, neither compound exhibited genotoxic properties.

The combination of GTME and vitamin C demonstrated a promising therapeutic potential for neurodegenerative diseases due to their synergistic effects**.**

Keyword: Antioxidant; anti-inflammatory; Green tea; vitamin C; Acetylcholine esterase; Alzheimer's disease.

1. Introduction

Green tea (GT), which is derived from the *Camellia sinensis* L. plant, is one of the most widely consumed beverages and dietary supplements globally. Due to the well-documented health benefits of green tea, it has become a valuable ingredient in the nutritional supplement and functional food product markets.

The extract of GT contains a number of polyphenolic components with antioxidant activities, but the main active components are the flavanol monomers known as catechins, where epigallocatechin-3-gallate and epicatechin-3-gallate (EGCG) are the most effective antioxidant compounds. Extensive evidence supports the multiple health benefits of green tea and its abundant EGCG including anti-inflammatory, antiarthritic, antibacterial, anti-angiogenic, antioxidant and neurotherapy effects (1-4).

The neurotherapy effects of green tea can be attributed to from its multifaceted mechanisms, including EGCG which can neutralize harmful free radicals and reactive oxygen species. This helps protect neural cells from the oxidative stress that contributes to neurodegenerative diseases(5). In addition, green tea polyphenols reduce neuroinflammation underlying various neurological disorders (6-8). They can also activate important signaling pathways, such as PI3K/Akt and MAPK, that promote neuronal survival and synaptic plasticity (9, 10). These pathways support the overall health and function of neurons, which is crucial for cognitive performance and neuronal integrity. Furthermore, green tea has been shown to improve mitochondrial function and energy metabolism within neurons (5, 11). This helps maintain neuronal energy homeostasis and supports their viability. Green tea compounds can also modulate the activity of key neurotransmitters, such as dopamine, serotonin, and acetylcholine, which are essential for cognitive function, mood, and neuronal communication(12, 13). Lastly, studies have shown that green tea stimulates neurogenesis, the development and differentiation of neural stem cells, as well as enhances synaptic plasticity, the formation and strengthening of connections between neurons (14).

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Vitamin C (ascorbic acid) emerges as another potential player in the protective effect against neurodegeneration (15, 16). This vital nutrient acts as a powerful brain antioxidant protecting neurons from harm caused by ROS molecules. Furthermore, vitamin C contributes in brain recovery after neurodegeneration by repairing and maintaining the myelin sheath, the protective layer around nerve cells (17). Beyond its antioxidant effects, Vitamin C's anti-inflammatory properties and influence on gene expression and epigenetics further strengthen its potential therapeutic role (18, 19).

Interestingly, some studies suggest that co-consumption of green tea with Vitamin C may enhance the bioavailability and potentially the neurotherapy effects of green tea catechins (5, 20).

Alzheimer's Disease (AD), the leading cause of dementia, presents a growing challenge due to the rising elderly population(21, 22). While the exact cause of AD remains elusive, the disease is characterized by the presence of beta-amyloid plaques and neurofibrillary tangles in the brain (23, 24). These pathological processes lead to progressive memory loss, cognitive decline, and ultimately, complete dependence on caregivers (25, 26).

Given the promising neurotherapy properties of both green tea and Vitamin C, this study aims to elucidate the mechanisms by which green tea, potentially synergistically enhanced by vitamin C may have neurotherapy effects against oxidative stress and inflammation, key contributors to AD.

2. Experimental

2.1. Preparation of Green tea methanol extract (GTME) by maceration method

Green tea leaves obtained from local Egyptian market were submerged in methanol and allowed to soak overnight at room temperature. A non-viscous, methanol extract was collected then underwent concentration using a rotary evaporator under vacuum at 40°C. This process effectively transformed the liquid extract into a dry, dense, and viscous form that could be stored at -20°C until use (27).

2.2. Vitamin C\Ascorbic acid

Ascorbic acid used in the present work is product supplied from Sigma chemical company, St., Louis, Mo, USA.

2.3. *In vitro approach*

Evaluation of the antioxidant and anti-inflammatory activities of GTME

2.3.1 Antioxidant activity

2.3.1.1. Free radical scavenging activity (DPPH).

The free radical scavenging ability of GTME, vitamin C and their combination was evaluated using the standard method described by Shimada et al.(28). Briefly, a solution containing 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was mixed with the GTME and incubated for 30 minutes. The lower the absorbance measured after incubation, the stronger the extract's free radical scavenging activity

Scavenging effect (%) = 100 − [((A0-A1)/A0) × 100]

Where A0 is the absorbance of the control, and A1 is the absorbance of extract.

2.3.1.2. Total antioxidant activity determination using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS Assay.

Total antioxidant activity of GTME, vitamin C and their combinationwasassessed following the method described by Hsu *et al*. (29). A pre-formed bluish-green complex of radical cation chromophore was generated by incubating a mixture of peroxidase, hydrogen peroxide, and ABTS in dark condition for one hour. Subsequently, the GTME was added, and its absorbance was measured at 734 nm. The total antioxidant activity was calculated as follows:

Total antioxidant activity (%) = [1- (A sample/ A control)] x 100

2.3.1.3. Superoxide anion scavenging activity using the PMS/NADH system.

The method published by Liu et al, was used with minor adjustments(30), to measure the superoxide anion scavenging activity of tested treatments GTME, vitamin C and their combination as follows :1 ml of the treatment was mixed with a solution containing 1 ml of nitrobluetetrazolium (NBT) (50 µM), 1 ml NADH (78 µM), and 3 ml of Tris-HCl buffer (16 mM, pH 8.0). The reaction was initiated by adding 1 ml of phenazine methosulfate solution (PMS) (10 µM) to the mixture. After incubation at 25°C for 5 minutes, the decrease in absorbance at 560 nm was measured. The percentage inhibition of superoxide anion generation was calculated using the following formula:

% inhibition =[(A0-A1)/A0] × 100

Where A0 is the absorbance of the control, and A1 is the absorbance of extract.

2.3.1.4. Scavenging of hydrogen peroxide

The ability of GTME, vitamin C and their combination to scavenge hydrogen peroxide was evaluated at various concentrations (25-75 µg/ml). Following Ruch et al. ,the extract was added to a prepared hydrogen peroxide solution and incubated(31). The remaining hydrogen peroxide was then quantified by measuring its absorbance at 230 nm.

A formula was used to calculate the percentage of hydrogen peroxide scavenged, reflecting the extract's antioxidant potential.

Percent scavenged [H_2O_2] = [(A0-A1) A0] × 100

Where A0 is the absorbance of the control, and A1 is the absorbance of the extract.

2.3.2. Anti-inflammatory activity assessment

2.3.2.1. Anti-inflammatory effects on nitric oxide production and cell viability in LPS-stimulated RAW 264.7 Macrophages

The *Mus musculus* macrophage cell line (RAW 264.7) was obtained from the ATCC (American type culture collection). The cells were cultured in RPMI, 1640 medium and supplemented with 1% pen/strep and 10% heat-inactivated fetal bovine serum. The cells were incubated, in a humidified incubator, in an atmosphere of 5% CO2at 37 °C.

Following 24 h of incubation1×105 cells per well (in 96 well plates), the cells were then treated with GTME, vitamin C and their combination at concentration range of 100, 50, 25 and 12.5, µg/mL and incubated for one hour. The cells were then stimulated with 10µg/mL of LPS for an additional 24 hours. The supernatant was transferred to 96-well plates for subsequent nitric oxide (NO) determination. The MTT assay was then used to evaluate the viability of the remaining cells(32, 33). The percentage of change in viability was calculated according to the formula:

((1-(Reading of extract / Reading of control)) x 100

2.4. *In vivo* approach

2.4.1 genotoxicity study

2.4.1.1. Genotoxicity experimental design.

Swiss albino mice (Mus musculus) BALB/c (8-10weeks) old (their weight range between (25-32 gm) were used, with controlled temperature (23±2) ºC and 12 hours light / dark cycle. All mice were provided with standard food pellets and regular drinking water *ad libitum*. The healthy mice received tenth of LD_{50} (oral) extracts of GTME, Vit C and their combination for 15 days (day after day).

2.4.1.2. Chromosome aberrations in somatic cells

The slides for chromosome analysis were prepared and stained as described by Disteche, C.M. and Adler, D. (34). In brief, both femurs were dissected and bone marrow was flushed from the femoral cavity with fetal calf serum. The cells were dispersed by gentle pipetting and collected by centrifugation at 1100 rpm, the harvested bone marrow cells were incubated in 10 ml of 0.075M KCl for 20 min at 37 °C. At the end of the incubation period, Carnoy's fixative solution (cold glacial acetic acid-methanol, 1:3, v/v) was added to each tube and then centrifuged for 10 min at 1100 rpm. The supernatant was discarded and 10 ml of fresh fixative was gently pipetted on to the cells without disturbing the pellet. Two to three changes in fixative were required before the preparation of slides. Finally the cells were suspended in small volume of fixative and burst open on a clean slide to release chromosomes. The slides were air dried and stained with 5% Giemsa and coded before observation. One Hundred well spread metaphases were analyzed per mouse.

2.4.1.3. Chromosome abnormalities in germ cells:

Chromosome preparations from spermatocytes (germ cells) were made according to the technique of Evans et al (1964). In brief, animals were I.P. injected with colchicines at final concentration of 3 mg/kg b.wt., 2-3 hours before sacrificing. Mice were anaesthetized using Ketamine/Xylazine mixture (Ketavet® 100 mg/mL (Zoetis Deutschland GmbH, Berlin, Germany)/ Rompun® 2% (Bayer Vital GmbH, Leverkusen, Germany) at a dosage of 80 mg/kg ketamine and 12.5 mg/kg xylazine through intraperitoneal injection. The testes were removed, transferred to a sterile petri-dish containing isotonic sodium citrate solution (2.2%) and cut into small pieces. The resulting cells were aspirated into clean centrifuge tube and centrifuged at 1500 rpm for 5 min. Hypotonic sodium citrate solution (1%) was added drop by drop and left for 20 min at 37ºC. Centrifuge at 1500 rpm for 5 min. The supernatant was discarded by gentle aspiration until a small volume remainder above the pellet. The pellet was re-suspended in the remained volume. The cells were fixed in freshly prepared 3:1 methanol: glacial acetic acid and added gently to the cells. Fixation was done twice and cell suspension was centrifuged at 1500 rpm for 5 min. Drops were taken from the sediment and were dropped onto dry slides. The slides were stained with 7% Giemsa in phosphate buffer pH 6.8. 100 well spread diakinase

metaphase I cells were analyzed per animal for chromosomal aberrations. Metaphases with X-Y univalents and autosomal univalents were recorded.

2.4.1.4. Scoring

All slides were scored under 1000× magnification using an Olympus Microscope. One-hundred well-spread metaphase plates per mouse (500 metaphases for each group) were scored for both structural and numerical aberrations (polyploidy) in bone marrow cells. Cells were classified according to the most severe damage which had occurred and were placed in one of five categories: cells with gaps only, cells with breaks, acentric fragments, centric rings and polyploidy. Gaps were defined as achromatic lesions in one or both chromatids not exceeding the width of a chromatid, and breaks as discontinuities greater than the width of a chromatid, irrespective of whether or not the distal fragment was dislocated. Cells with gaps were not included in the percentage of total aberrations. From the same slides 1000 cells from each animal were taken into consideration for the mitotic index study. The mitotic activity of bone marrow was evaluated by calculating the number of dividing cells in a population of 500 cells.

2.4.2. Alzheimer's animal model

2.4.2.1. Animal groups and treatment conditions

Male albino mice (30-35 g) for acute toxicity study and male adult wistar rats (200-250 g) for Alzheimer animal model experimental were purchased from animal housing unit of National Research Centre (NRC). All animal handling and experimental procedures were approved by the institutional Animal Ethics Committee, NRC (No. 04417082021). The experiments adhered to the guidelines set forth in the "Guide for the Care and Use of Laboratory Animals" and the regulations of the "Committee for the Purpose of Control and Supervision on Experimental Animals."

The animals were housed in groups within standard laboratory cages under controlled conditions. These conditions included a 12-hour light/dark cycle, room temperature (22-24°C), and access to a balanced diet throughout the study. Prior to the experiment's commencement, both rats and mice were acclimated to these laboratory conditions for at least five days.

Five groups of rats (n=6/group) were involved in this study. Group I functioned as the control, receiving no treatment. The remaining groups (II-V) were initially induced with Alzheimer's-like pathology with $AlCl_3$. Following $AlCl_3$ administration, these groups were subjected to different treatment regimens: Group II remained untreated ($AlCl_3$ only), Group III received GTME, Group IV received vitamin C, and Group V received a combined treatment of GTME and vitamin C.

2.4.2.2. Determination of maximum tolerated dose (MTD) of GTME

Three groups of six mice were given a single oral gavage at a dose of 5000 mg/kg body weight to determine the maximum tolerated dose of GMTE, vitamin C, and their combination. Following administration, the mice were monitored for any signs of toxicity or death for24 hours. In the absence of any adverse effects after 24, the 5000 mg/kg was considered the maximum tolerated dose of samples under test. Accordingly, a lower dosage of 500 mg/kg body weight (1/10th of the MTD in mice) was chosen for the rat experiment (35).

2.4.2.3. Induction of Alzheimer's-like pathology in rats using aluminum chloride

A daily oral gavage of $AlCl_3$ solution was administered at 17 mg/kg body weight per rat for duration of four weeks. This specific dosage was chosen to align with the established protocol outlined by Harakeh *et al.* (36).

2.4.2.4. Administration of GTME, vitamin C and their combination.

Animals in the treatment groups subjected to a fourteen-day course of daily oral gavage. Green tea extract (GTME) was administered at a dose of tenth of LD_{50} (37).

2.4.2.5. Assessment of memory function using Y-Maze behavioral test.

All rats in control and treated groups were subjected to the Y-maze behavioral test following a 12-hour fasting period. Each rat was placed at the end of one arm for five minutes and allowed to find the pre-positioned food pellets (38).The time taken by each rat to locate the food was recorded, completion times usually fell between two and five minutes.

2.4.2.6. Tissue and Blood Collection.

All rat groups were humanely euthanized via a decapitation protocol (ketamin/xylazine (50 and 7 mg/kg, respectively, ip). Blood samples were then collected from retro-orbital sinus and underwent centrifugation at a relative centrifugal force (RCF) of 1100 x g for five minutes. Sera and brain tissues were stored at -20◦C until further biochemical or histological analyses were performed.

2.4.2.7. Evaluation of serum acetylcholine esterase (AChE)

AChE activity in serum samples in all groups was measured using an ELISA assay kit from Acetylcholine esterase BTC/S.r.l. Biochemical Enterprise (Italy). The assay was carried out following the manufacturer's instructions.

2.4.2.8. Preparation of brain tissue sections for histological examination.

Animal brain tissues were carefully collected and subjected to fixation in a 10% formalin solution. Subsequently, the fixed tissues underwent a dehydration process using a graded series of ethanol solutions. Specimens were then cleared in xylene and embedded in paraffin at 56 degrees in hot air oven for twenty-four hours. Paraffin beeswax tissue blocks were prepared for sectioning at 4 microns' thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, Stained by hematoxylin and eosin stain for examination through the light electric microscope (39).

2. 4.2.9. Statistical Analysis

One-way ANOVA was used to evaluate the main effect of administered treatments across the various experimental groups. The analyses were carried out using the graph pad prism (version 9.0.1) software. A statistically significant difference between treatment groups was established based on a p-value of less than 0.05. All data are presented as mean ± standard deviation (SD).

3. Results

3.1. *In Vitro* approach

3.1.1. DPPH radical scavenging activity assay

The antioxidant activity of GTME, vitamin C and a combination (1:1 ratio) of them was measured by DPPH at 100µg /ml. The GTME was capable of neutralizing the DPPH free radicals by 76.3% while; vitamin C exhibited a higher radical scavenging ability, neutralizing 96.5% of the DPPH radicals. The most effective scavenging activity was the combined treatment with GTME and vitamin C achieving a 100% neutralization of the DPPH free radicals and a lower IC_{50} value compared to GTME alone (Figure 1).

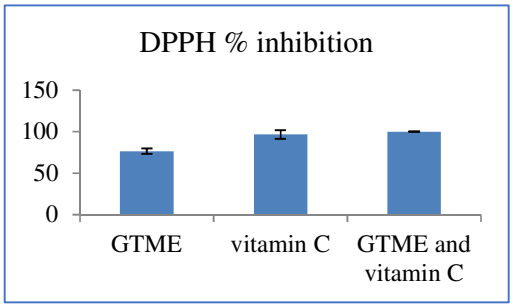

Figure 1: Radical scavenging capacity of GTME, vitamin C, and their combination (1:1 ratio) against DPPH free radical.

3.1.2. Total antioxidant activity (TAA)

TAA of GTME and vitamin C, both individually and in combination were evaluated at a concentration of 100µg/ml. The overall antioxidant capacity of the tested groups was assessed by measuring their ability to scavenge the 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation.

Figure 2 showed that most potent total antioxidant activity was GTME &Vitamin with a value of 100% ± 1.3% and the lowest IC_{50} of 18.0µg/ml. Individually GTME demonstrated 70.4% ± 3.2% TAA and an IC_{50} of 62.4µg/ml, while vitamin C showed a higher TAA of 82.3% ± 2.4% but a slightly less potentIC_{50} of 42.3µg/ml.

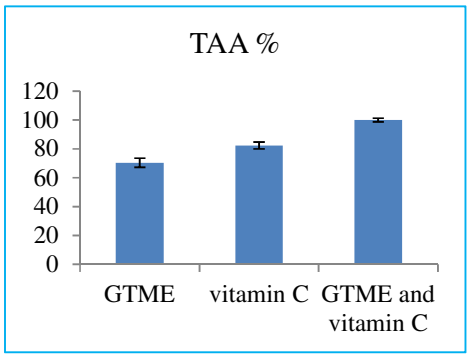

Figure 2: Total antioxidant activity of GTME and vitamin C and their combination (1:1 ratio) at 100µg/ml.

3.1.3. Superoxide anion scavenging activity using the PMS/NADH system

GTME and vitamin C showed the highest efficacy in neutralization the superoxide anion generated in the reaction mixture of the PMS/NADH. At the tested concentration of 100µg/ml of the combined treatment a complete scavenging activity (100%) with lowest IC_{50} value was achieved (Figure 3).

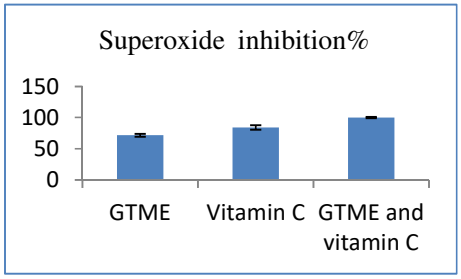

Figure 3: Superoxide radical scavenging activity of GTME, vitamin C and their combination (1:1 ratio) at 100µg/ml

3.1.4. Scavenging of hydrogen peroxide

The scavenging ability of the tested groups against hydrogen peroxide is shown in figure 4. Vitamin C has a stronger hydrogen peroxide scavenging activity, as evidenced by the higher inhibition percentage78.2 ±2.8 and lower IC_{50} value 57. The combination of GTME and vitamin C exhibited a moderate scavenging ability, with an inhibition percentage and IC_{50} value between the individual effects of the two compounds.

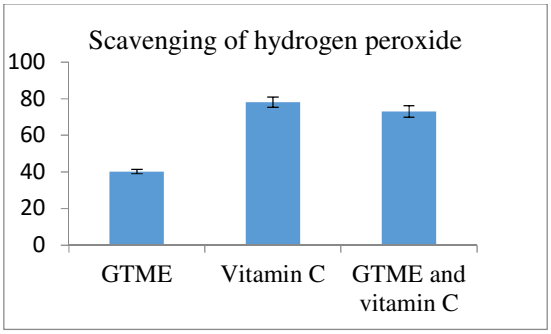

Figure 4: Hydrogen Peroxide Scavenging Ability of GTME, vitamin C, and Their Combination (1:1 ratio).

3.1.5. Anti-inflammatory activity on cell viability and nitric oxide production in LPS-Stimulated macrophages

3.1.5.1. Cell viability:

The ability of the tested treatments to protect the Raw cells from the damaging effects of inflammation was reflected in the cell viability results (Table 1). GTME exhibited the highest cell viability of 88.3 ± 3.4% at a concentration of 200 µg/ml, indicating it had the strongest anti-inflammatory effect among the tested groups.In contrast, vitamin C showed a lower cell viability of 64.8 ± 1.3%, suggesting it had a weaker anti-inflammatory activity.The combination of GTME and vitamin C resulted in a cell viability of 73.4 ± 2.4%, which was lower than GTME alone but higher than vitamin C alone.

Table 1: The viability percentage of treatment groups on RAW 264.7 macrophages.

Groups	**Cell viability % at 200ug/ml against Raw cells**
GTME	88.3±3.4
Vitamin C	64.8±1.3
GTME and vitamin C (1:1 ratio)	73.4±2.4
LPS (-ve control)	100±0

Table 1 illustrates the treatment effect of GTME and vitamin C, individually and together, on the viability of RAW 264.7 macrophage cells. Data is displayed as mean ± standard error.
LPS: Lipopolysaccharide

3.1.5.2 Nitric oxide (NO).

The effects of treatments on NO-mediated inflammation in RAW cells are illustrated in figure 5. The combined treatment GTME and vitamin C had the strongest protective effect (94.3%) with the lowest IC50 (34.7 µg/ml), while vitamin C had a relatively weaker anti-inflammatory activity. GTME fell in between the two, indicating a moderate anti-inflammatory potential.

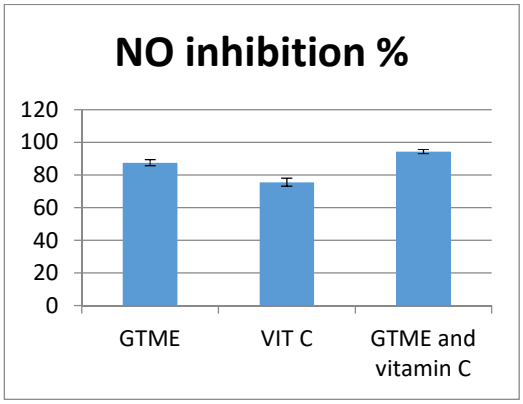

Figure 5: Inhibition of nitric oxide production by GTME, Vitamin C and their combination (1:1 ratio).

Encouraged by our *in vitro* findings demonstrating the antioxidant and anti-inflammatory properties of vitamin C and GTME, we attempted to further investigate their potential neurotherapy effects in an animal model. Therefore, we developed an Alzheimer's disease model to assess the ability of these treatments to reduce oxidative stress and inflammation in the brain.
The combination of GTME and vitamin C shows a significant reduction in IL-6, TNFα levels to 2.35±0.1 and 2.31±0.1, respectively, which is lower than the values observed with either GTME or vitamin C alone. Additionally, the combination resulted in the lowest MPP1 level at 2.12±0.7 (Figure 6).

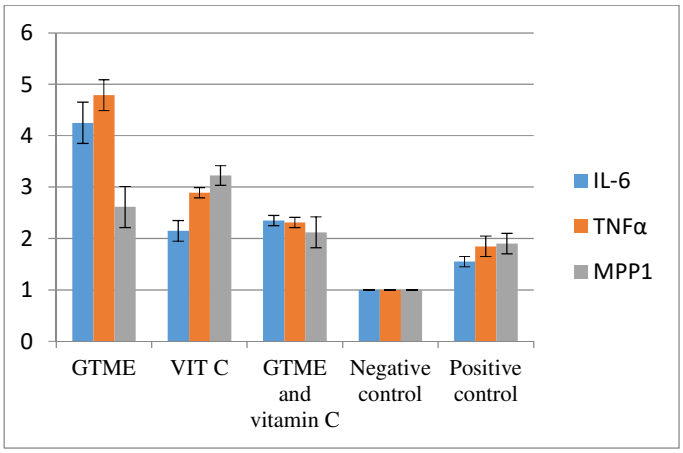

Figure 6: Inhibition of inflammatory mediators in LPS-stimulated macrophages by treatment groups

3.2. *In Vivo* approach

3.2.1. Genotoxicity effects of extracts of GTME, Green Tea and their combination (1:1ratio) in male albino mice.

Among short-term mutagenicity/genotoxicity assays, the chromosomal aberration test coupled with examining chromosomal abnormalities in spermatocytes represents a sensitive protocol widely used for detecting DNA damage and gives information on transmissible genetic damage (Table 2).

Table 2: Types and percentage of metaphases with chromosomal aberrations induced in mouse spermatocyte cells after treatment with different extracts.

Treatment (mg/kg b.wt)	Abnormal Metaphases		No. of different types of Metaphases Aberrations				
	No.	% Mean (±SE)	*XY-uni	*Auto – uni	Fragment and / or break	Chain IV	M.A.
GTME	26	5.8 ±0.4	18	8	0	0	0
Vit C	29	6.4 ±0.5	19	10	0	0	0
GTME+Vit C	31	6.2 ±1.10	20	11	0	0	0
DMSO	44	10.2 ±1.9	31	14	0	0	0
Negative control untreated	20	4.1 ±0.43	12	8	0	0	0
Positive control*	51	10.5 ±1.34	24	14	3	4	6

* Total number of examined metaphases =500 metaphase (5 animals/ group); XY-uni: XY- univalent; Auto- Uni.: Autosomal univalent
* Positive control 20mg/kg body weight

The results showed that DMSO induced statistically significant increase in chromosomal aberrations and sister chromatid exchange in mouse bone marrow after treatment with DMSO. The extracts were shown to have negligible effect on the genetic material. The percentage of chromosomal aberrations induced in somatic and germ cells after treatment with these extracts for 15 days was statistically not significant compared to the control group (saline). Furthermore, these extracts was capable of reducing the number of the chromosomal aberrations induced by the solvent DMSO after treatment for 2 weeks ($p<0.01$).

3.2.2. Alzheimer animal model

3.2.2.1. Behavioral assay in the $AlCl_3$-induced Alzheimer's in experimental animal model.

Following a four-week regimen of daily $AlCl_3$ injection at dose 17 mg/kg, rats exhibited a progressive behavioral and cognitive decline characteristic of AD pathology. This decline manifested as impaired memory function, and spatial memory damage that was demonstrated in the Y-maze test described below. Also, behavioral changes occurred as their activity decreased and increased fatigue was observed, in addition to gradual weight loss due to adipsia and aphagia. A shift from an initial state of alertness and focus (observed during the first week of injection) to a state of distraction, disconnection, and reduced environmental awareness in the final week. Test group rats showed significant behavioral variations in term of time spent to reach food billets. The rats treated with $AlCl_3$ alone took considerably longer time (4.8 minutes) than the negative control group, which had the fastest average time of 1.5 minutes. The $AlCl_3$-treated group behaved better after undergoing GTME treatment, with an average time decrease to 3.5 minutes. The vitamin C treatment had an average period of 2.5 minutes, the same as the negative control group. Furthermore, the combination of GTME and vitamin C produced an average time in the middle range at 2.2 minutes (figure 7).

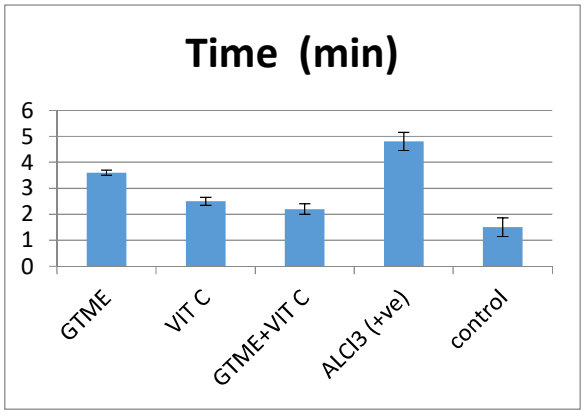

Figure 7: Time taken (minutes) by rat groups in the Y-Maze test to reach food pellets.

3.2.2.2. AChE assessment

AChE activity in all rat's serum following both individual and combined treatments with GTME and vitamin C was illustrated in figure 8. Control rats had an enzyme activity of 1775 U/L but $AlCl_3$ administration resulted in a statically significant increase in AChE activity reaching 2032 U/L. This represents an approximate 13% elevation compared to the control group. GTME partially reduced this increase (1940 U/L) with 4.5% inhibition activity comparing to $AlCl_3$ treated animals, while vitamin C fully normalized AChE activity (1820 U/L) with 10.4% inhibition activity comparing to $AlCl_3$. The combination of GTME and vitamin C significantly reduced AChE activity (1790 U/L) with 12% inhibition activity.

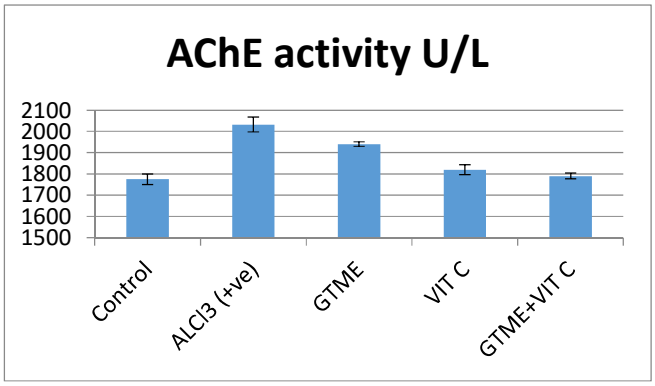

Figure 8: AChE activity levels in control, $AlCl_3$, GTME, vitamin C, GTME& vitamin C rat groups.

2.2. Histopathological assessment of brain tissues.

Histopathological examination of brain tissue showed that the control group's architecture was well-preserved in a number of different locations (Figure 9). Particularly, the striatum, fascia dentata gyrus of the hippocampus, subiculum hippocampus, and cerebral cortex all showed histologically normal cellular organization and neuronal architecture.

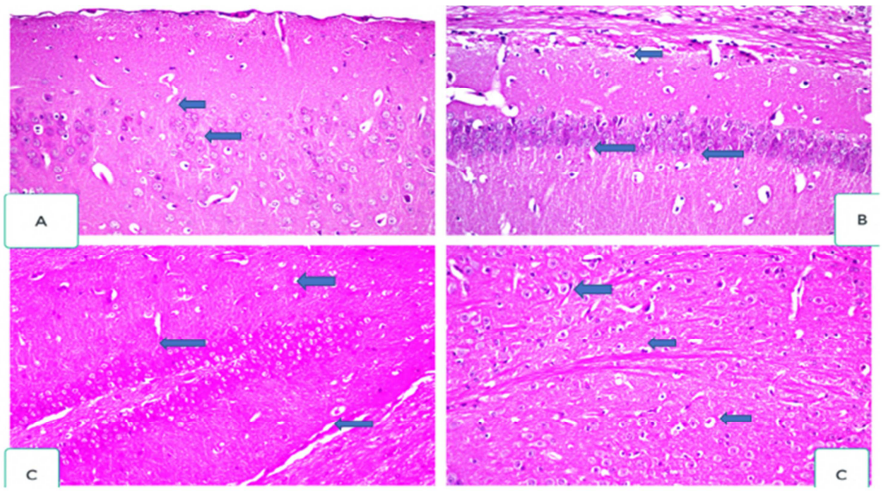

Figure 9.Photomicrograph of control: Histological analysis (H&E, x 40)

Whereas rats from the $AlCl_3$-induced group (II) showed localized areas of neuronal degenerationin cerebral cortex (A). The subiculum hippocampus (B) exhibited a notable deterioration, with most neurons showing shrunken and condensed nuclei (pyknosis) alongside degenerative changes. The fascia dentata gyrus of the hippocampus (C) also presented with pyknosis and degeneration, although to a lesser extent compared to the subiculum. Finally, the striatum (D) revealed focal areas of neuronal damage accompanied by the formation of eosinophilic plaques figure 10.

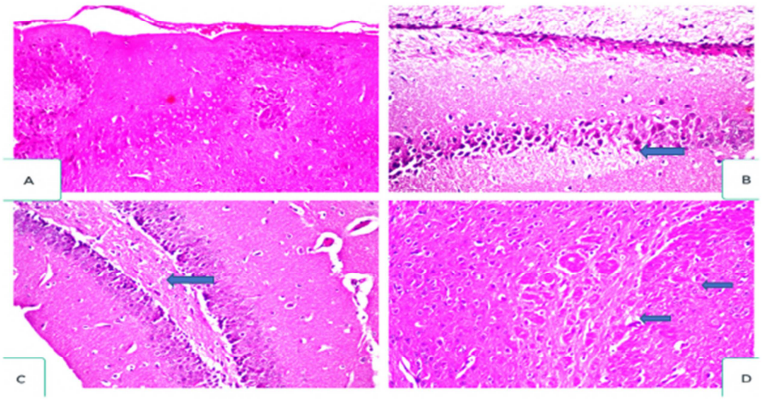

Figure 10. Photomicrograph of $AlCl_3$ -treated group: Histological analysis (H&E, x 40)

In contrast to the control group's well-preserved brain construction, and the $AlCl_3$-induced group (II), histological analysis of treated groups revealed distinct patterns of responses; firstly, in the group treated with GTME (III), cerebral cortex (A) showed nuclear pyknosis and degeneration was detected in most of the neurons, subiculum hippocampus (B) had no histopathological alterations, fascia dentate hippocampus (C) exhibited nuclear pyknosis and degeneration in some few neurons, and finally striatum (D) showed nuclear pyknosis and degeneration in few neurons (figure 11). Photomicrograph of GTME histopathological analysis (H&E , x 40).

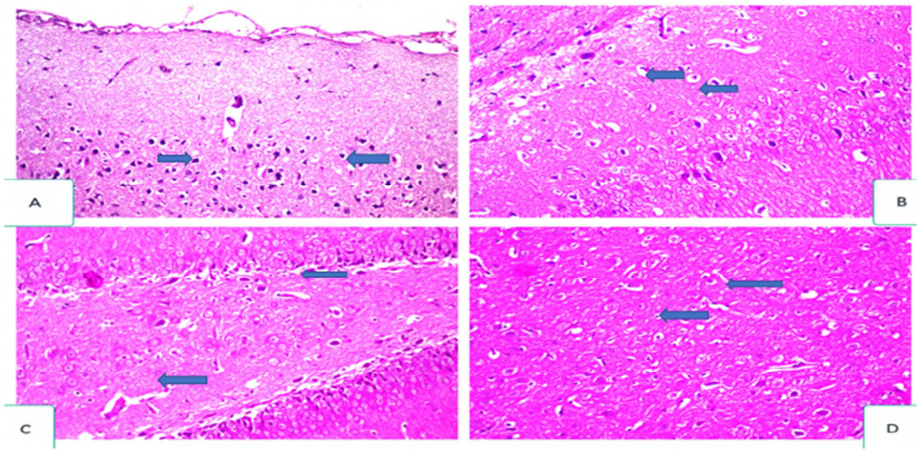

Figure 11. Photomicrograph of GTME-treated group : Histopathological analysis (H&E, x40).

Secondly, in the group treated with vitamin C,pen_sparkcerebral cortex (A), nuclear pyknosis and degeneration were observed in most of the neurons, Subiculum hippocampus (B) showed normal histological structure with no histopathological damage, fascia dentate hippocampus (C) most of its neurons indicated nuclear pyknosis and degeneration, and striatum (D) revealed neurodegeneration and nuclear pyknosis (figure 12).

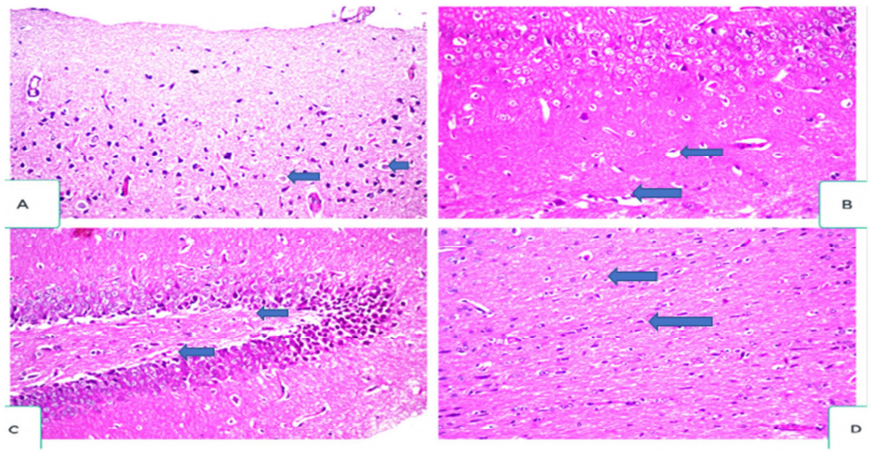

Figure 12. Photomicrograph of vitamin C-treated group :Histological analysis (H& E, x 40).

Thirdly, there was a significant ameliorative effect of the combined GTME and vitamin C treatment, group V, on $AlCl_3$-induced neurodegeneration. This was evident in the histopathological analysis where all examined brain regions, cerebral cortex (A), subiculum hippocampus (B), and fascia dentata hippocampus (C) represented no histopathological alterations, while the in the striatum (D) multiple eosinophilic plagues formation were detected (Figure 13).

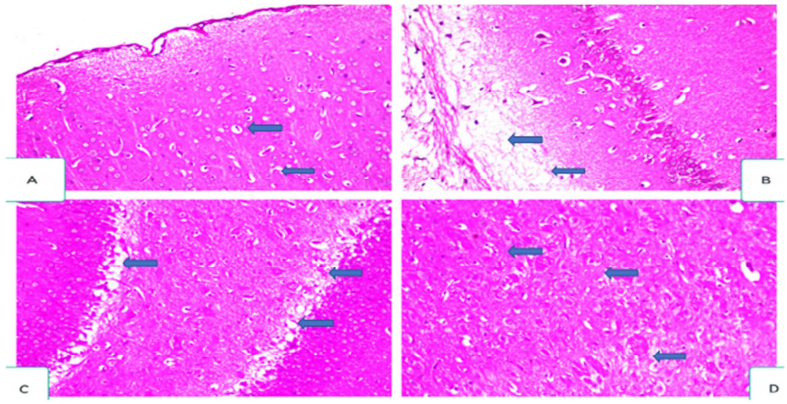

Figure 13.Photomicrograph of GTME &vitamin C –treated group : Histological analysis (H&E, x 40).

4. Discussion

Green tea flavonoids and vitamin C emerge as promising candidates for treating neurodegenerative diseases (6, 40-42). Despite some existing research exploring their antioxidants and anti-inflammatory pathways, a complete understanding of their role in AD is still unclear. Therefore, the present study aims to investigate the potential mechanism by which green tea and vitamin C ameliorate the symptomatic presentation of AD.

Our in vitro findings confirmed the anti-inflammatory and antioxidant activities of GTME and vitamin C separately (43-46). Interestingly, a synergistic effect upon their combination was revealed in our various assays including free radical scavenging (DPPH, ABTS, superoxide), hydrogen peroxide scavenging, and anti-inflammatory activity (nitric oxide production and cell viability) in LPS-stimulated macrophages. Vitamin C displayed a strong DPPH free radical scavenging ability indicated by its low IC_{50} value (47). However, combining it with GTME elevated the overall scavenging capacity. This potentiation likely

arises from the interaction between vitamin C and EGCG, the major antioxidant component of green tea extract (48, 49) (Figure 1).

Furthermore, the combined treatment exhibited the most potent total antioxidant activity neutralizing 100% of the ABTS radical cation at significantly lower IC_{50} than when these compounds acted alone (IC50 value of 18.0µg/ml) (Figure 2). A similar synergistic trend was observed in superoxide anion scavenging, where the treatment achieved complete neutralization (100%) at the tested concentration (Figure 3). The synergy between GTME and vitamin C extended to their anti-inflammatory properties on LPS-stimulated RAW 264.7 macrophages. It had the highest nitric oxide (NO) inhibition (94.3%) and the lowest IC50 value (34.7 µg/ml) compared to their individual effects (47) (Figure 5). Furthermore, the combination treatment significantly decreased levels of IL-6, TNF-α, and MPP1 compared to the control group, as shown in figure 6. This implies a potential mechanism of action involving the suppression of inflammatory signaling pathways and anti-inflammatory responses (50, 51) . This promising interaction needs further investigation in animal models to determine whether the combined treatment is more effective in reducing AD symptoms compared to either GTME or vitamin C administered alone.
The AD model treated with aluminum chloride for 30 days exhibited cognitive defects and symptoms in our study. These findings support the work of Exley and Clarkson (52), who concluded that aluminum is a neurotoxin capable of harming the nervous system. They further linked aluminum exposure to the progression of neurological diseases like Alzheimer's and Amyotrophic Lateral Sclerosis (ALS) by promoting neuronal apoptosis. Extended exposure to aluminum has been related to raised levels of oxidative stress, which might lead to cognitive impairment and enhance the expression of amyloid precursor protein (APP), thereby enabling the accumulation of amyloid beta plaques, a characteristic feature of Alzheimer's disease. Yellamma et al. (53) reported that both chronic and acute administration of aluminum in rats led to alteration in cholinergic transmission system and showed aluminum's neurotoxic properties, as evidenced by brain lesions in multiple regions, which likely had a role in the animals' apparent motor impairments.
Aluminum chloride induction in our study increased the level of AChE (Figure 8) resulting in poor neurotransmission and poor working memory on the Y-maze test (Figure 7). Furthermore, substantial cerebral cortex damages is seen in figure 9, which is consistent with their behavioral defects(53).
As demonstrated by previous research, GTME was associated with anti-AChE activity thus resulting in better neurotransmission(54), this agrees with the present study as GTME had an inhibitory effect on the activity of AChE as shown in figure 7.

A moderate enhancement of cognition in this group compared with aluminum chloride induced rats was observed, furthermore spatial working memory was slightly improved upon the end of the treatment that was shown in figure 7. While there was some neuroprotection provided by green tea extract (GTME) against aluminum chloride exposure, other hippocampal regions remained susceptible. This suggests that there was a localized protective effect on specific brain areas, leading to modest, rather than significant, and improvements in cognitive abilities. (Figure 11).
However, the literature on green tea's potential benefits is contradictory; when some studies have found it effective in minimizing oxidative stress and neuroinflammation, others do not consider it to be efficient in protecting against the development of Alzheimer's. The partially therapeutic benefits of green tea may be related to its inadequate pharmacological characteristics. Green tea has a limited bioavailability when it passes through the digestive system. Furthermore, pharmacokinetic research indicates low stability as a result of oxidative processes within the body, which ultimately impacts its efficacy (55).

Vitamin C, while improving Y-maze performance and inhibiting acetylcholinesterase following aluminum chloride treatment, failed to fully protect neurons (Fig 12). Degeneration remained in the cerebral cortex and fascia dentata. Despite this, vitamin C's potential anti-amyloid properties might still be beneficial in delaying Alzheimer's progression (37).
Rat group received the combination of GTME and vitamin C was improved in regard to behavioral aspects seen in the Y-maze test (figure 7) and AChE levels measurement comparison with rat group that received green tea only (figure 8). The histopathological analysis confirmed what was found by Granja et al., represented in figure 13, as the combination of GTME and vitamin C repaired the damage caused by aluminum chloride in different brain regions. Supporting our findings, Cano et al. showed that combining green tea and vitamin C in nanoparticles improved green tea's stability and effectiveness against Alzheimer's disease. This combination decreased beta-amyloid plaques, neuroinflammation, and improved memory(56, 57).
Neither GTME nor vitamin C exhibited mutagenic or chromosome-damaging properties this is in agreement with the previous data showing the safe effects of some plant extracts on genetic material e.g soybean (58, 59); Egyptian *plantago albicans* clove seed extracts (60, 61), green tea(59) . Moreover, many plant extracts were shown to have protective activity due to rich with bioactive phenolic compounds which have strong antioxidant activities that can protect cellular DNA(61) .

5. Conclusion

Our findings clearly indicate the synergistic combination of GTME and vitamin C as potential therapeutic intervention for slowing AD progression. This is evidenced by its potent antioxidant and anti-inflammatory effects, improvement in cognitive function, modulating release of AChE, and prevention of histopathological alterations in the rat brain. However, Future studies are still needed to fully elucidate the underlying mechanisms by which GTME and vitamin C exert their beneficial effects.

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