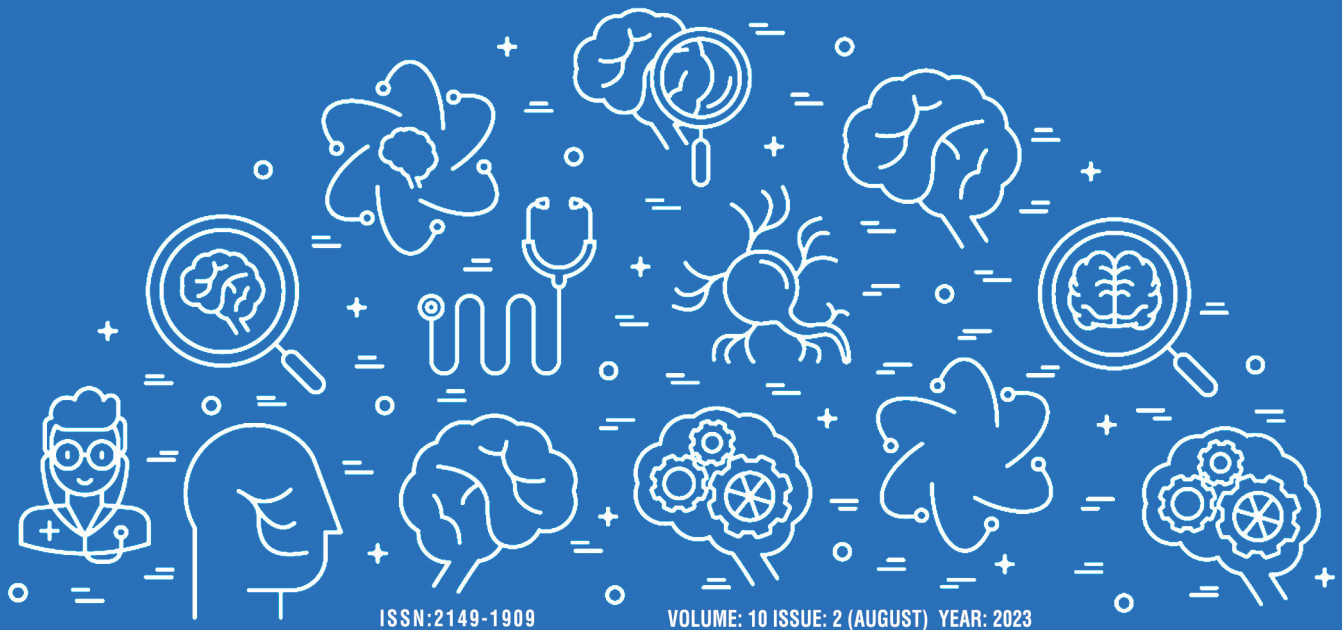

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THE JOURNAL OF NEUROBEHAVIORAL SCIENCES

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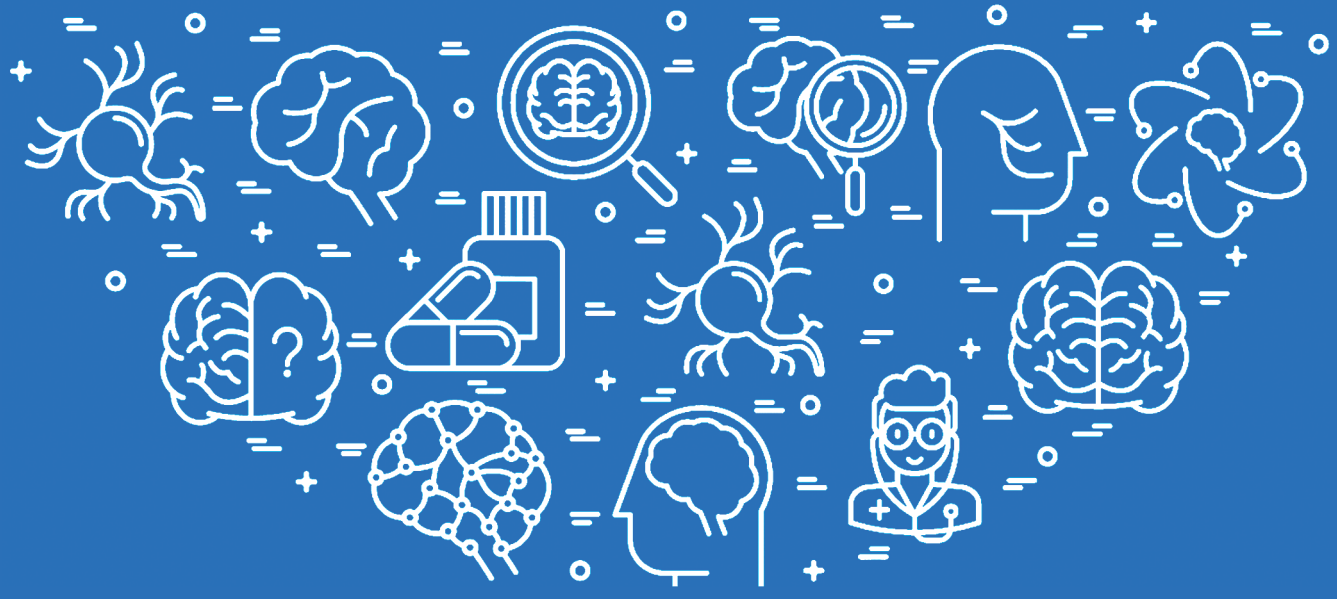

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JNBS (J. Neuro. Behav. Sci) is a comprehensive scientific journal in the field of behavioral sciences. It covers many disciplines and systems (eg neurophysiological, neuroscience systems) with behavioral (eg cognitive neuroscience) and clinical aspects of molecules (eg molecular neuroscience, biochemistry), and computational methods in health.

The journal covers all areas of neuroscience with an emphasis on psychiatry and psychology as long as the target is to describe the neural mechanisms underlying normal or pathological behavior. Pre-clinical and clinical studies are equally acceptable for publication. In this context; the articles and treatment results of computational modeling methods of psychiatric and neurological disorders are also covered by the journal.

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Submit manuscripts electronically (.doc format with including all figures inside) via the online submission system of our website (https://review.jow.medknow.com/jnbs).

Assoc. Prof. Dr. Turker Tekin Erguzel, Ph.D Co-Editor, Journal of Neurobehavioral Sciences Department of Psychology

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*Are some of the data used in the present study being analyzed for the first time? If yes, please identify data (constructs) that were not included in previously published or submitted manuscripts.

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Use Word's Insert Table function when you create tables. Using spaces or tabs in your table will create problems when the table is typeset and may result in errors.

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According to some estimates, the prevalence of ADHD has increased up to 30% in the last 20 years.[1]
S variant is associated with the lower transcriptional activity of the promoter when compared to the L variant.[4,7-9,11]

The Reference Section:

• Journal Article:

Russell FD, Coppell AL, Davenport AP. In vitro enzymatic processing of radiolabelled big ET-1 in human kidney as a food ingredient. Biochem Pharmacol 1998;55(5):697-701. doi: 10.1016/s0006-2952(97)00515-7.

Gonen, M. Planning for subgroup analysis: a case study of treatmentmarker interaction in metastatic colorectal cancer. Controlled Clinical Trials 2003;24 : 355-363. doi: 10.1016/s0197-2456(03)00006-0.

• Authored Book:

Lodish H, Baltimore D, Berk A, Zipursky SL, Matsudaira P, Darnell J. Molecular cell biology. 3rd ed. New York: Scientific American; 1995.

Millares M, editor. Applied drug information: strategies for information management. Vancouver: Applied Therapeutics, Inc.; 1998.

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The publication of an article in the peer-reviewed journal JNBS is an essential building block in the development of a coherent and respected network of knowledge. It is a direct reflection of the quality of the work of the authors and the institutions that support them. Peer-reviewed articles support and embody the scientific method. It is therefore important to agree upon standards of expected ethical behaviour for all parties involved in the act of publishing: the author, the journal editor, the peer reviewer, the publisher and the society of society-owned or sponsored journals.

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The corresponding author should ensure that all appropriate coauthors and no inappropriate coauthors are included on the paper, and that all co-authors have seen and approved the final version of the paper and have agreed to its submission for publication.

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The Journal of Neurobehavioral Sciences

Volume 10 ¦ Issue 2 ¦ April-August 2023

Contents

Original Article

Zingiber officinale Ameliorates Tramadol-induced Histopathological Distortions in CA1 and CA3 of the Hippocampus of Adult Wistar Rats

Ubong Udeme Ekpo, Uduak Emmanuel Umana, Abubakar Adamu Sadeeq

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Abstract

Background: Tramadol has a high potential for misuse resulting in cognitive impairment. *Zingiber officinale*, however, possesses neuroprotective qualities. **Objective:** Microscopically assessed hippocampal CA1 and CA3 following *Z. officinale* and tramadol treatment. **Materials and Methods:** Two milliliters/kilogram of distilled water was given to Group 1, Groups 2–5 were administered 50 mg/kg of tramadol while Group 3 was also administered 12.5 mg/kg of naltrexone, and Groups 4 and 5 were also administered 500, and 1000 mg/kg ethanol extract of *Z. officinale* (EEZO), respectively, orally for 21 days. The rats were euthanized and their brains were collected, fixed in 10% formal saline, and processed routinely using crystal fast violet (CFV) stain for the demonstration of Nissl substance, glial fibrillary acidic protein (GFAP) for the demonstration of astrocytes, and Hematoxylin and Eosin for general histoarchitecture and estimation of cell number and volume using physical dissector and Cavalieri estimator, respectively. **Results:** CFV stain revealed alterations in regions of CA1 and CA3 of the hippocampus presenting as indistinct staining intensity and peripheral Nissl substance accumulation in the tramadol-treated group. GFAP revealed numerous reactive astrocyte processes. The area of reactive astrocytes remarkably increased ($P < 0.05$) and the intensity of the Nissl substance remarkably reduced in the tramadol-exposed group. When compared to the control, the tramadol-exposed group's hippocampal volume considerably ($P < 0.05$) decreased (coefficient of error [CE] =0.050). The tramadol treatment group (CE = 0.090) relative to the control group (CE = 0.060) showed a striking decrease ($P < 0.05$) in the number of pyramidal cells in the CA3 region. The tramadol treatment group (CE = 0.090) compared to the control group (CE = 0.060) showed a striking decrease ($P < 0.05$) in the number of pyramidal cells in the CA3 region. Tramadol toxicity was attenuated in the groups treated with EEZO in a dose-dependent manner. **Conclusion:** *Z. officinale* possesses a potential neuroprotective effect against tramadol-induced neurotoxicity.

Keywords: *Cornu ammonis, histochemical, immunohistochemical, stereology*

Introduction

Tramadol is a prescription pain reliever mostly used to cure modest to mildly rigorous pain.[1] In addition, it is a preferred analgesic both during and after surgery, with a dose tailored to the patient's level of sensitivity and discomfort.[2,3] There are signs that the prescription opioid tramadol is addictive and prone to abuse.[4-7] Like other opiates, tramadol can cause both mental and physical dependence.[8] Tramadol has the ability to hinder the reuptake of serotonin and norepinephrine neurotransmitters, which can subsequently cause an increase in their levels in the brain. This may lead to oxidative stress and harm to neurons, particularly in the hippocampal region of the brain that is responsible for memory and learning.[9] In addition, tramadol has been discovered to stimulate the N-methyl-D-aspartate receptor, which can result in neuronal damage and excitotoxicity.[10] Moreover, tramadol can activate microglia, immune cells in the brain that produce pro-inflammatory cytokines, contributing to neuroinflammation.[11] Constant tramadol administration may cause the body to accumulate toxic metabolites, raise the contingency of pharmacokinetic interactions, and/or lower the clearance of tramadol, all of which raise the drug's possibility for toxicity.[12] The hippocampus is an outgrowth of the cerebral cortex's

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Ethics committee approval: The Ahmadu Bello University Ethics Committee on Animal Use and Care approved this work in accordance with ethical standards, with approval number ABUCAUC/2022/031.

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temporal region.[13] The hippocampus plays a significant part in memory and learning. It is a malleable and susceptible structure that is injured by many stimuli. Studies have revealed that it is also impacted by several neurological and psychiatric illnesses.[14]

Zingiber officinale has been reported to show neuroprotective effects due to the phenolic and flavonoid compounds present in it.[15] According to published evidence, *Z. officinale* accelerates the brain's antioxidant defense mechanisms and restores normal levels of melonlialdehyde (MDA) in rat models when exerting its neuroprotective effects.[16] Research has been done so far on the detrimental effects of tramadol on the hippocampus's neurotoxicity. Therefore, the purpose of this study was to investigate the effects of tramadol and *Z. officinale* on astrocytes, hippocampal volume, pyramidal cell number, and Nissl substances in adult Wistar rats.

Materials and Methods

This study's ethical endorsement was given by the Ethics Committee on Animal Use and Care, Ahmadu Bello University (ABU), with consent code ABUCAUC/2022/031.

Materials

Plant collection and identification

Locally grown ginger rhizomes were purchased in Samaru, Zaria, Nigeria. These rhizomes were recognized and verified at the ABU, Zaria, Herbarium unit of the Botany Department, Faculty of Life Sciences, where a voucher number (V/N) of ABU02261 was issued.

Preparation of plant extract

Z. officinale's ethanol extract was made in the Pharmacognosy and Drug Development Department, ABU, Zaria. Fresh ginger rhizomes were meticulously rinsed with fresh water to get rid of dirt, and then 2500 g of them were grinded and cold-macerated in 2 L of 70% ethanol while being meticulously agitated on occasion. For the aim of utterly dissolving the active ingredients, the mixture was left alone for 48 h. The extracted material was concentrated using a rotary evaporator and vaporized to dryness on a water bath at 50°C after being initially sieved through mesh cloth and then suction-filtered using Whatman no. 1 filter paper.[17] Before further experimentation and investigation, the dried ginger extracts were appropriately kept in a container.

Phytochemical assessment of ethanol extract of Zingiber officinale (ginger) rhizome

The Pharmacognosy and Drug Development Department, Faculty of Pharmaceutical Sciences, ABU, Kaduna, Nigeria, carried out a phytochemical screening of ethanol extract of *Z. officinale* (EEZO) rhizome. The phytochemical screening approach proposed by Evans[18], Sofowora[19] and Trease and Evans[20] was used.

Acute toxicity study (LD_{50}) of Zingiber officinale

The acute toxicity assessment for EEZO rhizome was carried out using the method of Lorke.[21]

Experimental animals

From the Animal House facility of the Faculty of Pharmaceutical Sciences at ABU, Zaria, Kaduna, Nigeria, 30 male Wistar rats (150–190 g) were collected. Before experimentation, they were moved and kept in wired cages at the Human Anatomy Department, Faculty of Basic Medical Sciences, ABU, Zaria, where they were given 2 weeks to acclimatize. The rats were given pelletized feed and water *ad libitum*.

Drugs

Tramadol

Tramadol hydrochloride (50 mg capsules) is produced by VADIS PHARM. LTD. Plot RD-14-Trans-Ekulu, Enugu State, Nigeria, was bought and used for the study.

Naltrexone

The standard medication for tramadol toxicity in this study was naltrexone (50 mg tablets), which was acquired from Healing Pharma India Pvt. Ltd. Shop No. 01, Plot No.: 25-B, Dev Industrial Estate, Gorwa, Vadodara-16.

Ketamine

For the experiment, ketamine (50 mg/mL ketamine hydrochloride injection USP) was utilized as the anesthetic. It was procured from Swiss Parenterals PVT Ltd., Gujarat, India.

Drugs administration

The dosage of the medication was determined by animal weight and then given.

Experimental design and treatment of animals

For this study, 30 male Wistar rats (150–190 g) were employed. They were shared into 5 groups, each containing 6 rats. As the control, Group 1 received 2 mL/kg of distilled water, while Group 2 received 50 mg/kg of tramadol.[22] Animals in Group 3 were administered tramadol (50 mg/kg) with 12.5 mg/kg of naltrexone. Group 4 animals were given tramadol (50 mg/kg) and 10% LD_{50} of *Z. officinale* (500 mg/kg) and Group 5 administered with tramadol (50 mg/kg) and 20% LD_{50} of *Z. officinale* (1000 mg/kg) orally consecutively for 21 days. The animals were euthanized 24 h after the last dose.

Animal sacrifice

The animals were anesthetized with ketamine and perfused transcardiacally with normal saline and 10% formal saline. After perfusion, the brains were carefully taken out from the skull and rinsed with normal saline. After the brains

were harvested, they were fixed in 10% formal saline for 48 h for proper fixation.

Histopathological studies

Cresyl fast violet

The method of Carson[23] was adopted. Hippocampal sections of the brain were deparaffinized and dehydrated to distilled water. The slides were quickly rinsed in one change of distilled water after being incubated in Cresyl Violet Stain Solution (0.1%) for 2 min. Following a brief dehydration in alcohol (absolute), the sections were immediately cleaned in xylene, mounted using synthetic resin, and cover-slipped with a DPX Mountant.

Glial fibrillary protein analysis

Hsu *et al*.[24] modified avidin-biotin-peroxidase complex method as stated by Yoshida *et al*.[25] was employed in this study. Hippocampal sections were cut into 5 µm-thick slices and preserved in 10% phosphate-buffered formalin for 6 h. The sections were treated with 1/100 anti-glial fibrillary acidic protein (GFAP) (Abcam, UK) primary antibodies, for 90 min after endogenous peroxidase had been blocked. The section was then treated with Mouse/Rabbit HRP (BioSB, Spain) for 45 min. The immunostained slices were dried, cleaned, and mounted in DPX after being mildly counterstained for 1 min with Mayer and Hematoxylin (Dako, Glostrup, Denmark). To perform the sections of the control for the immunohistochemistry of GFAP, the primary antibody was removed, and a nonimmune serum was substituted.

Quantification of Nissl substance distribution and astrocytes reactivity

Cresyl fast violet (CFV), a superb neuronal (cell body specific) stain that helps demonstrate Nissl substance in neurons, was used to assess the amount of Nissl substance in hippocampus pyramidal neurons.[26,27] The staining intensity from the CFV-stained micrographs was measured to quantify the reactivity of Nissl substances (digital micrograph imaging) as specified by the manufacturer.[28] Astrocyte reactivity was also assessed using a computer running image analysis program following instructions as recommended by the manufacturer (Image J, NIH, US).[28] Prejudice values arising from nonidentical picture quality (image acquisition setting) were reduced using the imageJ region of interest management device for an assessment of certain parts of the micrographs.

Stereological studies

Pyramidal cell number

To determine the pyramidal cell number, the hippocampus of the Wistar rat per group was isolated, processed, and sectioned at 5 µm after a pilot study on how many slices could be derived. Tissue sections of the hippocampus were designated using a systematic uniform random sampling method. The sections derived were stained using Hematoxylin and Eosin stain (H and E). Using the physical fractionator method described by Yurt *et al*.,[29] an unbiased estimate of the number of pyramidal cells in the regions of CA1 and CA3 of the Wistar rat was obtained. A transparent counting frame with an acceptance and rejection region was applied to the two succeeding sections to tally the quantity of pyramidal cells. The "look up" section was obtained from one of the section planes, while the "reference" section was taken from the other. Pyramidal cells were selected for the reference part, and counting was done in the look-up section. Pyramidal cells in the reference area but not in the lookup area were counted as the presence of the particle.[30-32] The sum-up of number of pyramidal cells was subsequently estimated using the formula:

$$N = Nv.\ V\ (ref)$$

$$\text{Where } Nv = \frac{\sum Q}{10.\ V\ (dis)}$$

$$V\ (dis) = \frac{\text{T. area of frame} \sum Q}{400.400\ (\text{Final Magnification})}$$ [32]

$$= \text{total number of cells counted}$$

Final Magnification = 400

Reference volume V (ref) was derived from the estimated volume calculated.

The coefficient of error (CE) was calculated as follows:

$$CE = \frac{SEM}{MEAN}$$

Where standard error of the mean (SEM) = $\frac{\sqrt{\text{Total variance}}}{\sum p}$

n = 10 (number of hippocampal sections).

Volume estimation

The absolute volume of the hippocampus was estimated using the Cavalieri estimator for volume estimation following the technique suggested by West *et al*.[33] Following a pilot study on the number of slices that could be derived and the number of slices that will result in the lowest CE, the hippocampus of the rats was isolated, processed, and sectioned serially using a microtome (Biobase Biodustry, Shandong, China), as stated by Gundersen and Jensen.[34] A method of systematic uniform random sampling was used to choose tissue samples from the hippocampus. H and E was used to stain the sections that were produced. Each hippocampus slice has a clear counting grid randomly placed on its surface. It was recorded how many points struck the hippocampus. Using Cavalieri's method,[35] the volume was calculated as follows:

$V = T.(a/p).\Sigma p$

Where ": =" indicates that the result is the estimated value rather than the true value, "V" is the total volume of the hippocampus, "T" = 0.05 mm is the average slice thickness, "a/p" ¼ is the area associated with each point in the counting grid (4 mm^2), and "Σp" is the total number of points hippocampus.

The CE was calculated as follows:

$$CE = \frac{\sqrt{\text{Total variance}}}{\sum p}$$

Statistical analysis

The data's standard error of the mean was displayed. The differences between and within the groups were assessed using the Kruskal–Wallis test and Dunn's *post hoc* test, respectively. In addition, analysis of variance and the Tukey *post hoc* test were used. The statistical significance was determined at $P \geq 0.05$. Utilizing statistical tools and services (IBM SPSS 26, Chicago and incorporated in Delaware), the data were evaluated.

Results

Acute toxicity analysis

The percentage yield of EEZO was calculated to be 1.72%. The acute toxicity assessment (LD_{50}) of EEZO was calculated to be above 5000 mg/kg bw. No visible behavioral changes were observed.

Phytochemical analysis

Phytochemical analysis of EEZO revealed positive and negative reactions for primary and some secondary metabolites: cardiac glycoside, flavonoids, saponins, tannins, tripenoids, carbohydrates, alkaloids, steroids, phenolic compounds were present, while anthraquinone was not seen.

Histochemical studies

Effects of ethanol extract of Zingiber officinale, naltrexone, and tramadol on Nissl substance

A histochemical study using CFV stain revealed the normal appearance of distinct intensely stained CA1 and CA3 regions in the control group. Regions of CA1 and CA3 of Group II treated with only tramadol (50 mg/kg) showed indistinct staining intensity with pathological changes such as karyolysis, cytoplasmic vacuolation, perineural vacuolation, dark neurons, and chromatolysis [Figures 1 and 2]. Group III (tramadol + naltrexone 12.5 mg/kg) revealed the reduced staining intensity of CA1 and CA3 with mild distortions in the histochemistry of the hippocampus presenting as chromatolysis [Figures 1 and 2]. Group IV treated with tramadol + 500 mg/kg of EEZO showed reduced indistinct staining intensity of the hippocampi regions (CA1 and CA3) with mild distortions in the histochemistry of the hippocampus presenting as karyolysis and chromatolysis while Group V (tramadol + 1000 mg/kg of EEZO) revealed reduced indistinct staining intensity of the hippocampi regions (CA1 and CA3) with mild distortions in the histochemistry of the hippocampus presenting as chromatolysis [Figures 1 and 2].

Effects of ethanol extract of Zingiber officinale, naltrexone, and tramadol on Nissl substance quantification

There was a remarkable decrease ($P < 0.05$) in the staining intensity of Nissl substance in Groups II (50 mg/kg of tramadol), III (tramadol + 12.5 0f naltrexone), and IV (tramadol + 500 mg/kg of EEZO) relative to the control. A notable increase ($P < 0.05$) in the intensity of Nissl substance was observed in Group V (tramadol + 1000 mg/kg of EEZO) in relation to the tramadol-treated group [Figure 3].

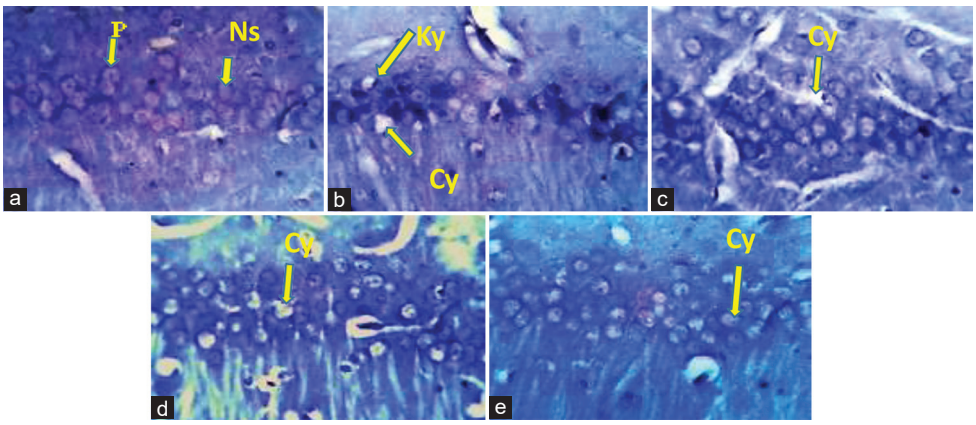

Figure 1: Photomicrograph of CA1 region of the hippocampal section of Wistar rat (CFV × 250). (a) Group I (2 mL/kg of distilled water) with normal appearance of distinct intensely stained CA1 and CA3 regions. Pyramidal cells (P); Nissl substance (Ns). (b) Group II (50 mg/kg of tramadol) showing indistinct staining intensity of CA1 region. Pyramidal cells (P); Karyolysis (Ly); Chromatolysis (Cy). (c) Group III (50 mg/kg of Tm + 12.5 mg/kg of Nalt) showing reduced staining intensity of CA1 region. Chromatolysis (Cy). (d) Group IV (50 mg/kg of Tm + 500 mg/kg of EZO) with reduced indistinct staining intensity of CA1 region. Chromatolysis (Cy). (e) Group V (50 mg/kg of Tm + 1000 mg/kg of EZO) showing reduced indistinct staining intensity of CA1 region. Chromatolysis (Cy). CFV: Crysl fast violet

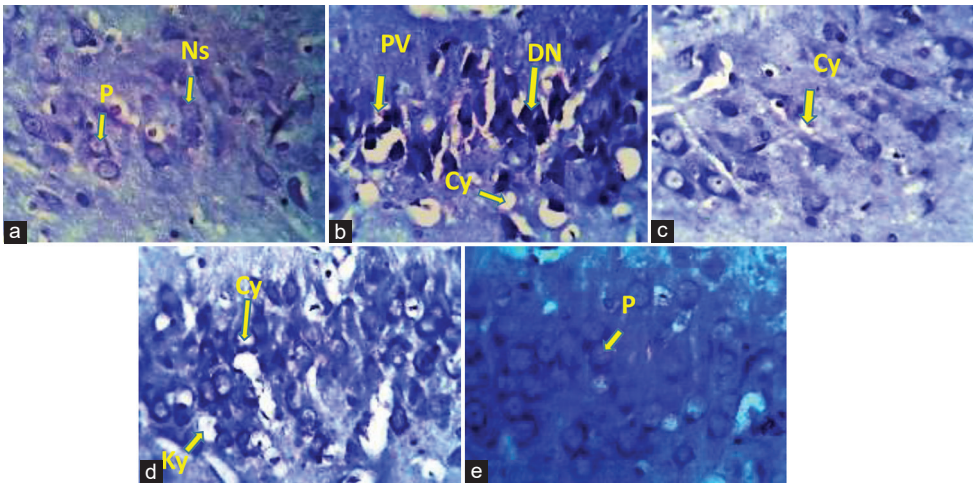

Figure 2: Photomicrograph of CA3 region of the hippocampal section of Wistar rat (CFV × 250). (a) Group I (2 mL/kg of distilled water) with normal appearance of distinct intensely stained CA3 region. Pyramidal cells (P); Nissl substance (Ns). (b) Group II (50 mg/kg of tramadol) showing the indistinct staining intensity of CA3 region. Cytoplasmic vacuolation (CV); Dark Neuron (DN); Perineural Vacuolation (PV). (c) Group III (50 mg/kg of Tm + 12.5 mg/kg of Nalt) showing reduced staining intensity of CA3 region. Chromatolysis (Cy). (d) Group IV (50 mg/kg of Tm + 500 mg/kg of EZO) with reduced indistinct staining intensity of CA3 region. Karyolysis (Ky); Chromatolysis (Cy). (e) Group V (50 mg/kg of Tm + 1000 mg/kg of EZO) showing reduced indistinct staining intensity of CA3 regions. Pyramidal cells (P). CFV: Crysl fast violet, EZO: Ethanol extract of *Zingiber officinale*

Immunochemical studies

Effects of ethanol extract of Zingiber officinale, naltrexone, and tramadol on reactive astrocytes

The control group displayed a confined presence of GFAP-immunoreactive astrocytes that had a regular spatial layout, size, and dark brown cytoplasmic fibers, constituting an orderly network arrangement in the neuropil, as observed during the astrocyte immunohistochemical demonstration using the GFAP stain. These astrocytes also had normal astrocyte processes that did not overlap. The majority of the astrocytes in the CA1 and CA3 areas of the hippocampal region do not exhibit measurable levels of GFAP expression. Group II treated with only tramadol (50 mg/kg) revealed numerous reactive astrocyte processes; extensive overlapping and interdigitation of astrocytes processes; astrocytes proliferation; and astrocyte cell bodies hypertrophy and thickening of astrocytes processes. Group III treated with tramadol and naltrexone (12.5 mg/kg) revealed moderately reactive astrocytes processes; some astrocytes processes overlapping and moderate astrocytes hypertrophy. Group IV treated with tramadol + 500 mg/kg of EEZO revealed less expression of detectable levels of astrocytes and astrocytes processes which do not overlap while Group V treated with tramadol 50 mg/kg + 1000 mg/kg of EEZO revealed moderate reactive astrocytes processes and moderate astrocytes hypertrophy [Figures 4 and 5].

Effects of ethanol extract of Zingiber officinale, naltrexone, and tramadol on quantification of reactive astrocytes

There was a remarkable increase ($P < 0.05$) in the area of reactive astrocytes in the tramadol-exposed group in relation to the control. While remarkable decrease ($P < 0.05$) in the area of reactive astrocytes was detected in groups IV (tramadol + 500 mg/kg of EEZO) and V (tramadol + 1000 mg/kg of EEZO) in relation to the tramadol exposed group [Figure 6].

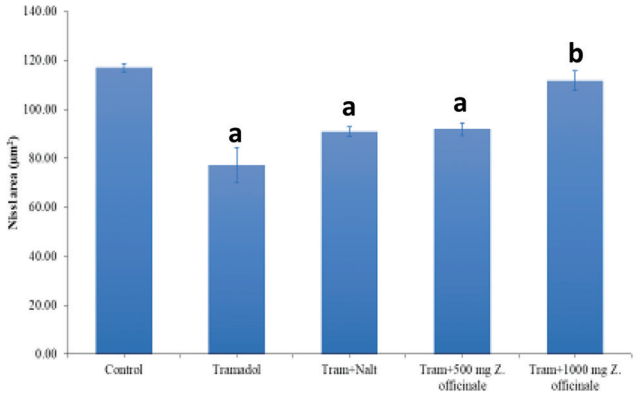

Figure 3: Quantification of Nissl substance reactivity of Wistar rats following oral administration of tramadol and *Zingiber officinale*. *n* = 6; mean ± SEM, one-way ANOVA, *Tukey post hoc test*, a = $P < 0.05$ when compared to control, and b = $P < 0.05$ when compared to tramadol treated group. Control = 2 mL/kg of H_2O, Tram = 50 mg/kg of Tramadol, Nalt = 12.5 mg/kg of Naltrexone, EZO: Ethanol extract of *Zingiber officinale*, SEM: Standard error of the mean, ANOVA: Analysis of variance

Effects of ethanol extract of Zingiber officinale, naltrexone, and tramadol on hippocampal volume

The volume of the hippocampus remarkably decreased ($P < 0.05$) in Groups II (50 mg/kg of tramadol) (CE = 0.050), Group III (tramadol + 12.5 mg/kg of naltrexone) (CE = 0.053), Group IV (tramadol + 500 mg/kg of EEZO) (CE = 0.051), and Group V (tramadol + 500 mg/kg of EEZO) (CE = 0.059) in relation to the control. Furthermore, there was a remarkable decrease in the hippocampal volume of Group III (CE = 0.053) and Group V (CE = 0.059) in relation to tramadol exposed group [Figure 7].

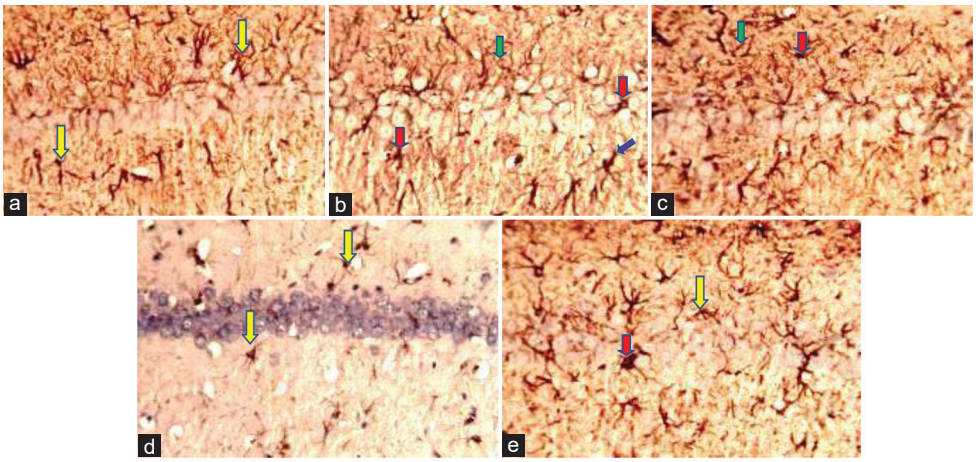

Figure 4: Photomicrograph of CA1 region of the hippocampal section of Wistar rat (GFAP × 250). (a) Group I (2 mL/kg of distilled water) with few expressions of detectable levels of astrocytes (arrows); no overlapping of astrocytes processes. (b) Group II (50 mg/kg of tramadol) with numerous reactive astrocytes processes; extensive overlapping and interdigitation of astrocytes processes (green arrow); astrocytes proliferation; astrocytes cell body hypertrophy (red arrows); thickening of astrocytes processes (purple arrow). (c) Group III (50 mg/kg of Tm + 12.5 mg/kg of Nalt) with moderately reactive astrocytes processes; some astrocytes processes overlapping (green arrow); astrocytes cell bodies hypertrophy (red arrow). (d) Group IV (50 mg/kg of Tm + 500 mg/kg of EZO) with less expression of detectable levels of astrocytes; astrocytes processes are not overlapping. (e) Group V (50 mg/kg of Tm + 1000 mg/kg of EZO) with moderate reactive astrocytes processes (yellow); some astrocytes hypertrophy (red). GFAP: Glial fibrillary acidic protein, EZO: Ethanol extract of *Zingiber officinale*

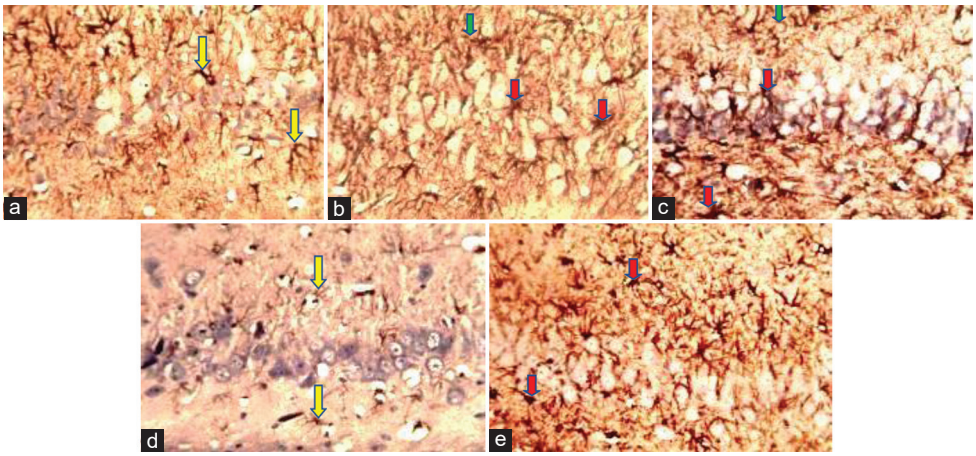

Figure 5: Photomicrograph of CA3 region of the hippocampal section of Wistar rat (GFAP × 250). (a) Group I (2 mL/kg of distilled water) with few expressions of detectable levels of astrocytes (arrows); no overlapping of astrocytes processes. (b) Group II (50 mg/kg of tramadol) with numerous reactive astrocytes processes; extensive overlapping and interdigitation of astrocytes processes (green arrow); astrocytes proliferation; astrocytes cell body hypertrophy (red arrows). (c) Group III (50 mg/kg of Tm + 12.5 mg/kg of Nalt) with moderately reactive astrocytes processes; some astrocytes processes overlapping (green arrow); astrocytes cell bodies hypertrophy (red arrows). (d) Group IV (50 mg/kg of Tm + 500 mg/kg of EZO) with less expression of detectable levels of astrocytes; astrocytes processes are not overlapping. (e) Group V (50 mg/kg of Tm + 1000 mg/kg of EZO) with moderate reactive astrocytes processes (yellow); some astrocytes hypertrophy (red). EZO: Ethanol extract of Zingiber officinale, GFAP: Glial fibrillary acidic protein

Effects of ethanol extract of Zingiber officinale, naltrexone, and tramadol on pyramidal cells number

There was a remarkable decrease ($P < 0.05$) in the pyramidal cells number in the CA1 region of the hippocampus of Group III (tramadol + 12.5 mg/kg of naltrexone) (CE = 0.043) and Group V (tramadol + 1000 mg/kg EEZO) (CE = 0.074) in relation to the control. A remarkable increase in the cells number in the CA1 region was detected in Group IV (tramadol + 500 mg/kg EEZO) (CE = 0.048) when compared to Group III and a remarkable decrease in Group V (tramadol + 1000 mg/kg EEZO) (CE = 0.074) in relation to Group IV [Figure 8]. In the CA3, a remarkable decrease ($P < 0.05$) in the number of pyramidal cells was detected in the tramadol-exposed group (CE = 0.090) in relation to the control (CE = 0.060) [Figures 8 and 9].

Discussion

The central nervous system's tissue and activities are impacted by tramadol, which readily penetrates the blood-brain barrier.[35,36] Given that it uses a lot of oxygen, elevated quantities of polyunsaturated fatty acids, and antioxidant concentrations that are not too high, the brain is predominantly prone to oxidative damage.[37] The evidence in favor of *Z. officinale's* neuroprotective properties points

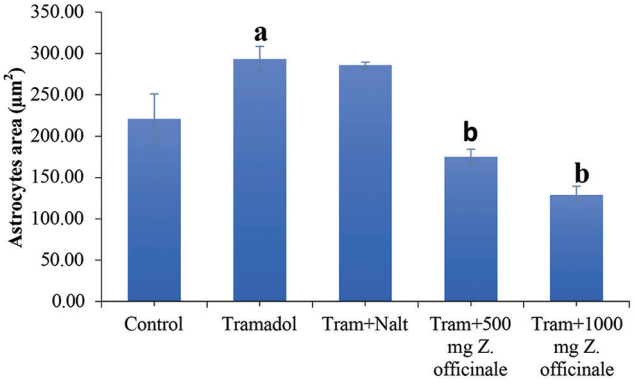

Figure 6: Area of reactive astrocytes of Wistar rats following oral administration of tramadol and *Zingiber officinale*. *n* = 6; Kruskal–Wallis, *Dunn's post hoc test*, a = *P* < 0.05 when compared to control, and b = *P* < 0.05 when compared to tramadol group. Control = 2 ml/kg of H_2O, Tram = 50 mg/kg of Tramadol, Nalt = 12.5 mg/kg of Naltrexone, EZO: Ethanol extract of *Zingiber officinale*

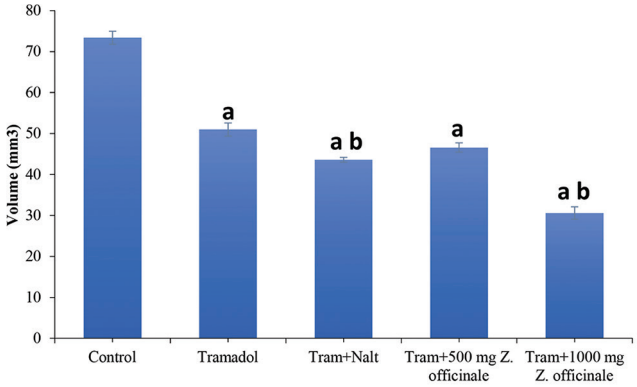

Figure 7: Hippocampal volume of Wistar rats following oral administration of tramadol and *Zingiber officinale*. *n* = 6; mean ± SEM, one-way ANOVA, *Tukey post hoc test*, a = *P* < 0.05 when compared to control, and b = *P* < 0.05 when compared to tramadol group. Control = 2 mL/kg of H_2O, Tram = 50 mg/kg of Tramadol, Nalt = 12.5 mg/kg of Naltrexone, EZO: Ethanol extract of *Zingiber officinale*, SEM: Standard error of the mean, ANOVA: Analysis of variance

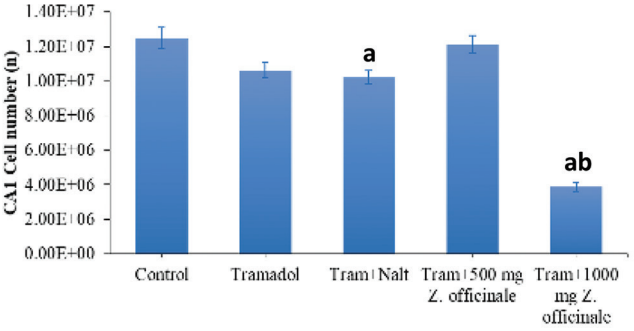

Figure 8: CA1 pyramidal cell number of Wistar rats following oral administration of tramadol and *Zingiber officinale*. *n* = 6; mean ± SEM, one-way ANOVA, *Tukey post hoc test*, a = *P* < 0.05 when compared to control, and b = *P* < 0.05 when compared to tramadol group. SEM: Standard error of the mean, ANOVA: Analysis of variance

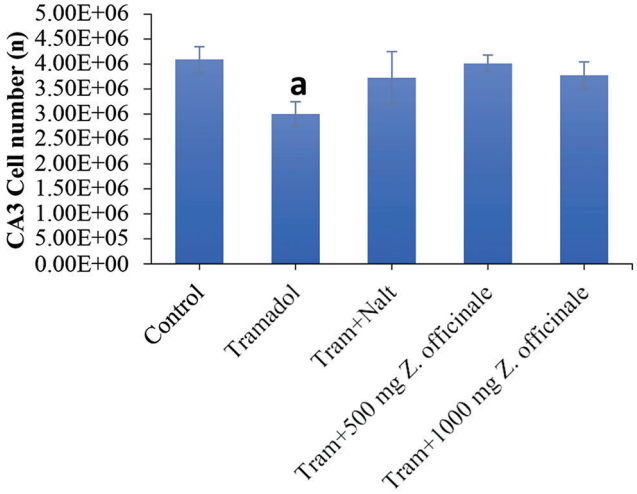

Figure 9: CA3 pyramidal cell number of Wistar rats following oral administration of tramadol and *Zingiber officinale*. *n* = 6; Kruskal–Wallis, *Dunn's post hoc test*, a = *P* < 0.05 when compared to control group. Control = 2 mL/kg of H_2O, Tram = 50 mg/kg of Tramadol, Nalt = 12.5 mg/kg of Naltrexone, EZO: Ethanol extract of *Zingiber officinale*

to the possibility that this plant accelerates the brain's natural anti-oxidant defenses while bringing MDA levels in rat models back to normal.[16] This study investigated the protective effects of EEZO on Nissl substance, reactive astrocytes, the quantity of pyramidal cells in the regions of CA1 and CA3 of the hippocampus, and the hippocampal volume in adult Wistar rats given tramadol.

Phytochemical screening of EEZO revealed that it contains tannins, flavonoids, saponins, steroids, cardiac glycoside, carbohydrates, alkanoids, tripenoids, phenolic compounds, and the absence of anthraquinone. This result is similar to several other studies which revealed positive and negative reactions for the above secondary metabolites.[38-42] *Z. officinale* and its components are crucial neuroprotectants. *Z. officinale*'s precise mode of operation in this view is not entirely understood. Nonetheless, it is believed to have neuroprotective properties as a result of the phenolic and flavonoid components.[43] Flavonoids are hypothesized to improve cognitive performance by increasing neuronal function, inducing neurogenesis, and having neuroprotective qualities.[44,45] They are capable of crossing the blood–brain barrier and have been detected in areas of the rat brain linked with memory and learning soon after being orally administered.[46,47] Numerous epidemiological studies indicate that consuming foods or supplements high in plant-derived polyphenols may halt the development and progression of neurodegenerative diseases.[48,49] Another discovery that supports *Z. officinale's* ability to act as a neuroprotective agent reveals that it has this ability by boosting the anti-oxidant defense mechanisms of the brain and bringing MDA levels back to standard in rat models.[16] Gingerol and derivatives of *Z. officinale* are reported to be effective at scavenging hydroxyl and superoxide radicals.[50-52] The protective impact of Z. officinale on rat models was observed by Sharma and Singh,[53] who reported that it diminishes LPO while increasing QR, SOD, GPx, GSH, GST, GR, CAT, and protein levels.

Histochemical assessment of the hippocampus (CA1 and CA3 regions) using CFV stain in this study, revealed histopathological changes such as karyolysis, cytoplasmic vacuolation, dark neuron, perineural vacuolation, chromatolysis and indistinct staining intensity of the hippocampi in tramadol-treated group. However, these histopathological changes were attenuated in the extract-treated groups in a dose-dependent manner. The loss of Nissl bodies and resulting decreased staining intensity of the Nissl bodies in the tramadol group, which are both signs of neuronal degeneration, could be attributed to the detrimental effects of tramadol administration, which suggests that opioids may be involved in neurodegeneration.

Neuronal degeneration leads to a decrease in Nissl granules.[54] The Nissl substance is affected by both chemical and toxic agents, which interfere with its metabolic function.[55] Awadalla and Salah-Eldin[56] reported alterations in Nissl granules in the cerebral cortex and hippocampus, where most neurons showed pale blue-colored granules at the rims of cytoplasm, indicating a decrease in Nissl substance. A study on the administration of both tramadol and alcohol in the medial prefrontal cortex of young male rats revealed alterations in the distribution of Nissl substance, suggesting either neuronal degeneration or impaired metabolism.[57] *Z. officinale* has the potential to increase the number of intracellular Nissl granules and neurons in the hippocampus, according to Jesudoss *et al*.[58] In a study on protection against Alzheimer's disease-induced behavioral impairment in rats, *Z. officinale* root extract improved Nissl granules in the rats' brains.[59]

According to Yang and Wang,[63] GFAP, a significant protein in glial filaments within astrocytes, which is responsible for supporting the cytoskeleton, can be a potential indicator of brain damage. Studies show that these protein biomarkers play a key role in observing brain injury and prognoses in individuals with traumatic brain injury or concussion.[61-63]

Hence, this report also studies the effect of *Z. officinale* extract on the expression of astrocytes using a GFAP stain. Exposure of rats to 50 mg/kg of tramadol resulted in numerous reactive astrocytes processes; extensive overlapping and interdigitation of astrocytes processes; astrocytes proliferation; astrocytes cell body hypertrophy and thickening of astrocytes processes. In addition, there was a notable upsurge ($P < 0.05$) in the area of reactive astrocytes in this group. The groups that were treated with the extract exhibited a reduction in the pathological changes brought on by tramadol toxicity. This discovery corresponds with the outcomes of the study performed by Adekomi *et al*.,[67] which evaluated the impact of concurrent treatment with alcohol and tramadol on the mental abilities and neuroinflammatory responses of male rats' medial prefrontal cortex in their early stages of development. Giving young rats 60 mg/kg twice daily for 30 days resulted in numerous reactive astrocyte processes and significant losses of neurons. Barbosa *et al*.[64] detected an upsurge in the expression GFAP in the cerebral cortex following varying doses of opioids. In another study, astrocytes showed enlarged cell bodies and thickened processes after intraperitoneally administering 50 mg/kg/day of tramadol for 4 weeks, with an increase in GFAP expression.[65]

GFAP overexpression is considered to be a factor in opioid tolerance and dependence, as supported by numerous studies.[66-68] Chronic drug misuse is believed to cause astrogliosis, which is a distinctive immune response to neurotoxicity and brain injury that can alter synaptogenesis and neurogenesis, or result in apoptosis and/or necrosis.[69,70] The increased GFAP expression found in this study is consistent with the histopathological evidence of glial proliferation and hypertrophy, which are themselves a response to opioid-induced damage. El-Akabawy and El-Kholy[70] demonstrated in their study on the neuroprotective effects of *Z. officinale* on the brains of streptozotocin-induced diabetic rats that ginger's therapeutic effects were facilitated by controlling the astroglial response to injury, bringing down AChE expression, and enhancing neurogenesis. Torkzadeh-Mahani *et al*.[68] found that *Z. officinale* extract significantly reduced GFAP levels to control levels following morphine administration. Another study reported a significant improvement in GFAP expression induced by nicotine following treatment with both ginger and cinnamon oil,[71] which is similar to the current study. The results of a study stipulated that the combined aqueous extract of ginger and garlic resulted in a reduction in GFAP expression after the administration of lead acetate.[72] Mabrouk *et al*.[73] observed moderately immune-reactive astrocytes in mice administered with Topamax and Ginger oil in sections from the animals.

Nowadays, in medical sciences, estimation of volume and cell number aids in diagnosis and therapy by studying tissues, cells, organelles, and the spatial placement of cells during the disease and treatment with advanced microscopic calculations. Also, it added to the advancement of science in the field of histology during health and disease through the study of, surface, length, volume, and number of biological objects.[32] In this present study, we estimated hippocampal volume and pyramidal cells number following treatment with tramadol and *Z. officinale*. Findings from this study showed a notable reduction ($P < 0.05$) in hippocampal volume and number of pyramidal cells in the region of CA3 in tramadol exposed group in relation to the control. Results from this study indicate that the pyramidal cell number in the hippocampus was moderately compensated by *Z. officinale*. The loss of pyramidal cells seen in this study may be attributed to apoptosis, which results in cellular death.[74] Programmed cell death, also known as

apoptosis, is an active process of natural cell death that takes place during development as well as a response to the cytotoxic effects of certain neurotoxins.[75] Long-term opioid use may cause structural changes and neuronal death.[71] Numerous investigations have demonstrated a connection between prolonged opioid use and elevated reactive oxygen species (ROS) generation.[76-81]

Oxidative stress is a result of an imbalance between the body's ability to produce ROS and its capacity to eliminate them. Large doses of reactive intermediary cause the breakdown of cellular constituents and the production of dangerous compounds. Large amounts of the reactive intermediate lead to the breakdown of cellular constituents and the production of subsequent dangerous compounds. Nuclear factor-kappaB (NF-B), a redox-sensitive transcription factor, can be activated by ROS. NF-B then causes the transcription of inflammatory genes and the production of several inflammatory mediators.[54] In addition to producing ROS, exposure to opioid receptor agonists has been related to stimulate apoptotic processes.[82]

The findings from this present study are similar to that of Ezi *et al*.[83] who compared the experimental group to the control group and observed a notable reduction in the amount of white matter, Purkinje neurons, molecular layer, and granular layer in the cerebellum. Thus, the finding from the study revealed that tramadol treatment resulted in cerebellar atrophy in rats. Another study also reported that the tubular diameter and epithelial height mean values significantly decreased after using tramadol for 30 days.[84] Rafati *et al*.[85] observed that the motor prefrontal cortex's volume and its divisions were found to have dropped by about 15%, its total number of neurons by about 44%, and its whole number of oligodendrocytes by about 41% in the morphine-treated group compared to that in the saline group (P = 0.05). Adekomi *et al*.[60] also reported a remarkable decrease in the number of normal neurons and the number of degenerating neurons in the motor prefrontal cortex was raised.

In vitro and *in vivo* research has demonstrated the powerful antioxidant effects of *Z. officinale*. With its high antioxidant activity, it has a strong impact on scavenging DPPH radicals and regulating the peroxidation of lipids.[86] Bordbar *et al*.[87] reported that *Z. officinale* enlarged the volumes of the seminiferous tubule in the 100 mg/kg treatment group relative to the control group, based on stereotypical studies on the herb's impact on the testicles in rats with busulfan-induced infertility.

Conclusion

The findings of this investigation showed that *Z. officinale* has the propensity to preserve the structures and cells of the nervous system. To manage and cure opiate-induced neurodegeneration, it may be employed.

Patient informed consent

There is no need for patient informed consent.

Ethics committee approval

The Ahmadu Bello University Ethics Committee on Animal Use and Care approved this work in accordance with ethical standards, with approval number ABUCAUC/2022/031.

Financial support and sponsorship

No funding was received.

Conflict of Interest

There is no conflict of interest to declare.

Author Contributions subject and rate

- Ekpo Ubong Udeme (40%): Design the research, data collection, and analyses.
- Umana Uduak Emmanuel (30%): Supervision and research organization.
- Abubakar Addamu Sadeeq (30%): Supervision and research organization.

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Review Article

Risk of Tumor Necrosis Factor Alpha Inhibitors Usage and Related Adverse Effects

Abstract

Tumor necrosis factor (TNF) plays a role in host cell defense. TNF-alpha (TNF-α), secreted from macrophage has an important role in proinflammatory response mechanism. TNF-α levels increase in autoimmune, systemic inflammatory diseases, especially rheumatological diseases. Therefore, TNF-α inhibitors are alternant in the treatment of many inflammatory diseases. TNF-α inhibitors are not the first choice of clinicians due to their important adverse effects, despite the fact that successful results in diseases treatments. Treatment with TNF-α inhibitors causes different adverse effects including many bacterial, viral and fungal infectious diseases, lung diseases, demyelinating diseases, and malignancies. One of the most important adverse effect is tuberculosis (TB) by *Mycobacterium tuberculosis bacillus*. TB occurs through reactivation in Latent TB infection. Thus, TB screening tests appliedbefore TNF-α inhibitors treatment have an importance. In this review, TNF-α inhibitors and their important adverse effect TB flaming were discussed, and also genetic background features of these molecules have been explained.

Keywords: *Adverse effect, tuberculosis, tumor necrosis factor-alpha inhibitors, tumor necrosis factor-alpha*

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Introduction

Inflammatory and autoimmune diseases are very common. Substantial treatment options are sometimes being insufficient to cure diseases. Recent years new treatment options have been applied in patients that did not respond to substantial treatment. The technological advancement on DNA hybrid subject led to diverse biologicals which aims especially tumor necrosis factor (TNF)-alpha. The body produces TNF and it takes place on normal inflammatory and immune responses. Cytokines such as interleukin (IL)-1, IL-6, IL-8, platelet-derived growth factor-B, eicosanoids, platelet-activating factors, and granulocyte monocyte colony-stimulating factor that induce both autocrine and paracrine cell signaling systems. Anti TNF-alpha (TNF-α) therapy reduces the capacity of monocytes to generate proinflammatory cytokines and also stimulates an alteration to generate more anti-inflammatory TH2 cytokine. Transforming growth factor ß which is generated by diverse cell types such as T-cells and monocytes, soluble TNF receptor (TNFR)-1 that stimulates apoptosis by reverse TNF signaling and autocrine transforming growth, soluble IL-1 receptor, IL-4, IL-10, IL-11, IL-13, and IL-16 are anti-inflammatory cytokines. On the advancement on biologicals against TNF-α, therapeutic access to inflammatory diseases has significantly altered.[1] The most used TNF-α inhibitors in clinical medicine include infliximab, adalimumab, etanercept, golimumab, and certulizumab pegol. These inhibitors were thought as a new option for the cure of inflammatory and autoimmune diseases.[2] However, over time, it has been seen that, besides their therapeutic properties, they have unwanted side effects.

Tumor Necrosis Factor-alpha and Molecular Function

TNF- α is a polypeptide cytokine with autocrine and paracrine properties. It is generated mainly by monocytes and macrophages. It is believed that tumor necrotizing activity was caused by endotoxin and the concept of TNF-α was first defined in 1975. Two structurally

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different cytokines were found in 1985. These are macrophage derived TNF and lymphocyte-derived lymphotoxins. These cytokines were subsequently named TNF-α and TNF-beta, respectively, and associated with these cytokines, 17 more ligands were identified, together with the concept of the TNF superfamily. Pro-inflammatory activity, apoptosis, proliferation, morphogenetic changes, and differentiation are the important physiological features of the TNF superfamily, including TNF-α [Table 1].[3,4]

TNF-α's structure is different from other cytokines and provides lipopolysaccharide inactivation. TNF-α is mostly secreted by activated natural killer, activated macrophages, mast cells, and also antigen-stimulated T-lymphocytes. Besides that, it was seen that TNF-α is less secreted by glomerular mesangial cells, fibroblasts, endothelial cells, Kupffer cells, and astrocytes.[5,6]

There are two forms of TNF-α as membrane-embedded protein (transmembrane) and soluble cytokine. TNF-α interacts with two receptors, TNFR-I and TNFR-II. Although TNFR-I is widely found in the body, TNFR-II is predominantly found in immune system, nervous system, and endothelial cells. TNFR-I is activated by the soluble ligand (soluble TNF-α), while TNFR-II is mostly activated by the membrane-resident form (transmembrane TNF-α). TNF-α regulates the local inflammation through cell-to-cell contact with cell specific manner through binding target cells receptors.[7-9]

Transmembrane TNF-α has played essential act in host defense against various viral, parasitic, and bacterial infections such as Human Immunodeficiency Virus (HIV), *Listeria monocytogenes*, *leishmania* and *Mycobacterium tuberculosis*, as well as fine-tuning the immune response. It shows activity against acute infection of tuberculosis (TB) by initiating T-cell and macrophage migration and granuloma formation. Soluble TNF-α, which is proteolytically separated from the transmembrane form by TNF-α converting enzyme, also shows pro-inflammatory and apoptosis triggering effects after cleavage. The soluble form of TNF-α is delivered in reply to lipopolysaccharides, other bacterial products, and IL-1 and mediated pathological responses including autoimmune, neurological, cardiovascular, metabolic diseases, cancer, and lung diseases.[8,10-13] Many kinds of cells discharge TNF after that, it keeps going to induce other immune cells. Macrophages, dendritic cells, T-cells, fat cells, and fibroblasts release TNF and TNF influence various notable cells. Especially, it will make a difference to cells which line our blood vessels that give reason to vascular problems such as angiogenesis and hypervascularization. Shanmugam *et al*. also stated that TNF-α has a significant role in many diseases linked with oxidative stress, concluding cancer, diabetes, cardiac hypertrophy, and cardiomyopathy. In medicine, over 5 anti-TNF drugs are now being utilized and normally behave as deactivating antibodies or a soluble TNFR. It has been shown that chronical use of TNF-α blockers and anti-TNF-α therapies is destructive in diverse cells and organ systems and is united with threat of improving cancer, demyelinating irregularities, and cardiovascular complexities. TNF-α inhibition cause adverse effects are not clear. One probability is that extended TNF-α blockade may push Reactive oxygen species generation down the threshold which is wanted for physiological regulation of the Kelch like ECH associated protein 1 (Keap1)/Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. The expression of Nrf2 regulated proteins (NAD [P] H Dehydrogenase, Quinone 1 [NQO1], Heme oxygenase-1 [HO-1], Glucose-6-phosphate dehydrogenase [G6PD]) were considerably down regulated in hearts of the Double knock out (DKO). *In vivo* experiments with TNFR1/2-DKO show that the expression of Nrf2-regulated proteins (NQO1, HO-1, G6PD) were considerably down regulated in hearts of the DKO when crosschecked to wild type mice pointing out a weakened antioxidant system under basal conditions. Through, these effects point out that TNF-α display has a bimodal impact on the Keap1/Nrf2 system and when a condensed inflammatory activation pushes down the expression of antioxidant proteins at a low grade come in sight to be preventive. In this research, it is also demonstrated that the activity of Nrf2 and its nuclear translocation enhanced by exposure of TNF-α to cells at concentrations well below the threshold linked with sub-inflammation. Therefore, the transcriptional induction of Nrf2 and its next goals takes place in response to a low-dose of TNF-α, whereas concentrations higher than 10 ng/mL were significantly suppressive and linked with cell death. Other researchment has demonstrated that an exact neutralization gave reason to damaged immune function and enhanced threat of cancer more over to these, cardiovascular difficulties.[14] TNF also negatively influences intestinal cells, giving reason to cell death, leaky gut, Irritable bowel syndrome and Inflammatory bowel disease. TNF induces macrophages and effector T cells that cause more inflammatory cytokine production and apoptosis resistance that attends to cancer.[15]

TNFR1 and TNFR2 are the two main regulatory factors of TNF-α. TNFR1 activation initiates inflammatory and apoptotic mechanisms, whereas TNFR2 activation

Table 1: Physiological roles of tumor necrosis factor superfamily members (Aggarwal *et al*., 2012)

Morphogenesis	Proliferation	Apoptosis
TNF-alpha	TNF-alpha	TNF-alpha
EDA-A1	TNF-beta	TNF-beta
EDA-A2	LT-beta	CD40L
CD40L	CD27L	CD30L
TRAIL	RANKL	FasL
FasL	BAFF	VEGI

TNF: Tumor necrosis factor, EDA: Ectodysplasin A, TRAIL: TNF-related apoptosis-inducing ligand, FasL: Fas Ligand, RANKL: Receptor activator of NF-κB ligand, BAFF: B-cell activating factor receptor, VEGI: Vascular endothelial growth inhibitor

initiates anti-inflammatory and cytoprotective and repair mechanisms of cellular processes. Soluble TNF-α interacts with TNFR1 receptor and induces apoptosis mechanism via mitogen activated protein kinase, Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), Phosphoinositide 3-kinase signaling and caspases cascade. In turn, these cascades initiate cellular oxidative stress, necrosis and/or apoptosis.[16]

When TNF binds to a receptor, varied dissimilar facts can occur, depending on the cell type and receptor type. Transcription factor NF-κB, which controls cell survival and inflammatory response, gets activated. Primarily, TNF alpha has been aforethought as a pro-inflammatory molecule. On the other hand, preclinical and clinical instructions have demonstrated that it also remotes a paradoxical anti-inflammatory and immunomodulatory effect [Figure 1].[17-19]

Tumor necrosis factor-alpha inhibitors

TNF increases in various autoimmune diseases. In 1990, it turned into a famous therapeutic target for the treatment of diseases like rheumatoid arthritis (RA). An engineered monoclonal antibody, fusion proteins are the biological agents that target TNF. There are 5 types of TNF-α inhibitor drugs as etanercept, sertolizumab, adalimumab, golimumab and infliximab.[20] Etanercept is a fusion protein that contains two p75-soluble TNFR and binds to the Fc portion of human Immunoglobulin (Ig) G1. It inactivates by binding directly to TNF-6alpha. As a result of this binding, interaction with cell surface TNFR blocked and target cell activation via TNF is inhibited.[21] Etanercept show less affinity for TNFR1 than TNFR2. Etanercept's binds to TNF-α with lower affinity and reversible. Etanercept's elimination half-life is 68 h and it is excreted in bile or urine after metabolism with preteolytic enzymes. Widely distributed in tissues and reaching steady-state concentration before 12 weeks.[22]

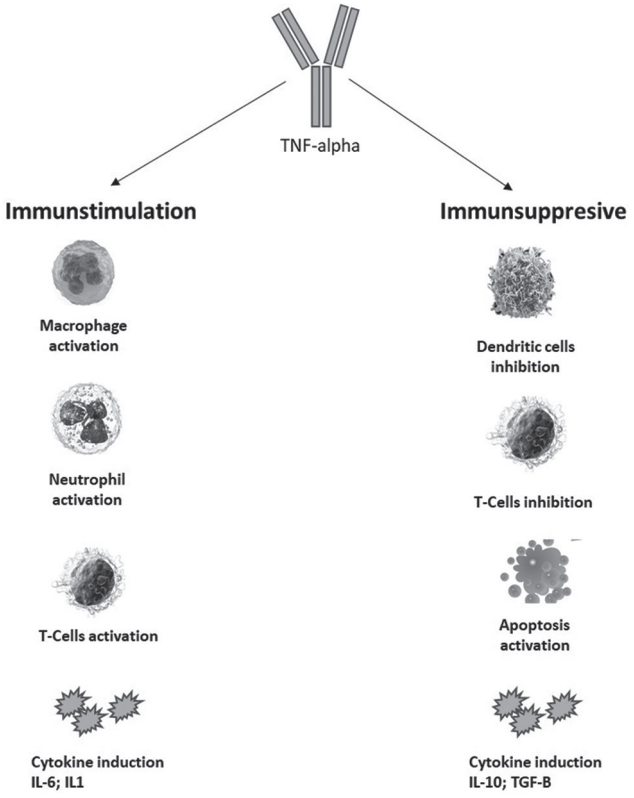

Figure 1: Suppressive and stimulatory effects of tumor necrosis factor-alpha on immune cells. IL: Interleukin, TGF: Transforming Growth Factor-β

Certolizumab pegol is a precise for TNF-α that it is a humanized monoclonal antibody. There is a Fab fragment conjugated with a 40-kDa polyethylene glycol chain that binds to TNF-α[23] and neutralizes transmembrane and soluble TNF-α. Due to certolizumab lacks a fixed Fc region, it does not cause antibody-dependent cell-mediated cytotoxicity.[24,25] Infliximab is a chimeric anti-TNF antibody combined with the human constant region. It prevents inflammatory cells' TNF's interaction with TNFR by binding them. It shows high affinity for transmembrane and soluble TNF. Therefore, it may show a cytotoxic response in macrophages and monocytes expressing the antibody on the surface. It has a half-life of about 8.5–9 days. Although it depends on the dose of the drug and the duration of treatment, it can be seen in serum up to the 28th week.[25,26]

Adalimumab is a fully recombinant human IgG1 monoclonal antibody precise for human TNF-a. It prevents TNF activity by binding to TNF-a.[27] Similar to infliximab, the two have the same mechanism of action.[25] However, it is less antigenic than infliximab. It has a half-life of 10–20 days.[28] Golimumab; It is a fully humanized monoclonal antibody. It has a similar treatment of action to adalimumab and infliximab.[26] Its half-life is 14 days.[24,29]

In graft-versus-host disease (GVHD) in aiming TNF-α expectation and disillusion. Anti-TNF therapies can block the effect of TNF at varied steps of acute GVHD pathophysiology which includes initial host antigen–presenting cell activation, effector T cell support and activation in target tissues and direct cell necrosis. Anti TNF therapies also may have a harmful impact on suppressive cells by restraining TNF ligation to TNFR2 which are stated by regulatory T cells. TNF alpha inhibitors are broadly used for therapy of inflammatory illness. One third of patients who are in pain from inflammatory diseases like RA and Crohn's disease are nonresponder to TNF alpha inhibitors. In pharmacogenomics studies related to anti-TNF drug response, single nucleotide polymorphisms (SNPs) in TNF linked genes, HLA, IL related genes, signal network related genes have been examined.[30] Advanced genotyping technology can detect a great number of SNPs which play role anti-TNF drug response.[31] TNF-α G-308A polymorphism is the most common studied polymorphism related to TNF-inhibitor therapy.[32] TNF-α-308A locates in the promoter region of

gene and upregulates transcriptional activity and increases TNF alpha protein level.

TNF-α-308A is located in the gene promoter region, upregulating transcriptional activity, and increasing TNF alpha protein level. Additionally, Netz *et al*. described Fas ligand (rs763110) and the TNF gene-308 (rs1800629), as functional SNPs, for prediction of anti-TNF drug response in Crohn's disease.[33] Meta-analysis study of Salimi *et al*. underlined the powerful relationship between TNF-α-308A genotype and chronic obstructive pulmonary disease risk.[34] As non-TNF-α related genes, Bek *et al*. reported that genetic variants in CTCN5, CHUK, FCGR2A EYA4, IRAQ NFKBIB, NUBPL, PD2D2, and TEC and PTPRC, TRAF1/C5, and may influence the response to anti-TNF drugs in RA.[35]

In an 2014 report demonstrates anterior proof for an unification among the SNP +489, which is placed at the TNF locus and both sensitivity to the progress of PsA and to therapy answers to TNF-a blockers.[36] Although a considerable amount of targeted or genome wide genotyping studies, a biomarker has not been validated yet in clinical use. Mechanisms that cause differences in drug response may be highlighted by holistic approaches such as transcriptomics, proteomics, and metabolomics studies.[31] Applying anti TNF therapy to responders only may prevent adverse effects of inefficient drugs in nonresponder patients. Clinicians expect biomarker panels for optimal therapy management.[35]

Adverse effects of tumor necrosis factor-alpha inhibitors

In the treatment of various illnesses like RA, ankylosing spondylitis, psoriatic arthritis, juvenile idiopathic arthritis, TNF-α inhibitors have become the standard.[37] However, TNF-α inhibitors cause side effects because of TNF-α's normal functions as normal immune response, granuloma formation and its maintenance, macrophage activation and differentiation, as well as viral immune responses.[38,39] The reported lateral impacts are injection site reactions, Infusion reactions, Neutropenia, Infections, Lung diseases, Demyelinating diseases, Heart failure, Cutaneous reactions, Malignancy, induction of autoimmunity.[1]

The most common side effects of TNF-α inhibitors on the lungs are diffuse interstitial lung disease and pulmonary fibrosis that can be seen in 0.5% to 0.6% of risky cases.[40] Autoimmune and inflammatory demyelinating diseases associated with anti-TNF-a therapy aremultiple sclerosis and neuropathy. In most of the studies, it was observed that neuropathies improved after the discontinuation of the TNF-a agents.[41]

It has been reported in studies that, TNF-α inhibitors may cause an increase of bacterial, fungal and opportunistic infections during the treatment.[42] With the utilization of TNF-α inhibitors, the most common respiratory tract infections are seen as bacterial and fungal infections. TB is caused by *M. tuberculosis* as bacterial infections and legionellosis (leyyoner's disease) is caused by Legionella pneumophila. Fungal infections are TB and histoplasmosis, which are caused by Pneumocystis jiroveci and Histoplasma capsulatum, respectively. Other bacterial and fungal infections are rare.[25] Also, apart from these infections, the most common toxicities on the lung of TNF-α inhibitors are diffuse interstitial lung disease and pulmonary fibrosis.[43]

However, (TNF-α) inhibitors have different important adverse effects. One of the important adverse effect is TB. TNF-α is released from macrophages and one of the important mediator of body defense. TNF-α induce antimicrobial activity in macrophages, T-cell activation and T-cell migration with interferon gamma (IFN-gamma). And also, trigger granuloma formation against to mycobacteria. Thus, with the use of TNF-α inhibitors, TNF-α expression levels decreases and the threat of developing TB disease increases.

Tuberculosis

In the worldwide, TB disease is seen as among the most extensive infectious illnesses. It is estimated that TB infects a third of the world's population. And most of this has a latent stage, which we call latent period, which can later progress to active TB disease. There are risk factors for activation of TB after a long latent period. The most important of these risk factors are contact with active TB patients, HIV co-infection, silicosis, diabetes, and initiation of anti-TNF therapy.[44]

TNF is alpha is the key cytokine in the preventive host guard counter to *M. tuberculosis* and has a very signifficant role in the cellular immune response generated against this *bacillus*. Basic contamination begins when bacilli attain the pulmonary alveoli and invading of the alveolar macrophages to infection area. TNF-α stimulates immature dendritic cells to become mature, thus contributing to the presentation of *bacillus* epitopes to T helper 1 cells and the formation of cellular immune response, and by providing apoptosis of macrophages, enabling the destruction of TB *bacillus* without spreading. In addition, lymphocyte proliferation and cell migration of lymphocytes (B and T cells) in the inflammation zone are caused by increasing cytokine (IFN-gamma) and chemokine release. As a result of this event, granuloma is formed and the bacilli that cannot be destroyed are locked into the granuloma and prevented from multiplying and spreading. With the effect of TNF-α, the active macrophages in the granuloma turn into epiloid cells and these cells are fused to form giant cells. Providing granuloma continuity depends on TNF-α release. Thus, TNF-α is necessary for the control of TB infection by ensuring the formation and continuation of granulomas.[18,44,45] When TNF-α is overexpressed, the systemic inflammatory process is

triggered by increased mesenchymal cell proliferation. With the continuation of this chronic inflammatory response, autoimmune systemic inflammatory diseases are occurring. Therefore, TNF-α inhibitors are used in the therapy of these illnesses. By the utilization of these, TNF activity is prevented; thus granuloma formation is suppressed and they can lead to the occurrence of TB disease.[11,46]

TB is one of the most significant infections linked with anti TNF treatment. It has been reported in many studies that the risk of TB and Latent TB infection (LTBI) increases along therapy with TNF-α inhibitors. In addition, it was observed that fatal reactivation occurred in the TB-infected animal, which received a large number of TNF blockages agents.[47-49] With the reactivation of the latent *Mycobacterium tuberculosis bacillus*, pulmonary TB can develop and as a result of this reactivation, extrapulmonary TB can also occur.[50] It is accepted that TNF-α inhibitors facilitate the development of TB. However, studies have shown that infliximab, adalimumab and etanercept do not show the same risk of developing TB.[51] Due to the existing throughput, it is seen that the threat of etarsept to cause TB is lower than infliximab. This is thought to be due to the difference in mechanism of action between the two drugs. These differences can be listed as follows; Both infliximab and etanercept have a high affinity for TNF, but the binding of infliximab to TNF is irreversible, whereas binding of etanercept is reversible. Down-regulation of IFN production by T cells by infliximab may contribute to loss of resistance to infection with *M. tuberculosis*. Etanercept has less effect on suppression of IFN production. Infliximab has been demonstrated to cause apoptosis and can also induce cellular cytotoxicity by causing cell lysis.[3] In addition, while infliximab directly inhibits TNF-α, etanercept demonstrates its impact according to linking to receptors.[52] TNF-α inhibitors have different active TB generation incidences as well as different active TB generation times. In a study by Gómez-Reino *et al*., active TB generation times were determined after anti-TNF therapy was initiated. Infliximab was found to induce active TB in 1–8 months, adalimumab in 12 months, and etanercept in <2.5 months.[24] Studies on TB risk increase that related TNF-α inhibitors usage listed in Table 2.

Discussion

TNF-α has an effective role in body defense. It provides lymphocyte migration to the primary focus of the patient infected with *M. Tuberculosis* and lymphocytes etc. gets a significant role in the creation of granulomas surrounded by cells. In addition, TNF-α is wanted for the continuation of granulomas. By anti-TNF treatment, the continuity of the granuloma is suppressed and the threat of TB development increases. This risk has been determined in many researches.[53,54] WHO recommends that all patients who will begin Anti-TNF therapy be tested systematically and treated for LTBI if indicated. Tuberculin skin test (TST) or IFN gamma release assay (IGRA) tests can be used to diagnose LTBI. In patients who does not show any symptoms of TB, active and positive for TST or IGRA, chest radiography ought to be demanded to exclude active TB. If the radiography is negative, LTBI therapy is specified.[55]

TB is a bacterial infection gave rise to the *M. tuberculosis bacillus*. It is forecasted that one third of the world is contaminated with the *bacillus M. tuberculosis*.[56] There

Table 2: Studies list of tuberculosis risk increase that related tumor necrosis factor-alpha inhibitors

Drug	Study population disease	Tuberculosis occurred cases/total anti-TNF used patients	References
Infliximab	Rheumatoid arthiritis	525/100.000	[49]
Infliximab, etanercept and adalimumab	Rheumatoid arthiritis	230/62.321	[53]
Infliximab, etanercept	Rheumatoid arthiritis	2/90 - Infliximab 0/103 - Etanercept	[54]
Infliximab, etanercept and adalimumab	Rheumatoid arthiritis, ankylosing spondylitis, psoriatic arthritis	2/179	[44]
Infliximab, etanercept and adalimumab	Rheumatoid arthiritis, ankylosing spondylitis, psoriatic arthritis	3/192 (2 infliximab and 1 etanercept)	[55]
Infliximab, etanercept, adalimumab, anakinra, rituximab	Rheumatoid arthiritis, ankylosing spondylitis	14/53 (5 infliximab, 2 etanercept, 1 adalimumab, 6 others)	[45]
Infliximab, etanercept and adalimumab	Rheumatoid arthiritis	144/100.000 adalimumab 136/100.000 infliximab 39/100.000 etanercept	[49]
Infliximab, etanercept, and adalimumab	Rheumatoid arthiritis	51/100.000 infliximab 47/100.000 adalimumab 20/100.000 etanercept	[56]

TNF: Tumor necrosis factor

is an asymptomatic silent phase when the *bacillus* enters the body extend over diseases symptoms occur that called as LTBI. During LTBI phase, there is no active disease in the body. LTBI can in turn active TB with the impaired of general resistance of the body with diabetes, stress, etc. TNF-α inhibitors, suppresses the TNF-α release and this cause impairment of mycobacterium granuloma formation via TNF-α and results with TB flaming. Many studies have found that the rate of TB enhances by TNF-α inhibitors. For this reason, TB diagnostic tests are performed before anti-TNF treatment, and treatment is not initiated in active TB patients. Chemo-prophylactic treatment is started in patients with LTBI findings before anti-TNF therapy.[1,12] TB, which is one of the serious adverse effects of TNF-α inhibitors and the risk of its occurrence compared to TNF-α inhibitors, LTBI diagnosis and treatments were emphasized in this review.

As a result; the increased risk of TB with the use of TNF-α inhibitors in many diseases, especially rheumatological diseases, has been proven by studies. TB diagnostic tests should be performed before anti-TNF treatment, and anti-TNF therapy should not be started in patients with active TB. If TB improves along anti-TNF therapy, TNF-α therapy should be discontinued. Screening should be done routinely, especially in countries with high TB incidence. Chemoprophylactic treatment should be started 1 month before anti-TNF treatment in patients with LTBI. In this study, it was aimed to underline the subject that it is important to get attention to related side effects of anti-TNF therapies. Before to begin treatment of targeted diseases, necessary tests should be done cautiously.

Patient informed consent

There is no need for patient informed consent.

Ethics committee approval

There is no need for ethics committee approval.

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Conflict of Interest

There is no conflict of interest to declare.

Author Contributions subject and rate

- Şükran Erik (21%): Study conception and design
- Esmanur Bülbül (24%): Data collection, Draft manuscript preparation, reviewing results, approving final version.
- Çiğdem Sevim (19%): Analysis and interpretation of results.
- Seda Eren Keskin (18%): Draft manuscript preparation.
- Mehtap Kara (18%): Data collection.

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Review Article

The Toxic Effect Ways of Allicin on Different Cell Lines

Abstract

In this review, the biological structure of garlic, its active ingredients, especially allicin and its way of antimicrobial effects on cells evaluated and also the toxic possibility of allicin on diverse cells was investigated. Various academic papers have been found in reliable literature. It is stated that garlic includes lots of phytoconstituents with activities against cells. The toxic action of allicin on different cell lines such as bacteria kinds such as Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumoniae*) and Gram-negative bacteria (*Klebsiella pneumoniae*, *Helicobacter pylori*, *Escherichia coli*, *Pseudomonas aeruginosa*) for all that fungi like *Candida albicans* and parasites, virus, glioma and human neuroblastoma cell lines and also oral tongue squamous cell carcinoma, cancer cell lines which are characterized as malignant (leukemia, colon, gastric, and breast cell lines) cancer. It has been revealed that, on those cells, allicin has also been demonstrated toxicity mechanisms on cells like canceling deed of nitric oxide synthase, the peroxidation of lipids, nuclear factor, kappa B, arranging cell period, modulating the activity of redox precision proteins and influence cellular signaling.

Keywords: *Allicin, cells, cytotoxicity, garlic, toxicity mechanisms*

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Introduction

In prohibiting and treating diverse beneficial diseases, native herb yields are acquiring popularity. It has been seen that garlic is the furthest broadly investigated native yield. Its potential health benefits and treatment properties take its source from the bioactive ingredients of garlic. The significant functional act of garlic is its anticancer, antimicrobial, antioxidant, antidiabetic, antifibrinolytic, immune enhancing, antiplatelet collected effect and its possible act in prohibiting cardiovascular illnesses. Declared well-being advances of components of garlic in healing diverse irregularities have been researched in animals and humans.[1] Garlic (Allium Sativum L.) shows wide series of nutritional and medicinal features. Lemma *et al*.[2] investigated oil extracts of garlic's effects of biological and physicochemical. It has been stated that, oil essences of garlic bulb releases indicated important biological effects which can be utilized as resources for nutraceutical and pharmaceutical components. Allium, several species (spp) containing garlic, demonstrates a wide spectrum of pharmacological effects. Sulfur including components of garlic such as alliin, allicin and ajoene, aminoacids (methionine, glutamine,cysteine and isoleucine), enzymes,minerals (zinc, germanium and selenium), solvable sugars, flavonoids (allistains I and II,quercetin and cyanidin) and vitamins (A,E,C,B,B2), β-carotene have therapeutic qualities. Counter to infections and free radicals waited and cooked garlic essences and oils have been demonstrated as preferable preservation to fresh garlic. The bioactive ingredients of garlic are primarily for recovering features.[1] First, in the 1940s, it was reported that the antimicrobial activities demonstrated by garlic were due to its active component allicin. Allicin is an evaporating ingredient removed from uncooked garlic. Thenceforth, it has been researched broadly counter to wide series of microorganisms. It has indicated an ability like aiming thiol, including proteins and enzymes in microorganism. Furthermore, it has been shown that allicin has an ability such as arranging lots of genes necessary for microorganisms intensity. Furthermore, allicin demonstrates lots of bioactive qualities, which cover diverse areas of investigations containing

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anti-inflammatory, antimicrobial, anticancer, and immunomodulatory activities.[3]

The biological and chemical components of garlic

The construction of garlic is seen as fairly complicated. In all untouched garlic, the S-alk(en)yl-l-cysteine sulfoxides and γ-glutamyl-S-alk(en)yl-l-cysteines are revealed as main sulfur components that contain alliin. It is an advanced own preventive contraption toward microorganisms and other insults. The γ-glutamyl peptides are interceders that are biosynthetic for communicating cysteine sulfoxides. All garlic ordinarily includes ~1% alliin, in common with (+)-S-methyl-l-cysteine sulfoxide (methiin) and (+)-S-(trans-1-propenyl)-l-cysteine sulfoxide. S-(2-Carboxypropyl) glutathione, γ-glutamyl-S-allyl-l-cysteine, γ-glutamyl-S-(trans-1-prop enyl)-l-cysteine, and γ-glutamyl-S-allyl-mercapto-l-cystei ne are stated as available in garlic cloves. It has been seen that when garlic bulbs were preserved at cold temperatures, alliin was gathered inherently. On average, whole garlic includes until 0.9% γ-glutamylcysteines and till 1.8% alliin. Moreover, primary sulphur complexes and untouched garlic bulbs include few quantity of S-allyl cysteine but they do not contain allicin. In Figure 1, it is demonstrated that by catabolism of γ-glutamyl cysteine, S-allyl cysteine is composed. It has been informed to conduce to the well-being profits of some garlic arrangements.

Afterward, treating like mashing, cutting, masticating, or dehydration processes, allinase enzyme arises. Allinase enzyme quickly comminutes alliin which is a cytosolic cysteine sulfoxide to create alkyl alkane-thiosulfinates. These have toxic effects on cells and also has a scented as allicin. Allicin and other thiosulfinates immediately dissociate with another complexes such as diallyl trisulfide, ajoene, diallyl sulfide, dithiins, and diallyl disulfide.[4] These complexes of garlic that are bioactive ingredients and organosulfur ingredients in garlic, demonstrate health features of it. Moreover to those compounds, garlic is also symbolized according to phenolic complexes. They also show amazing features that are pharmacological. Great plyphenolic ingredient, a count of native antioxidants, and numerous dissimilar bioactive complexes can straightly and implicitly improve the production of antioxidant enzymes

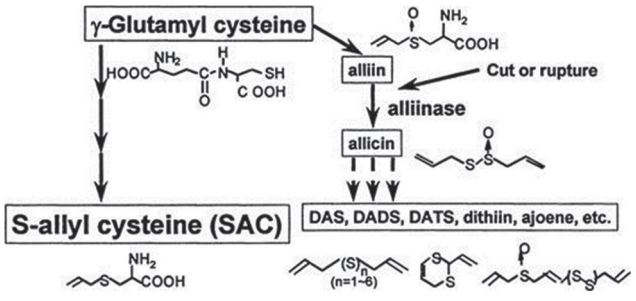

Figure 1: Garlic chemical modification

that guard regular cells. Genetic unsteadiness is provided by reactive oxygen species (ROS) that contain oxygen radicals and some nonradical involutions of molecular oxygen. These molecules can diffuse into the nucleus and assault Deoxyribonucleic acid (DNA). The excess output of ROS breaks functional homeostasis. This event causes to apoptosis, so it activates the mitochondrial pathway. Elevated getting foods that are plenty in natural antioxidants or foods that give rise to the enhanced output of enzymes that shows antioxidant property. It considerably decreases the thread of a few kinds of cancer, containing breast, bladder, prostate, and colon cancers. It is also approved that garlic substances and complexes which are acquired from garlic intercept oxidative alteration of DNA, lipids, and proteins according to clean ROS and improve the production of cellular antioxidant enzymes and increase glutathione grades within regular cells. The mechanism of enhancing ROS metabolizing enzymes is linked on insignificant inducement of ROS output in cells. Besides these, depending on their manufacturing processes, trading garlic preparations vary in their sulfur-containing compounds. Depending on their producing operation ways, treated garlic includes a diversity of sulfur-including complexes rather than complexes that are discovered inherently in full garlic. In addition, scented oil-solvable complexes, less scented water-solvable organosulfur complexes were showed to be effective in diverse fields biologically. S-allyl cysteine provides declining in bioactivity as carcinogenic and also it provides collapsing in oxidational harm. The nonevaporating sulfur-including complexes that S-allyl cysteine and S-allyl mercaptocysteine has been available in lots of garlic providences, even though the ingredient alters significantly. It has been shown that garlic preparations demonstrate hypolipidemic, antiplatelet, and procirculatory effects. Throughout with other garlic preparations, aged garlic extract (AGE) has been indicated to have hepatoprotective, immune, improving, anticancer, and chemoprotective actions. In addition, AGE shows antioxidative effects, while unrefined garlic induces oxidation. Therefore, whole garlic providences cannot be accepted same in their combination, and more significantly, they may sediment.[5]

Allicin

Garlic has a defensive molecule called as Allicin. It has a broad effect area in biology. When tissue is harmed, it has an enzyme called allinase catalyzes a reaction. By this reaction, alliin that is nonproteinogenic amino acid, has been produced. Allicin is from reactive sulfur kinds called as thiosulfinates. It carries a redox reaction with thiol groups in glutathione and proteins which are required for the effect of biological properties. Allicin is physiologically effective in cells that are microbial, mammalian, and plant. It is revealed that allicin restricts the reproduction of bacteria and fungi and also slay those cells completely dependent to dosage-related act with the inclusion of

antibiotic counteractive kinds such as Methicillin-resistant *Staphylococcus aureus*. In addition, it causes cell mortality moreover diminishes cancer cell reproduction. Allicin embarrasses ovum sprouting and also diminishes root growth in herbs. The plurality of allicin's actions is relied on to be intervened over redox-linked contraptions.[6]

In general, allicin does not exist in garlic and allinase which is an enzyme that is required to activate to generate the allicin by the help of water. The mechanism is shown in Figure 2. Solvent extraction, ultrasound-assisted extraction, pressurized liquid extraction, and supercritical carbon dioxide are the mutual techniques to take out allicin. The enzymatic process, subtraction process, using organic solvent, and long extraction time are accepted as the drawbacks of these techniques. Subcritical water extraction is presented to improve the traditional treatment with the elevated refinement of yield, water exposures, an imitation of the qualities of organic dissolvent, and the enzymatic and exclusion treatment pass on in one system.[7]

Cytotoxicity Effect Ways of Allicin

Allicin provides the mechanism of toxicity of garlic. An organosulfur complex, Allicin is set to behave like wide-spectrum antibiotics that contain effects which are bactericidal on both Gram-positive and Gram-negative bacteria. Hence, extreme input cause to death of regular bodily floral by reasoning the increment of strange microbes. Another and substantinal toxicity mechanism of allicin shows similarity to that of organophosphate ingredients that is discovered in a specific plant. Allicin has an impact on the autonomic nervous system through its inhibiting activities on cholinesterase; thence, it makes the action as an indirect-acting muscarinic agonist. Sympthoms such as sweating, miosis, salivation, vomiting, cognitive disturbances, coma, bronchial constriction, diarrhea and convulsion start with the presence of extra toxicity of cholinergic reseptor agonist. In investigations, allicin's parasympathetic effects cause diarrhea and fast evacuation are declared. The gastrointestinal system has been stimulated by this parasympathetic effect of allicin and that leads an increment in gastrointestinal contractility and movements. Therefore, this may cause to hydration and further hypovolemic shock. By using allicin additions, bronchoconstriction may be very dormant, due to extra acetylcholine, which can not be destroyed withal cholinesterase, the central nervous system is effected.[8]

Anticancer effects of garlic

In another research, the toxicity of garlic on three flagitious cancer cell lines (breast, gastric, and colon) as well a nonmalignant cell line were investigated by using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test. According to these test results, gastric and breast cells were delicate to garlic extract. Despite a lot of other reports, this study demonstrated that these impacts are neoplasm precise and dosage related. Significant molecules and their mechanisms are required to be explained by any more analysis on animal models and humans.[9] In the opposite, lots of investigations revealed that cancer cells show an enhanced grade of ROS that is the impact of elevated metabolic and peroxisome action, mitochondrial dysfunction, improved cellular receptor signaling, elevated action of oxidases, lipoxygenases, thymidine phosphorylase, and cyclooxygenase and oncogene action. This quality makes them, particularly to an additional rise in the number of ROS. Because of that, a little premonition of ROS generation that is good for regular cells, but it may be mortal against cancer cells. Ossama *et al*.[10] appraised allicin's cytotoxic potential on dissimilar cancer cells, especially such as lung, hepatic, prostatic, and breast cells. Predisposed allicin-loaded gelatin nanoparticles have been arranged where the ideal formulation was surface-inosculated to glycyrrhetinic acid. To our knowledge, glycyrrhetinic acid gelatin which is used for ligand carrier pair was not discovered before, and the advanced array provides an accomplished liver cancer therapy.

Mösbauer *et al*.[11] showed by their study, cell line Vero E6 as well Calu-3, which are human lung cell lines were reproduced in the primate kidney, allicin at bioconvenient dosages reduces infectious viral particles and viral RNA of SARS-CoV-2. The infection of SARS-CoV-2 cause to proteome changes and the impact of allicin on these host tracks has been defined. Meanwhile, the gene which

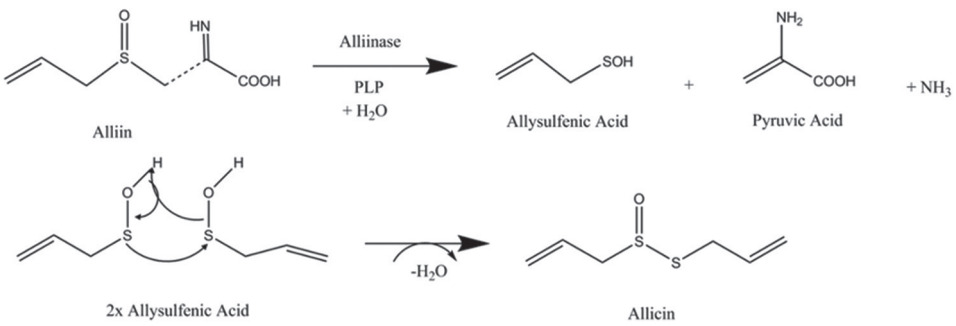

Figure 2: Biosynthesis of allicin

is stimulated with interferon has been significantly upregulated in Calu-3 cells that are transmitted by SARS-CoV-2.Thence, the impact of allicin which are antiviral and immunomodulatory were applied *in vitro* cell cultures that is assisted at the proteome grade. In another study, Rauf *et al*.[12] stated that garlic includes lots of phytoconstituents with effects con cancer and also they revealed the ingredients such as diallyl sulfide, diallyl disulfide, diallyl trisulfide, allicin, and allyl mercaptan. In according to this investigation, samples taken from the countless source of garlic as well the effect of its nanoformulations and phytochemicals on liver, breast, oral, colorectal, pancreatic and prostate cancers have been researched. The molecules in garlic which are bioactive have been discovered to diminish the diverse phases of cancer. Furthermore, ingredients of garlic also cancel the effectiveness of nitric oxide synthase and lipid peroxidation mechanism and also epidermal growth receptor, protein kinase C, and nuclear factor kappa.

Effected cells by allicin

In an investigation, Allicin (diallythiosulphinate) has been demonstrated to reveal antimicrobial features counter to a wide range of microbes containing pattern strains and clinical isolates of bacteria called *S. aureus*. Further, it has been concluded in that research, Allicin aims Fem enzymes to sense the reason of spaces on cell walls and simplifies fragmentation of peptidoglycans and access of hydrolases access into them. The capability of allicin to influence cell wall performs it as an ideal assembling therapy cum alcohol, including bactericides, so it is presumed to have a synergistic effect on cures together with decreasing the risk of extending resistance of antibiotics.[13] Allicin which is diallyl thiosulfinate derived from garlic, is used as a wide spectrum of antimicrobials to threat bacterial infections. Allicin can also break up diallyl disulfides and diallyl polysulfanes to seven sequential sulfur atoms with heat. Allicin and diallyl polysulfanes demonstrate a wide range spectrum of antimicrobial effect to multi-resistant bacterias, including *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *S. aureus*, *Helicobacter pylori Streptococcus pneumoniae* and *Pseudomonas aeruginosa* with fungi such as *Candia albicans* and parasites. Allicin can be implemented above the pulmonary way as a steam because of its volatile nature that also ensures it is appealing on antimicrobial therapy for respiratory pathogens to fight pneumonia infection. Allicin can pass through the cellular phopholipid membrane with ease as an evaporating complex. This property conduces to its antimicrobial way of act.[14]

In another study, Nakamoto *et al*.[15] summarized the impacts of hydrophobic complexes of garlic on bacteria. Diverse hydrophobic antimicrobial complexes were isolated by extracting from garlic. The structures of them are shown in Figure 3. Among these complexes, allicin has been thought to get a main part of the biological act of garlic. However, allicin molecule is unstable and slants to be transformed into lots of complexes like diallyl polysulfides and ajoenes. These compounds were indicated to demonstrate antimicrobial activity. Therefore briefly, allicin is a typical sulfur-including complex discovered in unprocessed garlic generated from alliin and shows antimicrobial activity con to Gram-negative and Gram-positive bacteria. Borlinghaus *et al*. demonstrated that allicin exhibits dose-dependent antimicrobial activity. Allicin can stimulate apoptosis or necrosis at higher doses while biocompatible ingredients can arrange the activitiy of redox precision proteins and influence the signaling of cells. Allicin obviously reacts as an oxidant, especially in diet. Allicin has a mutual effect with the mechanism that is oxidative stress sensing on a few sample systems. Allicin has an amazing impact underlined by giving harm on the cellular envelope as throughput on upregulated transcripts or proteins. Allicin can form openings in the cell wall and so the plasma membrane might have been extracted. In the experiments, the impact of allicin by the propert of its cathching thiol reagent is indicated obviously. Futhermore, it was seen that dissimilar cell lines have been demonstrated to reply in specific mechanisms mutually. For example, Escherichia Coli represents that allicin exhalation have not only been used for bacteriostatic impacts to avoid expansion it is also have been used to kill bacteria cells. Besides these, frequently in cultured cell investigations, plenty of times it was declared that allicin shows antitumorigenic and proapoptotic and antitumorigenic qualities counter to lots of cancer types. As also it is stated, in human cells, allicin has been showed multiple cellular destinations such as enzymes which are glycolytic and cytoskeletal proteins which are nominees due to cancer cures. Lots of research have demonstrated that significant signaling mechanisms on neoplasm cells can be connected cum allicin therapy. The great issue of targeting allicin to tumor tissues immediately needs to cope and also it is stated that cautiously checked future investigations on animal models and clinical experiments including humans are required.[16] Luo *et al*.[17] revealed that, allicin was discovered to be powerful in cancer therapies. It is an

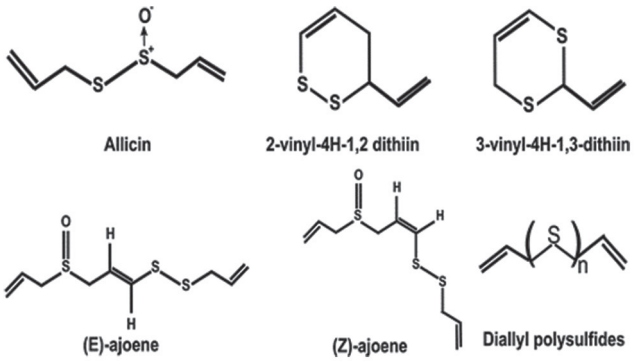

Figure 3: Hydrophobic compounds in garlic

assertive material with a wide range of biological qualities such as obstruction on the growth of fungus, virus and bacteria tested hypertension, diabetes, and chemosupression on lots of cancers. Trials also have been demonstrated that by embarrassing the development of cancer cells, allicin can be chemopreventive to gastric cancer. Allicin shows this effect as apprehending cell cycle at mitochondria and G2/M phase intervened apoptosis which contains caspase-linked and unlinked mechanisms and endoplasmic reticulum stress. These pathways likely include arranging enzymatic action, obstructing DNA creation, cleaning independent radicals and influencing cell multiplication and also even the development of tumor. In another research, it is stated that Myobacterium tuberculosis is obstructed by allicin with the mechanism of embarrassing receptors on the top and removes bacteria according to the use allicin's antimycobacterial lethal mechanisms. Researchers stated that allicin does not just destroy the bacteria withal it demonstrates an action as an immunomodulator stimulating host preventive immune reply that defends the host by reducing the side effects.[18] Furthermore, Rehman[19] indicated that garlic demonstrates a wide antibiotic act counter to Gram-positive and Gram-negative bacteria which have become durable to antibiotics and powerful against many mutual pathogenic bacteria. The antimicrobial activity of garlic is the only account of thiosulfinates compound. It detoxifies the body cleansing the kidney and increase the urine flow. It was demonstrated that a few garlic components changes the activation of cancer as well they cause to inhibit the growth of tumor cells. It has been finalized from this study that allicin comes in sight to fulfill the criteria as antifungal agent. In another study, it is stated that allicin has beneficial effects on the human neuroblastoma cell line that is induced neurotoxicity with amyloid β in terms of cell viability, oxidative stress, and apoptosis.[20] Diverse garlic species have been demonstrated antibacterial activity on a wide spectrum area against to Gram-positive and Gram-negative bacteria such as *Klebsiella, Streptococcus, Clostridium*, *Salmonella*, *E. coli*, *Staphlyococcus and Bacillus*. In fact, it has been seen that *Mycobacterium tuberculosis* which is acid-fast bacteria are delicate to garlic. The extracts of garlic showed powerful antifungal action, liable from the prevention of fungal development. These investigators demonstrated the eventual antibacterial and antifungal activities of garlic openly. Therefore, it would be good to utilize of uniform providences of garlic. It has been revealed that mouthwash including garlic is not cytotoxic on human gingival fibroblasts. Moreover, it was required to state the actions of garlic on oral illness.[21] In another study, it was suggested that allicin can kill cancer cells by cell cycle retention and apoptosis, and allicin kill cancer cells. Besides, according to real-time polymerase chain reaction analysis and western blot analysis protein and gene expressions were examined. Allicin encourages apoptotic cell death by the activation of caspase proteins with intrinsic pathways.[22] Jian *et al*.[23] tried to discover a curing way of allicin in ratty bladder tumor. They concluded in their experiment that Allicin has an evident restrictive impact on bladder tumor. Perchance that impact could be linked to cytotoxicity and activation of immune reply straightly and also it is stated that for cursory bladder cancer allicin could verify to be an efficient intravesical cure agent. Ilic *et al*.[24] also studied on allicin's cytotoxic and antimicrobial activities and its conversion substances. They stated that Allicin has been demonstrated powerful action counter to proven Gram-positive and Gram-negative bacteria by minimum restrive concentration. Further, the conversion yields of allicin showed powerful antifungal and antibacterial action. However, this action was less than the action of allicin. Studies appraising allicin mostly reported cytotoxicity and apoptosis as contrary results which are intermediated by dissimilar resolution incidents such as modified genes and expression of protein or cell cycle alterations. It is revealed in three dissimilar cell lines, ajoene was more poisonous than allicin. Distinct investigators made researchments on this complex, ajoene. They watched cytotoxicity on dissimilar cell lines demonstrating varied tenderness and apoptosis.[25] Miron *et al*.[26] revealed that according to dosage adjustment, allicin was demonstrated to be poisonous in diverse mammalian cells. These investigators provided the use of this cytotoxicity property to improve a new attempt to the therapy for cancer based upon conducted production of allicin. Guo *et al*.[27] also pointed out that in traditional medicine, allicin is a great component of mashed garlic. Allicin has plural pharmacologic acts that lend to a broad spectrum of anticancer activities. To give an instance, according to the consuming intracellular grade, allicin embarrasses cell production in colon cancer cells. In addition, it has been demonstrated that the allicin molecule causes apoptosis of gastric cancer cells by a signaling mechanism called protein kinase stimulated with p38 mitogen. Furthermore, it was seen that it improves apoptosis on human renal cancer cells. It was discovered that cell multiplication and advanced cell apoptosis in oral tongue squamous cell carcinoma were blocked by allicin. In another study, it is stated that allicin has various biological effects and it has been implied that anti-inflammatory factor. It is known that in intestinal inflammation epithelial cells have a significant act.[28] Jung *et al*.[29] revealed that it was known in comparison with its antimicrobial and anti-inflammatory effects, allicin has been accelerated wound healing. Investigators aimed to evaluate that if allicin coated tracheal tube can obstruct tracheal stenosis by virtue of improving wound healing after tracheal damage. It was demonstrated that covering the tracheal tube with allicin has anti-inflammatory and antibacterial effects on harmed tracheal mucosa. Based on these studies, tracheal tube covered with allicin can be used to support physiological wound healing to prevent laryngotracheal stenosis. Catanzaro *et al*.[30] stated that the development of dissimilar kinds of tumor has been pushed

down by allicin. Especially, it was goaled that lots of signaling cells combined with cancer growth. Furthermore, further exploratory ways were summarized to define this encouraging native crop. In a study, it was stated that Tamoxifen (TAM) is broadly used on therapy for breast cancer which is related to hormones. Nevertheless, it may cause to hepatic damage. It is suggested that in cancer therapy, allicin takes a beneficial significant role like an adjuvant to TAM according to relieve liver harm. In another study, it has been demonstrated that in a dosage-linked way *in vitro*, allicin revealed an action as being poisonous to diverse mammalian cells. This cytotoxicity property of allicin was used to improve the new way for cancer therapy area conducted production.[31] Li *et al*.[32] studied on the cytotoxicity effect of allicin which was viewed on glioma cells in a dosage-linked way. The capability of the colony formation and the growth of glioma cells prevented by allicin in a time and dosage-linked way. Hence, allicin has demonstrated cytotoxicity act on glioma cells by dosage-linked way. The reproduction and apoptosis of glioma cells *in vitro* were stimulated by allicin. Therefore, in curing glioma, allicin has been indicated as a new antitumor factor. In other study, the effects of allicin on leukemic cell lines have been studied. It was seen that allicin has shown growth on these cells. According to glutathione discharge and alterations in the intracellular redox condition, the antiproliferation function of allicin mechanistic basis includes the act of mitochondrial apoptotic way.[33] Sultan *et al*.[34] stated that allicin is cytotoxic to monocytic leukemia cells (THP-1 cells) and stimulates calcium-linked hemolysis and eryptosis in human red blood cells. Investigations demonstrated that Allicin advances calcium grades in cells, reasons to oxidative stress and also induces CK1α, caspase, p38, mitogen-activated protein kinase. In chemotherapy, utilization of allicin needs attentive thought, and it is stated that the inhibitors of symptosis were defined and the utilization of these agents was accordingly restricted its harmful effects on red blood cells. Jung *et al*. stated that tracheal tubes coated with allicin demonstrated antibacterial and anti-inflammatory actions for harmed mucosa that belongs to tracheal cells.[29] In another study, sulfur ingredients of the herb may diminish the growth of dissimilar protozoan parasites containing *Leptomonas colosoma*, *Giardia lamblia*, *Tetratrichomonas gallinarum, Crithidia fasciculata*, *Leishmania majör*, *Cryptosporidium baileyi*, *Trypanosoma* spp., Histomonas meleagridis, and Plasmodium berghei. *In vitro*, the reproduction of L. Infantum and Leishmania donovani were obstructed. It was seen that Allicin demonstrated antileishmanial effect thus *in vitro* and *in vivo* circumstances, this effect was evaluated.[35] Chen *et al*.[36] also researched the actions of allicin on *S. aureus* stimulated mastisits in rats. It is showed, according to the advancing LXRα effect and decreasing lipid mass nascency, allicin carried out anti-inflammatory actions counter to *S. aureus* mastitis. In another investigation, according to determine the apoptotic and oxidative ways in human embryonic kidney cells, it was goaled to state the toxical property of allicin. It enhances reactive nitrogen and oxygen species and gives to reason to oxidative stress. Transcription of superoxide dismutase 2 and upregulation of Nrf2 were investigated on allicin's antioxidant properties. However, according to allicin's oxidant capability, this impact was covered at the IC50 value. Consequently, the fate of the cell is formed over apoptotic pathway by courtesy of killer caspases and the precursor activation. Moreover, this researchment showed that allicin-induced proapoptotic proteins which are detached from p53 in kidney cells called HEK293. Segmentation of Poly [ADP-ribose] polymerase 1 (PARP-1) and decreased production of p53 which are responsible from the disintegration of DNA in those kidney cells, pointing out that allicin has toxic effect on cells at high amount concentrations. To sum up, through this study, it was demonstrated that by the transaction of allicin the expression of p53 was downregulated meantime PARP-1 was upregulated.[37] Sheppard *et al*.[38] evaluated the chemical reactivity of allicin to look its antimicrobial effect counter to the board of pathogenic bacteria. It is restricted to apply allicin-like medications assembled with drugs which are used as antibiotics that are used for Gram-positive bacterial infections that show decreased sensibility to vancomycin. In another study, allicin's toxicity on cells and virucidal effect has been linked to the virus cell membrane and envelope. It has been demonstrated that allicin inhibits getting or grasping viruses into cells. In addition to that, diverse combination of merchant garlic yields, containing mellow garlic which was dissolved in alcohol and oil of garlic that is fermented, powder formed tablets and capsules which were stated to reveal virucidal effects of yields counter to herpes simplex virus 1 and human parainfluenza virus type 3. The effects of virucidal of merchant yields have been linked to their provision procedure. Allicin and other thiosulfinates in great amounts had been shown as the best virus cell toxicity effects.[39] Khakbaz *et al*.[40] demonstrated that the propagation of gastric cancer cells that show resistance to chemotherapy may be obstructed with mutual therapy process of 5-fluorouracil and allicin according to compress cells that express CD44. For this reason, those researches severaly encourage the utilization of allicin-established mutual chemotherapy medical treatment in lower dosages has importance in gastric cancer clients. In another research, the antimicrobial effect of allicin has been decoded and the enzyme DNA gyrase has been approved. The antibacterial effect of allicin has also demonstrated encouraging antifungal effects counter to diverse yeasts that are pathogenic. Counter to diverse herb pathogenic fungi, the broad set of effects of allicin that are antifungal were approved. In prior investigations, according to suggest a diverse effect pathways, allicin's antifungal effect has been clarified. Nevertheless, allicin's mechanism and effect pathways related to its sanitary utilities are not

still fully known. The potential effects of allicin that are antifungal counter to a diversity of pathogenic fungi were exposed by that investigation. Allicin demonstrated encouraging effects which are antifungal against pathogens that are counter to herbs. Apparently, more researchments were required to discover the cytotoxic and antimicrobial effect of allicin.[41] Wu *et al*.[42] clarified that allicin applies hepaprotective action counter to hepatic toxicity of cells that are acetaminophen-stimulated perchance owing to the molecular pathways of inhibiting endo hepatic metabolism purulent stress linked with intrahepatic TLR4/NF-κB pathway.

Conclusions

In this comprehensive review, it is stated that allicin has lots of significant effects on diverse cell lines such as Gram-positive and Gram-negative bacteria, fungi, and yeast. The cytotoxic effects and their pathways are summarized by gathering studies that worked on that subject. More investigations of allicin which are about toxicity properties of allicin are needed to provide users security and should primarily be focalized on the assessment of toxicity effects that are long-term and genotoxicity.

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Patient informed consent

There is no need for patient informed consent.

Ethics committee approval

There is no need for ethics committee approval.

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Conflict of interest

There is no conflict of interest to declare.

Author contributions subject and rate:

- Esma Nur Bülbül (85%): Design the research, data collection and analyses and wrote the whole manuscript.
- Emel Serdaroğlu Kaşıkçı (15%): Contributed with comments on manuscript organization and write-up.

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Review Article

Review Article on "Importance of Behavioral Genetics on the Psychological Development of the Person"

Abstract

The study of behavioral genetics holds great promise for revealing the genetic and environmental factors that impact both typical and abnormal behavior. The ideas and techniques that have been used to identifying the constituent parts of complex human characteristics serve as the foundation for behavioral-genetic procedures. To analyze the genetic component of these complex features, new tools are now accessible. We can start investigating how certain genes interact with environmental variables in development as they are discovered. Important factors to take into account include how we interpret these results, how we pose fresh questions, how we celebrate the information, and how we make use of or abuse this knowledge. These problems are prevalent in all human research fields, but they are particularly evident in human behavioral genetics. In this article, we review the results of studies and theories, explore their implications for our knowledge of adult personality development, and highlight outstanding issues that require more investigation.

Keywords: *Behavioral genetics, environment factors of personality, genetic, impact of environment on personality, impact of genetic factors on personality development, personality development, psychological development*

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Introduction

As believed by various scientists, the genotype of the person influences almost all the traits of a person. However, the point of interest or question is that "Are the genes, only the triggering factor for the expression of various traits or are there any other factors which work in conjugation with genes and influence its action." The answer to this question can be explained by "behavioral genetic studies," which aim at understanding the genetic and environmental influences on behavior.

In this article, the topic of interest picked to consider is the "importance of behavioral genetics on the psychological development of a person." This article provides you a "bird's eye point of view" of the effect of genotype on personality and how the environment shows its effect on the expression of a particular gene.

As Cole and Bruce described, "from the moment of conception it grows. 'Whole,' having within itself possibilities of developing into a dynamic, active, walking, talking, thinking, feeling human being, provided that the environment furnishes the raw materials and the appropriate conditions of stimulation."[1] This perspective clearly suggests that the environment triggers or suppresses the action of genes under certain circumstances.

Genes versus Environment

It is a well-established fact that genes are the basis for the expression of any trait in living organisms. Talking more concisely, the development of psychological personality in human beings, there are various debates so far regarding the extent of impact or the contribution of genes and the environment. Although the impact of genes is likely on the person, it is often shaded and/or masked by various environmental factors that they are surrounded with.

According to the "first law" of genetics, "Each human conduct feature is heritable." Researchers in psychology, sociology, political science, economics, and industry have proven that all conduct studied by their disciplines is inherited by the validity of this first law and using statistical methods. In addition, they say in several

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instances that unique genes have been found that play a part.[2] This is interpreted as the source for the development of personality of the person coming from genes-the genetic factors. Further, the quality of the scientific methodology used to justify these arguments will decide the various genetic conduct as a science.

It is also interesting to know that as per Kandler and Papendick, genetic variations are no longer contentious, as they are reflecting the differences in all individual characteristics such as personality traits, which account for above 40%–50% of diversity due to genetic sources.[3] The various tests of personality traits provide sufficient data to prove the heritability estimates stand way higher. The combined observations of multiple family genetics and molecular genetics studies reveal minor effects of genes that communicate with each other in a complicated way and with the numerous environmental factors leading to its contribution to the genetic basis of personality traits. An important point here to be noted is that the genetic variations caused by the individual interactions led to the creation of individual traits within the environmental opportunities.

As cited above, there is various scientific evidence which strongly supports the influence of environmental variables on the development of psychological personality. Data from adoption research suggests that the shared environment affects the cognitive ability of children at an early point of life, but this effect eventually deteriorates as hereditary factors grow in their developmental impact. This indicates that upbringing and other family influences such as financial status, and educational background have an effect on the success of younger children in intelligence quotient assessment but are shortlisted. Biology, i.e. genetic factors are crucial to human variation in the cognitive skills in adulthood rather than nurturing them.[4] However, a word of caution is justified. The findings involve children residing in the usual anticipated spectrum. The exploitation or neglect of children has very genuine harmful long-term effects.

The Various Psychological Theories Explaining the Personality of the Person

About what personality means, psychologists disagree with one another. The word "personality," which most people think refers to as a theatrical mask used by Roman players in Greek tragedies, was first used in Latin. The masks (personas) used by these historical Roman actors were used to create a character or fake look.[5] Naturally, this superficial description of personality is inadequate. The word "personality," as used by psychologists, refers to more than just the roles that people perform.

There is not a specific definition of personality that all personality theorists agree on, though. Since they disagreed on the essence of mankind and each viewed personality from a different perspective, they really developed original and important theories.

All theories are a mirror of the individual backgrounds, early life experiences, philosophies of life, interpersonal interactions, and distinct worldviews of their writers. Because each observer's frame of reference affects how they interpret data, there may be a wide range of ideas.[5] Divergent theories can still be helpful, though. A theory's utility is determined by its capacity to produce research and to describe research data as well as other observations, not by its applicability to common sense or by its conformity with other theories.

Viewpoints in Personality Theories

Psychologist's attempt explaining how people's ideas, feelings, motivations, and behaviors function. There are five kinds of perspectives:

Psychodynamic theories

Since Freud, psychodynamic and then more broadly psychoanalytic methods have emphasized the significance of early events and parental interactions as forces influencing personality development.[5] This viewpoint holds that the unconscious mind and motivations are far more potent than conscious awareness. As a primary method of treatment for mental disease and neurosis, psychoanalysis typically employed dream interpretation to elucidate unconscious ideas, feelings, and impulses. Following Freud, these thinkers shifted their focus from the significance of sexuality to social and cultural causes.

Humanistic-existential theories

The main tenet of the humanistic approach – currently referred to as "positive psychology" – is that individuals seek for purpose, development, well-being, happiness, and psychological health. Happiness and good emotional states promote prosocial conduct and psychological well-being.[5] Studying these adaptively beneficial features of human behavior offers just as much insight into human nature as understanding the problematic ones. Existential theorists believe that in addition to our desire for meaning, we are also motivated by unpleasant events that can lead to psychological development. These experiences include failing, facing mortality, losing a loved one, and experiencing anxiety.

Dispositional theories

Dispositional theorists contend that the distinctive and enduring propensities to act in certain ways make up the core of our personality. The term "trait" refers to these particular tendencies, including such extraversion or anxiousness.[5] The discipline has come to the consensus that there are five main characteristic dimensions in individual personality. Certain actions are made more probable in some persons by traits.

Theories of biological evolution

Individual variations in fundamental genetic, epigenetic, and neurological systems have an impact on behavior, thoughts, feelings, and personality. Some persons have distinctive characteristics, attitudes, and modes of thought because of variations in their chromosomes and central nervous systems (neurochemistry and brain structures). Human intellect, conduct, and personality have been influenced by processes of evolution (natural and sexual selection) over millions of years since they are founded on developed brain systems. Environment, body, and brain interact and develop together, and this viewpoint stresses that what we experience psychologically is more important than everything else, therefore, nature (biological) and nurture interact to shape what we think, feel, and do.[5]

Theories of learning-(social) cognition

Focus solely on conduct, not hypothetical situations, if you want to comprehend behavior and internal, imperceptible states including emotions, motivations, urges, and ideas.

All actions are taught by association and/or their results (whether it is reinforced or punished). To mold desired behaviors, we must first comprehend and then create the circumstances that lead to those specific actions.[5]

Sexual Psychology and Personality

Eysenck was the first to seriously examine the connections between sexuality and personality (1976). We may try to frame our knowledge of the connections between sexual psychology and personality as a result of sexual selection by following the advice provided from an evolutionary perspective.[6] According to the parental investment theory (Trivers, 1972), human females would invest more resources in raising their children, whereas males will prioritize mating.

Genes and Well-Being

Happiness is no exception to the rule that genetic variables tend to play a significant effect in the majority of human characteristics.[7] Heritability estimates generally vary from 0.30 to 0.50.[8-13] Various conceptualizations of well-being. A meta-analysis of 13 research from 7 distinct nations with more than 30,000 twins found a weighted average heritability of 0.40 for happiness.[14] Further supporting the theory that there is no set heredity for well-being, this meta-analysis discovered significant variation in heritability estimates among studies, above and above that predicted by random fluctuations. Instead, the proportion of variation explained by hereditary variables differs among cultures, age groups, and the specific well-being phenomena addressed. The average heritability for well-being was 0.36 according to a separate meta-analysis by Bartels, which used somewhat separate inclusion criteria, samples, and analytical methodology.[15]

There is evidence of genetic impacts that are particular to the various components[13,16] as well as genetic factors that are common to the many well-being components, such as subjective happiness, life satisfaction, subjective well-being, and psychological well-being. The genetic impacts on social support[17] and, in reverse, depression[18] and internalizing disorders,[19-21] are partially connected to the genetic influences on well-being. Longitudinal studies have furthermore demonstrated that genetic variables are largely responsible for the stability in well-being, with heritability for the stable variation, or dispositional well-being, predicted in the range of 70%–90%.[22,23] In contrast, the primary cause of variance in well-being is environmental factors.[23,24]

Despite abundant evidence of significant genetic implications on welfare generally, results on life satisfaction are inconsistent, with heritability estimations varying from 0 to 0.59.[12,21,24-27] The meta-analysis by Bartels[15] particularly looked at the heritability of life satisfaction and found that it had an average heritability of 0.32. Thus, compared to other aspects of well-being, life satisfaction seems to be considerably more impacted by environmental circumstances. Furthermore, although rates of life satisfaction often only minimally change with age, genetic, and environmental influences may also moderate with age. Given that life satisfaction is an assessment of one's life so far, older age presumably entails more life experiences, challenges, and victories than younger age, indicating greater environmental than genetic influences.

Environmental Factors

Societal influences on personality

When an individual has group experiences and contacts with others, his or her personality is influenced by others in a give-and-take relationship that may be positive or negative depending on the associations, in which he or she maintains. Every person has a unique position and status in society.

Toward instance, younger people in our culture are expected to show respect for seniors. Numerous additional social elements, such as the environment, social interactions with others, family, and media, shape people's personalities on a daily basis. It is possible to state that anything that comes into touch with a person's social life has an impact on their personality and determines whether they have a good or poor personality.

Culture's influences on personality

An individual's personality is influenced by both material and nonmaterial culture. People who are immersed in their culture either deliberately or unintentionally embrace the qualities and behave appropriately. An individual's personality and actions are determined by the culture of

the society, in which they live, and they are expected to behave accordingly. When someone conforms to all of the social standards of a society, a good personality is formed, but when someone does not, an abnormal or bad personality is formed. Thus, a person adjusts to and develops their personality in the society, in which they seek happiness.

Geographical Setting

In addition, a person's physical surroundings affect their personality. Land, rivers, hills, mountains, forests, plains, and other environmental aspects can influence a person's personality and whether it is strong or weak, good, or negative. A person's physical environment, to which they belong, determines all of their thoughts, feelings, opinions, attitudes, routines, and behavior as well as their physical makeup. As an illustration, rural and urban populations differ in terms of body type, physique, color, and health. These individuals grow into a range of personalities as a result of their varied environments. Contrary to rural residents who lack these amenities, city dwellers have facilities and sophisticated lifestyles that help sensitive bodies and minds grow.

Situational Factors of Personality

A person's personality development is also entirely influenced by situational conditions. Situational personality traits change in response to social circumstances. Every individual encounters a variety of circumstances during their lives that provide them the opportunity to alter their behavior. For instance, a teacher could be harsh and strict with pupils but not with his or her own family. An officer could act differently among subordinates than they would with friends. Personality is not the outcome of just one thing; each component contributes equally to its creation. When a person interacts with their surroundings, culture, society, parents, friends, and random strangers, their behavior and personality come into being.

According to behavioral genetics research, environment influences personality, yet siblings are not identical. This has been taken as a challenge to the notion that raising children and family gatherings are significant. Reviewing behavioral genetics and developmental psychology research, the following conclusions are drawn: due to methodological, measurement, and outcome variable limitations, sibling dissimilarity may be overestimated; however, developmental psychology conceptualizes the family as involving interactions between the person and their environment, and personality as being multidetermined, so sibling dissimilarity is not surprising; additionally, objective and subjective family experiences differ for siblings due to age differences, birth order, genetics, gender, and idiosyncrasies; and finally, sibling dissimilarity is not surprising.

Introduction and Definition of the Multidimensional Personality Questionnaire

The Multidimensional Personality Questionnaire (MPQ) is a self-report questionnaire used to measure personality using 11 lower-order ("main") trait scales that load onto three higher-order variables that represent temperamental characteristics. The entire edition of the inventory has 276 items; there are also shorter versions with standard and simplified wordings (155 and 157, respectively). The MPQ only accepts "true" or "false" responses for each question. Along with this, the inventory includes validity scores that evaluate social desirability, acquiescence versus "Nay-saying," and random response. The MPQ has been especially helpful in elucidating the connections between psychological dispositions related to temperament and both normal and unhelpful conduct.[28]

The MPQ is a normal-range personality test that evaluates a variety of fundamental characteristics such as emotional sensitivity and motivation, interpersonal style, and more. To estimate higher-order broad dimensions such as positive emotionality, negative emotionality, and constraint, these trait scores can be differently weighted. Broad trait scores, on the other hand, must be almost entirely administered since they must be calculated using proprietary regression algorithms.[29] Through the development of condensed item-based estimates of these weighted scores, we intended to improve measurement effectiveness. Classical test theory and item response theory methodologies were utilized to choose five items from each primary trait scale that approximated the weighted estimates while still retaining the breadth of MPQ content coverage to parsimoniously define and separate the broad qualities.[29]

Conclusion

Every person's behavioral and psychological traits – including intelligence, personality, and susceptibility to mental illness – are influenced by genes. These traits can have an impact on both parents and children within a family.

There are three major findings. First, throughout the adult life span, there is a strong and largely stable genetic basis for individual differences in personality; second, environmental influences become more significant and contribute to increasing rank-order stability of personality traits; and third, both genetic and nonshared environmental influences influence both the stability and change of personality traits. For future research, (i) Identification of quantifiable environmental elements and (ii) to depict the interaction of hereditary and environmental factors on personality stability and variation throughout adulthood will supposedly be critical subjects.

Patient informed consent

There is no need for patient informed consent.

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- Bhavyaja Chakrala (100%): Design the research, data collection and analyses and wrote the whole manuscript.

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A Trajectory of Psychosocial Needs of the Caregivers of Persons with Spinal Cord Injury: A Mental Health-care Professional Standpoint

Dear Editor,

Spinal cord injury (SCI) is a calamitous condition that causes temporary or permanent changes in its function resulting either from trauma or nontrauma.[1] The abrupt nature of injury infers a radical change in various domains on both persons with SCI (PwSCI) and their family caregivers (FCGs).[2] The unanticipated nature of this condition leaves the FCGs to adopt new roles and responsibilities that they were not aware of it before. The FCG's psychosocial and vocational outcomes are negatively impacted by taking on this additional caregiver responsibility. This caregiving role puts them in a phase where they often neglect their needs and health.

The FCGs of PwSCI are unprepared for the caregiving task as they face mysterious and startling psychosocial sequelae. The FCG's psychosocial needs are secondary to the PwSCI care and recovery, and meeting their Psycho-Social (PS) needs timely are very substantial. The pattern of metamorphosis in PS needs among the FCGs is unique for the PwSCI. The trajectory of the PS needs of the FCGs of PwSCI evolves along different phases, such as during the rehabilitation, at the time of discharge, and postdischarge.[3] The rehabilitation professionals must address the PS needs of the FCGs timely by providing tailor-made PS interventions. The FCG's forms of adjustment and adaptation skills are varied before the crisis, during and after the crisis; thus, their PS needs will vary as well. Hence, it is pivotal to assess the PS needs of the FCGs at different phases and to provide tailor-made PS interventions. As mental health-care professional, we would like to present the FCG's PS needs at different phases of rehabilitation, which can help the rehabilitation professionals to understand the PS needs of the FCGs to meet their needs, thus helping in the PwSCI recovery as well.

As a psychiatric social worker, we see many PwSCI and their FCGs in our routine clinical practice at the department of neurological rehabilitation in a tertiary care center in India. Psychiatric social workers provide their expert tailor-made PS interventions during inpatient and outpatient neurological rehabilitation as SCI has associated psychosocial sequelae. As mental health-care social workers, we have listed the various PS needs of FCGs of PwSCI at different periods. We carry out routine PS assessments as part of the daily clinical practice for both inpatient and outpatient FCGs. The PS needs from the FCGs have listed here based on the findings from the above-mentioned PS assessment. The contents mentioned in Table 1 disseminate insights about the psychosocial needs of the FCGs of PwSCI at different periods of rehabilitation.

Table 1: Psychosocial needs of the family caregivers of persons with spinal cord injury at different time periods of rehabilitation

	Psychosocial needs during the rehabilitation		Psychosocial needs after the rehabilitation
	At the time of admission	At the time of discharge	In the community
Expert's perspective	Treatment Financial Emotional	Financial Physical Family Welfare Therapeutic	Therapeutic Financial Emotional Family Vocational Community Welfare Marital
Family caregiver's perspective	Financial Treatment Physical Family	Financial Vocational Physical Welfare Family	Financial Vocational Welfare Marital Family Community
Common psychosocial needs		Financial Treatment Vocational Welfare Emotional	

Addressing the PS issues faced by the PwSCI, and their FCGs are significant in the rehabilitation goals. The rehabilitation professionals must include therapeutic strategies which should address the PS needs of the FCGs in their treatment plans. Prolonged exposure to these PS issues will adversely affect the quality of life and well-being of both PwSCI and their FCGs. Thus, appropriate psychosocial interventions should be planned and implemented to reduce the distress in the PwSCI and his/her family and also to imwprove their biopsychosocial wellness (physical and mental health). A multidisciplinary approach will help in the assessment, treatment, and management of physical, psychological, social, and vocational issues of the FCGs of PwSCI effectively.

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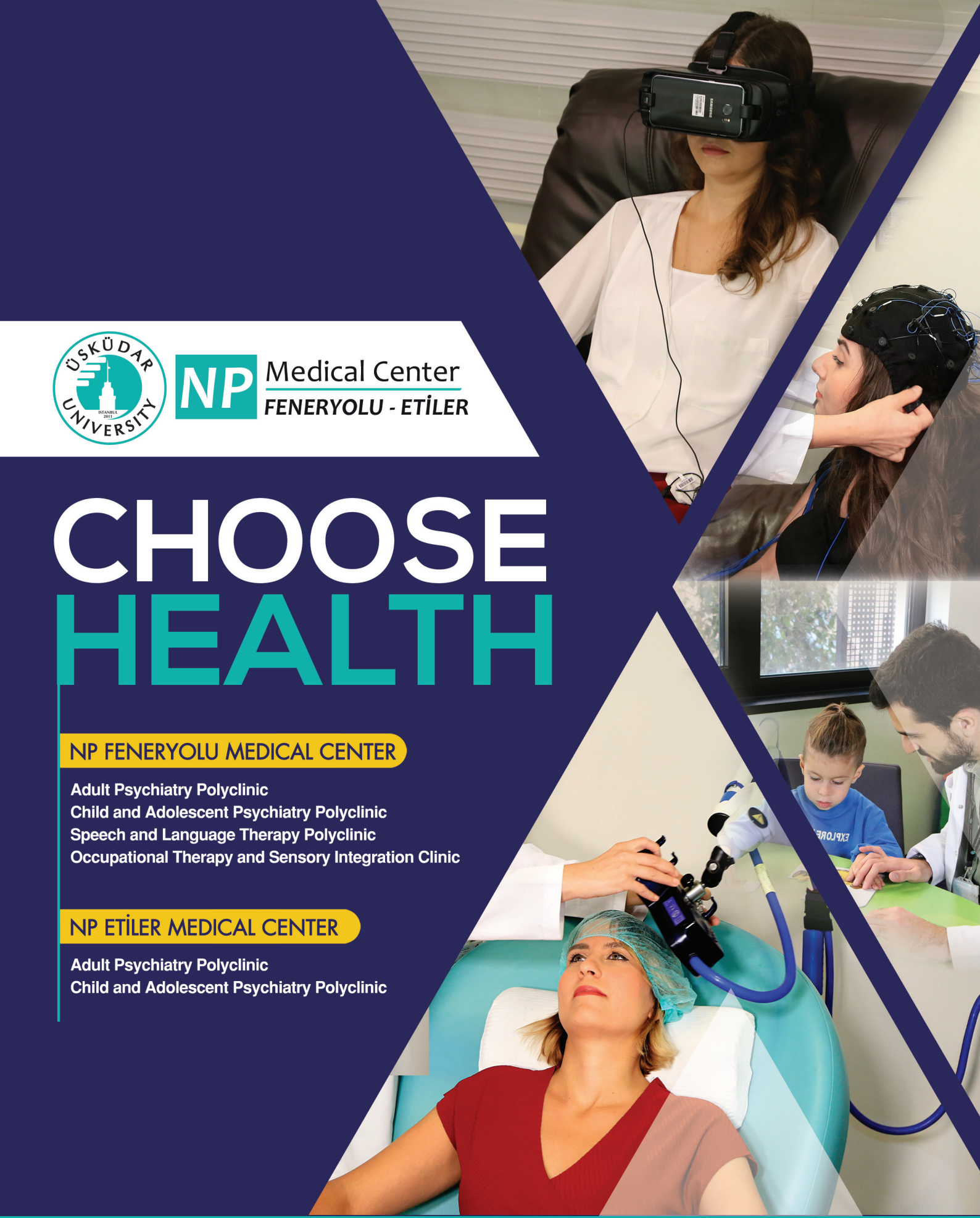

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