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# Neurotoxicity Induced By Zinc Oxide Nanoparticles And Mureer Plant with the Promising Protective Role of Gallic Acid in Albino Rats

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**Abstract**

Zinc oxide nanoparticles (ZnO NPs) are the most used nanoparticles in the profitable arenas. Mureer (Senecio glaucus L. plant) (SP) is one of the natural plants in the deserts. Gallic acid (GA) deeds as an important antioxidant used in the treatment of various diseases. This study intended to gauge the lethal impacts of either single or dual treatments of zinc oxide nanoparticles (ZnO NPs) and mureer (*Senecio glaucus* L. plant) (SP) in neural tissue via scrutinizing the biochemical indices and histological examination to assess the convenient outcome of gallic acid (GA) against them for 30 days in rats with oral injection, and to ascertain the chemical constituents of SP, using Liquid Performance Mass Liquid Chromatography (HPLC-MS) method. Rats were alienated into eight groups: Control, GA (100 mg/kg), ZnO NPs (150 mg/kg), SP (400 mg/kg), GA+ZnO NPs (100,150 mg/kg), GA+SP (100,400 mg/kg), ZnO NPs+SP (150, 400 mg/kg), and GA+ZnO NPs+SP (100,150,400 mg/kg). Our results revealed that single or dual treatments of ZnO NPs and SP decreased the activity of acetylcholinesterase (AChE) and upraised the activity of lactate dehydrogenase (LDH), as well as the level of total lipids (TL) compared to the control group. Likewise, they prompted inflammation, neuronal degeneration, and cerebral hemorrhage. Our facts also revealed that the toxic effect of the dual group of ZnO NPs and SP was more than the effect of the single group. Contrariwise, our results estimated that GA amended neural injury. To sum up, this study presented that ZnO NPs and SP parade as neurotoxic agents; yet, GA acts as a neuroprotective agent.

*Keywords:* Zinc oxide nanoparticles; Mureer plant; gallic acid; biochemical investigation; histological study; brain tissue.

## 1. Introduction

Currently, the required use of insecticides causes spreading of the cancerous diseases all over the world, which has been widely used in the agricultural control programs [1-2]. Nanotechnology currently befits the furthermost emergent discipline that is universally applied to diverse extents, including ecology, pharmacology, medication, chemistry, and astronomy. Because of the minor bulk with a defined variety (1-100 nm) to the used nanoparticles (NPs) in the marketplace, they could be convinced the various risky influences for the living cells [3-4].

Zinc oxide nanoparticles (ZnO NPs) have expansively used to the plentiful fabricators, including maquillages, sunburn rub, drug delivery, electrical sensors, batteries, porcelains, fly sprays, and dyes. Inaptly, some studies have displayed that ZnO NPs convinced cytotoxicity appliance in the intracellular tissues [5-6]. The solubility of ZnO NPs has the principal character for the stimulation of its cytotoxicity. The accumulation of zinc ion ($Zn^{2+}$) can cause irregular zinc hemostasis equilibrium, leading to initiation of the programmed cell death manner and liberating lactate dehydrogenase enzyme (LDH) from the injured tissues [7].

NPs can badge effortlessly through the blood-brain barrier (BBB), which persuades hypoxia and

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cerebral complaints. The instigation of oxidative phosphorylation in the neural mitochondria incites cell death in the brain [8].

To use a promising standard bio-insecticide, natural plants are broadly used because of producing slightly lethal properties from chemical ones toward living tissues. Mureer (Senecio glaucus subsp. coronopifolius (Maire) C. Alexander L. plant) (SP), (Senecio species, Asteraceae family), is a natural plant distributed in the coastal deserts [9]. The accumulation of the phytochemical compounds may be convinced the cell death in the exposed living organisms. Epidemiological and uncertain modifications of the living tissues have presented an enlarged risk for pulmonic and hepatic analyses [10-11].

Largely, the brain is the main organ affected by some ecological substances in the mammals. It remits a stimulus of the biochemical symptoms, such as intellect, strength, feelings, communiqué, philosophy, memory, reproduction, and others. It involves of cerebrum, cerebellum, and brain stem, which is responsible for its energetic functions through treatment of the flow of nerve signal distribution and synaptic work by controlling neurotransmitters [12].

Exclusively, the acetylcholinesterase (AChE) enzyme is measured as the principal neurotransmitter used in an indication of the neural injury. It is a vital enzyme for the extent levels of acetylcholine that is responsible for regulating the abundant central nervous difficulties, such as synaptic plasticity, sleep-wake cycle, and hormone secretions [13]. Although more neurotoxins mainly persuade neurotoxic effects due to shifting o AChE activity, resulting in the cholinergic neurotransmission fracas [14].

Remarkably, it is necessary to determine more effective strategies against the complications from the lethal amalgams while conserving or augmenting the cheerful properties. Amongst the lucrative amalgams used as cytoprotection and antioxidant properties in the different biological cells is gallic acid (GA). GA is 3,4,5-trihydroxy benzoic acid that is a plant-derived natural polyphenolic composite, dispersed in various fruits and vegetables. It is involved via the gastrointestinal tract into the bloodstream, taking part in a broad sort of the metabolic events [15]. There are a relation between the structure of GA and its neuroprotective effect; it has an aromatic ring with hydroxyl and carboxyl groups in its assembly. These carbon-based groups can prompt antioxidant, anti-inflammatory, and anti-apoptotic effects in living cells [16-17]. Due to the brusque penetrability of GA through BBB, it has an ability for an ion chelating that prevents cell decease [18]. The aim of this study was estimated to gauge the toxic effects of either single or dual treatments of ZnO NPs and SP via studying biochemical and histopathological investigations in the neural tissue and describing the vital chemical constituents, using Liquid Performance Mass Chromatography (HPLC-MS) method. Consistently, it intended to evaluate the supposable prophylactic outcome of GA against neurotoxicity prompted by ZnO NPs and SP in the male rats.

## 2. Materials and Methods

*2.1. Chemicals and reagents:*

Zinc oxide nanoparticles (ZnO NPs), sodium carboxymethyl cellulose (Na-CMC) salt, and gallic acid (GA) were acquired from (Sigma Aldrich Enterprise, St.Louis, Missouri, USA). 70 % of ethanol solvent was procured from (EL-Naser Firm, Egypt). Total lipids (TL) kit was come from (Biodiagnostic Firm, Egypt). LDH kit was credited from (Egyptian Establishment for Biotechnology, S.A.E, Egypt). Other substances were used to the best formulas for the biochemical measurements.

*2.2. Plant extraction:*

SP was obtained from Cairo-Ismailia Road, Egypt that was identified by Dr. Abdel-Halim Abdel-Mogaly Botanist (Herbarium of Horticultural Research Institute, Agricultural research center, Egypt). 1500 g of the entire quotas of the plant (leaves, stems, roots, and flowers) were dehydrated in the workroom, crumpled, and strongly inundated in 70% of ethanol for 3 days in glass containers. At the finale of the maceration age, the gotten extract was filtered with *whatman* paper (90 mm) and concentrated the formed solution from the additional solvent at 60˚C in a rotary evaporator. Then, the enthusiastic solution was dried in an aired oven at 45˚C to progress to a greenish extract. The harvest of extraction was involved at -20 ˚C for the tryout at Plant Protection Institute, Agricultural Research Center [19].

*2.3. Liquid Performance Mass Chromatography (HPLC-MS/MS) method for plant analysis:*

The ethanolic extract of SP was examined, using High-performance liquid chromatography (HPLC) (LC-2040 Controller model) that was worked by ESI-MS positive and negative ion acquisition [20].

*2.4. .Protocol of trial study:*

Forty albino male rats (*Rattus norvegicus*), weighing 180-220 g (b.wt.) with 6-7 weeks age-old, were used in the trial. It was approved by the Animal Ethics Commission of Zagazig University under “Principles of Laboratory Animal Care” as an approval number (ZU-IACUC/1/F/42/2019) in the Faculty of Medicine. The rats were placed in plastic birdcages at an organized temperature (23±1˚C), humidity (55±5%),

and (12 h dark/12 h light) cycle with *ad libitum* access to food and water. After a week of acclimation, the rats were disjointed into eight groups and each group contained five rats:

(1) Control group: rats were treated with 0.5% Na-CMC as a vehicle or suspending agent (5 ml/kg) [21-22].
(2) GA-treated group: rats were treated with (100 mg/kg of GA), suspending in 0.5% Na-CMC [23].
(3) ZnO NPs-treated group: rats were treated with (150 mg/kg of ZnO NPs), suspending in 0.5 % Na-CMC [24].
(4) SP-treated group: rats were treated with (400 mg/kg of SP) [25].
(5) GA+ZnO NPs-treated group: rats were treated with (GA plus ZnO NPs) at similar used doses.
(6) GA+SP-treated group: rats were treated with (GA plus SP) at the like used doses.
(7) ZnO NPs+SP-treated group: rats were treated with (ZnO NPs plus SP) at similar used doses.
(8) GA+ZnO NPs+SP-treated group: rats were treated with (GA plus ZnO NPs plus SP) at similar used doses.

GA complement was administrated before the management of other ingredients. The managing of all toxic and amending agents was applied for 30 days via oral administration method, suspending in 0.5% Na-CMC (w/v) at 5 ml/kg. The rats were injected after one night from the up-to-date dose; they were sacrificed by cervical dislocation and distinguished: serum and brain tissues that were alienated into two fragments:

1) The fragment was instantly detached, crinkled, and homogenized with 0.9% NaCl in a homogenizer. The homogenates were centrifuged by 3.000 r.p.m and the following supernatant was engaged at –80 ̊C.
2) This fragment was fixed in 10% formalin buffered saline for the histopathological inquiry.

*2.5. Biochemical investigation:*

*2.5.1. Neural acetylcholinesterase activity (AChE) analysis:*

AChE activity was scrutinized in the brain samples, using the spectrophotometric according to [26]. It has hydrolyzed acetylthiocholine iodide into thiocholine and butyric acid. The thiocholine has reacted with 5,5′-dithiobis-2-nitrobenzoic acid to form 5-thio-2-nitrobenzoic acid. The yellow color was dignified using a spectrophotometer at 412 nm.

*2.5.2. Neural lactate dehydrogenase activity (LDH) analysis:*

20 µl of brain homogenate was added to 1 ml of working solution, mixed, and recited at an initial absorbance after 30 sec. Then, it must be read again after 1,2, and 3 min, and noticed the absorbance difference per minute to divide its activity [27].

*2.5.3. Serum total lipids level (TL) analysis:*

Serum retorted with sulfuric, phosphoric acids, and vanillin to form a pink-colored complex. The absorbance of this color was appraised at 545 nm using a spectrophotometer [28].

*2.6. Histopathological investigation:*

Brain samplings were fixed by using 10% neutral buffered formaldehyde. After proper fixation, the samplings were dehydrated in mounting rankings of ethyl alcohol, and then embedded in the paraffin wax. 5-µm thick segments were censored using a rotatory microtome, deparaffinized, desiccated, tarnished with hematoxylin and eosin (H&E.,) stain for studying the histological structure of the brain and perceived under a light microscope [29].

*2.7. Statistical investigation:*

Data were pigeonholed as a mean±standard deviation (mean±SD), using the statistical software package SPSS that was used one-way ANOVA trial to create a comparison between the biochemical indicators followed by Tukey's post hoc assessment. The level of significance was done at ($p<0.05$) [30].

## 3. Results:

*3.1. The organic compounds of ethanolic fraction of SP using UPLC-MS:*

**Table 1** displayed main organic amalgams of ethanolic fraction of SP, using UPLC-MS, which recognized the mass of the precursor ion and their fragments, together with neutral mass loss and identified fragmentation arrangements for the expected classes of *compounds*. Seventeen compounds were comprised from phenolic acids, flavonoids, alkaloids, and fatty acids that exposed their retention times (RT), m/z in both negative and positive ionization modes, and MS/MS fragments.

Phenolic acids appeared in the negative ion mode. Caffeic acid (1) demonstrated a deprotonated molecular ion at m/z 179 and MS2 fragment at m/z135 ($[M-H]^{--}$ $CO_2$). Fragment ions at (m/z 173, m/z 127, and m/z 85) were shaped by the precursor ion at m/z 191 $[M–H]^-$. This fragmentation pattern led to the recognition of quinic acid (2) and its isomer (12). The precursor ion of chlorogenic acid (3) at m/z 353 in $[M-H]^-$ and it was renowned by an illustrative fragment at m/z 191, signifying the certainty of quinic acid moiety after a neutral loss of caffeic acid (162 Da). Dicaffoelyquinic acid (10) and its isomer (11) exhibited a deprotonated molecular ion m/z at 515 ($[M-H]^-$) and a detailed fragment ion at m/z 191, proposing the presence of quinic acid moiety after the neutral loss of two caffeic acid moieties (2x162 Da). Two MS2 fragments at m/z 179 and m/z 135 were resembled to caffeic acid, which was identical to the testified facts of caffeic acid moiety.

Chlorogenic acid, the furthermost copious isomer amongst caffeoylquinic acid isomers, is a biologically active polyphenol that induces several biological activities, such as antioxidant Seneciphyllic acid (5) and its isomer (6) displayed a deprotonated molecular ion at m/z 213 and MS2 fragment at m/z 169 ($[M-H]^-$-$CO_2$).

Alkaloids appeared at the positive ion mode. Senecionine (7) possess a molecular weight of 335. It created fragment ions at m/z 120 and m/z 138 in MS2 spectrum, and peak at m/z 308 ($[M+H-28]^+$). From the MS data, the compound (4) at m/z 352 corresponding to $[M+H]^+$ was achieved. From the data of MS2 of the m/z 352, a sequence of distinguishing fragments ions at m/z 120 and m/z 138. Compound 4 has an unsaturated structure at 1, 2 sites and was recognized as monocrotolinine.

Flavonoids appeared through the negative ion mode. Five flavonoids were discovered in the scrutinized fraction. Quercetin hexoside was acclaimed for compound 9 at RT 6.97 min (m/z 463,$[M-H]^-$). In the MS2 spectrum, a fragment ion at (m/z 301) contains quercetin in the structure after hexose unit loss. Quercetin (15) was renowned for comparing its MS/MS fragmentation pattern with the previously informed records in the negative ionization mode. The compound related to peak 16 was recognized as 7,3'-dimethoxyquercetin. It revealed a deprotonated molecular ion at (m/z 329). The following MS/MS fragmentation of m/z 329 permitted the incidence of a base peak at (m/z 299), due to the loss of 30 Da (M-H-$2CH_3$)$^-$. Peak 13 at Rt 8.29 min gave a pseudo molecular ion at m/z 331 for 7-methoxymyricetin that was fragmented to give fragmention at (m/z 271) by losing one methoxy residue while the other two peaks (m/z 179,151) were indication for myricetin. One compound with a pseudomolecular ion $[M-H]^-$ at (m/z 609) was supposed and construed as rutin (quercetin-3-O-rutinoside) (8) with the MS2 daughter ion peak at m/z 301 for quercetin aglycone after the loss of rutinosyl moiety $[M-H-308]^-$.

Fatty acids appeared through the negative ion mode. There is a compound (17) that is lanopalmitic acid.

**Table (1):** Spectroscopy analysis of major metabolites identified in the ethanolic fraction of mureer (*Senecio glaucus* L.) using (HPLC-ESI-MS/MS) analysis in negative and positive ionization modes:

| m | Rt | Name | $[M-M]^-$ *(m/z)* | $[M-M]^+$ *(m/z)* | Fragment | Reference |
|---|---|---|---|---|---|---|
| **1** | **0.77** | **Caffeic acid** | **179** | | **135** | [31] |
| **2** | **2.49** | **Quinic acid** | **191** | | **191,173,155,147,127,111,85** | [32-33] |
| **3** | **2.52** | **Monocrotalinine** | **353** | | **191** | [31] |
| **4** | **3.49** | **Monocrotalinine** | | **352** | **322,278,156,138,120,94** | [34] |
| **5** | **5.16** | **Seneciphyllic acid** | **213** | | **213,169** | [31] |
| **6** | **5.30** | **Seneciphyllic acid isomer** | **213** | | **213,169** | [31] |
| **7** | **6.17** | **Senecioine** | | **336** | **308,290,138,120** | [34] |
| **8** | **6.82** | **Rutine** | **609** | | **301,300,285,271,255,179,151** | [35-36] |
| **9** | **6.97** | **Quercetin hexoside** | **463** | | **301,300,271,255,179,151** | [36] |
| **10** | **7.45** | **Dicffeoylquinic acid** | **515** | | **191,179,135** | [31-36] |
| **11** | **7.71** | **Dicffeoylquinic acid isomer** | **515** | | **191,179,135** | [31-36] |
| **12** | **7.73** | **Quinic acid isomer** | **191** | | **191,173,155,147,127,111,85** | [32-33] |
| **13** | **8.29** | **7-Methoxymyricetin** | **331** | | **271,197,151** | [31-36] |
| **14** | **14.03** | **unknown** | **675** | | **415,397,277,235,161,125,119,113, 101,89** | |
| **15** | **15.75** | **Quercetin** | **301** | | **213** | [31] |
| **16** | **16.07** | **7,3 Dimethoxyquercetin** | **329** | | **299** | [31] |
| **17** | **20.80** | **Lanopalmitic acid** | **271** | | **225** | [37] |

*3.2. Biochemical assessment:*

**Table (2)** revealed that either single or dual treatments of ZnO NPs and SP may be persuaded neural toxicity and the pretreatment of GA induced a neural-protective influence against them through exploring of neural function biomarkers: [neural acetylcholinesterase activity (AChE)(IU/g tissue), neural lactate dehydrogenase activity (LDH) (U/mg protein), and serum total lipids level (TL)(mg/dl)].

On a hand, our results found that a significant upsurge in neural LDH activity, and serum TL level; however, they prompted a significant diminution in the activity of AChE compared to the control group ($p<0.001$). Additionally, there was no significant change between control and GA-treated groups in all

established strictures. Moreover, the vicissitudes in these strictures of the dual treatment of ZnO NPs and SP (ZnO NPs+SP-treated group) were harder than the vicissitudes of the single treatment of them (ZnO NPs-treated group or SP-treated group). Besides, the disparities in these factors of ZnO NPs-treated group were stronger than the disparities of SP-treated group. There was no significant modification in all factors between the control group and GA-treated group.

On the other hand, the addition of GA to ZnO NPs and SP significantly reduced neural LDH activity and serum TL level; hitherto, they significantly increased the activity of AChE enzyme relative to either single or dual treatments of ZnO NPs and SP as follows: (GA+ZnO NPs-treated group relative to ZnO NPs-treated group, GA+SP-treated group relative to SP-treated group, and GA+ZnO NPs+SP-treated group relative to ZnO NPs+SP-treated group), ($p<0.001$).

**Table (2):** Influence of zinc oxide nanoparticles (ZnO NPs), Mureer (*Senecio glaucus* L.) (SP) plant, and gallic acid (GA) on biochemical analyses: The activities of [neural lactate dehydrogenase enzyme (LDH) (U/mg protein) and neural acetylcholinesterase enzyme (AChE) (IU/g tissue)], and serum total lipids level (TL) (mg/dl).

| Groups | LDH (U/mg protein) | AChE (IU/g tissue) | TL (mg/dl) |
|---|---|---|---|
| **Control** | **34.31±1.03** | **0.16±0.01** | **400.16± 2.45** |
| **GA** | **33.41±0.92** $^{n.s.g}$ | **0.17±0.01**$^{n.s.g}$ | **393.79± 3.59** $^{n.s.g}$ |
| **ZnO NPs** | **181.56±1.25**$^{***a}$ | **0.13±0.02**$^{***a}$ | **1164.43± 3.71**$^{***a}$ |
| **SP** | **88.19±1.39**$^{***}$ | **0.12±0.01**$^{***}$ | **792.64±1.89**$^{***}$ |
| **GA+ ZnO NPs** | **132.05±2.12**$^{***d}$ | **0.16±0.02**$^{***d}$ | **845.03 ± 2.89**$^{***d}$ |
| **GA+ SP** | **51.74±1.28**$^{***e}$ | **0.17±0.02**$^{***\ e}$ | **552.58±2.18** $^{***\ e}$ |
| **ZnO NPs+SP** | **188.13±1.13**$^{***b,\ c}$ | **0.08±0.05**$^{***b,\ c}$ | **1341.81±7.23**$^{***b,\ c}$ |
| **GA+ZnO NPs+SP** | **105.07±1.01**$^{***\ f}$ | **0.27±0.11**$^{***f}$ | **772.04±2.29**$^{***f}$ |

Values were given as mean ± SD, (n=5 rats per group). Statistical analysis was done by using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons between groups. Compared to the control group, highly significant$^{***}$ ($p<0.001$) and $^{n.s.}$ (p is non-significant). a, b, c, d, e, f letters represent the relations between treated groups at $p< 0.05$: [$^{a}$ZnO NPs relative to SP, $^{b}$ZnO NPs+SP relative to ZnO NPs, $^{c}$ZnO NPs+SP relative to SP,$^{d}$GA+ZnO NPs relative to ZnO NPs, $^{e}$GA+SP relative to SP, $^{f}$GA+ZnO NPs+SP relative to ZnO NPs+SP, $^{g}$GA relative to control].

*3.3. Histopathological assessment:*

**Figure 1 (a-h)** revealed that the histopathological clarifications of neural tissue in the diverse groups. Our records showed that the lethal effects of either single or dual treatments of ZnO NPs and SP and revealed the protective effect of GA against their effects with induction an improvement in the tissue integrity. There was a typical histological structure of the cerebral cortex neural cells, including astrocytes, pyramidal cells, and ordinary blood vessels appeared in the control group **(Figure 1a)**. There was a healthy histological arrangement of the cerebral cortex neuroglia cells parallel to the control group in GA-treated group **(Figure 1b)**. On a hand, the profoundly stained shrunken nuclei or pyknosis of neurons, the entrance of a hefty extent of neural degeneration, and the arrival of vacuolization in the cytoplasm occurred in ZnO NPs-treated group **(Figure 1c)**. Still, pyknosis of neurons and vacuolated cytoplasm transpired in the SP-treated group **(Figure 1d)**. On the other hand, the co-administration of GA prompted a perfection in the anomalous neural cells with a trivial vacuolization acted in the cytoplasm of GA+ ZnO NPs-treated group, **(Figure 1e)**. Moreover, a slight vacuolization zone and slight lesions of neuronal degeneration ensued in GA+SP-treated group, **(Figure 1f)**. Regrettably, dual treatment impelled the atypical attendance of neural cells, including focal gliosis, smattering distorted cells with genuinely tarnished shrunken nuclei in the cytoplasm, dilation of the blood vessels, and dissemination of regions of a cerebral hemorrhage executed in ZnO NPs+SP-treated group, **(Figure 1g)**. Lastly, slight gliosis expanses and marginal vacuolated lesions arose in ZnO NPs+SP-treated group, **(Figure 1h)**. Microscopically, our pamphlets also offered that the neurotoxic impact of SP was more than the impact of ZnO NPs and GA regards as a neuroprotective agent.

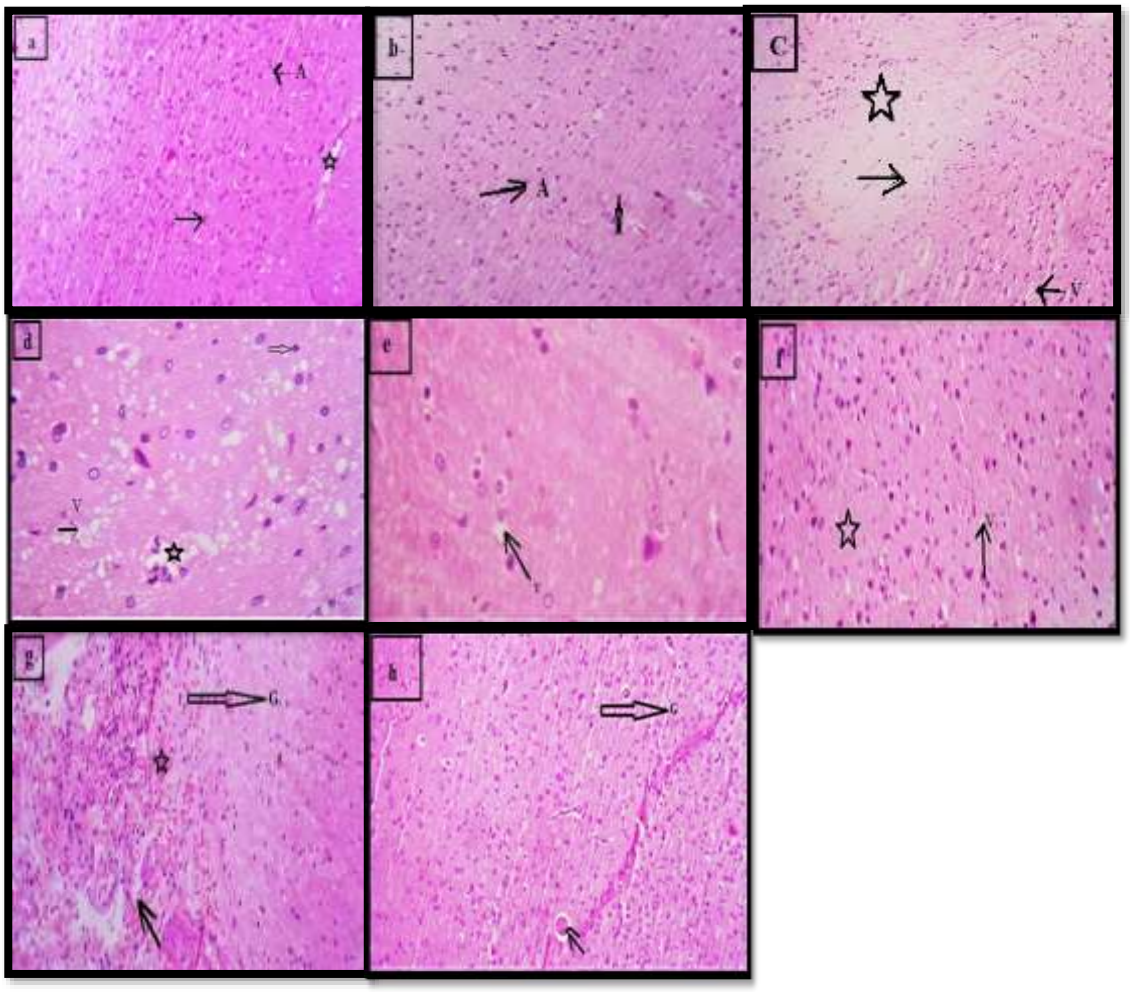


**Figure 1(a-h):** Photograph of influence of zinc oxide nanoparticles (ZnO NPs), Mureer or *Senecio glaucus* L. plant (SP), and gallic acid (GA) on the histopathological structure of neural tissue in different groups. **a)** Control group showing a normal histological structure of the cerebral cortex of neuroglia cells: astrocytes (A), pyramidal cells (arrow), and normal cerebral blood vessels (star). **b)** GA-treated group showing a healthy histological structure of the cerebral cortex of neuroglia cells: astrocytes (A) and normal cerebral blood vessels (star). **c)** ZnO NPs-treated group showing abnormal neural cells with deeply stained shrunken nuclei or pyknosis of neurons (arrow), a large area of neural degeneration (star), and an appearance of vacuolization in the cytoplasm (V) (H&EX200). **d)** SP-treated group showing pyknosis of neurons (arrow), vacuolated cytoplasm (V), and congestion of cerebral blood cells (star). **e)** GA+ZnO NPs-treated group showing a mild vacuolization in the cytoplasm (V)(H& E.,X400). **f)** GA+SP-treated group showing mild vacuolization (V) along with minimal lesions of neuronal degeneration (star). **g)** ZnO NPs+SP-treated group showing a scattering of focal gliosis to neural cells (deeply stained shrunken nuclei in the cytoplasm)(G), dilation of blood vessels (arrow), and distribution of areas of cerebral hemorrhage (star). **h)** GA+ZnO NPs+SP-treated group showing mild gliosis (G) along with minimal vacuolated lesions in the cytoplasm (arrow) (H&E.,X200).

## 4. Discussion:

From the biochemical investigations, either single or dual treatments of ZnO NPs and SP induced a disturbance in the biochemical and histological analyses of the neural tissue. Furthermore, the secondary phytochemical amalgams were showed in spectroscopy analysis that strewed the reasons for lethal effects of SP in neural cells. These results endorsed that they persuaded an interruption in brain function for the neurotransmitter synthesis level and neural mutilation. These consequences were in the corresponding line with, Zia et al. [38], Balážová et al. [39] who showed that NPs handlings prompted brain mutilation in the mammalian cells due to initiation of the programmed cell death in brain cells.

The histopathological analyses were confirmed by the biochemical analyses in which either alone or dual treatments of ZnO NPs or SP exhibited as neurotoxic

agents, and GA was proceeded as a neuroprotective agent. In precise, the brain is an imperative portion of the central nervous system for the mammalian body. It comprises from BBB, which is composed of the endothelial cells connected to elaborate snug junctions and other neural cells, such as astrocytes and pericytes [40]. The materials can pass through BBB by one of three foremost transport alleyways (passive diffusion, active transport, and endocytosis) that have a selective eccentric for passing ions and materials to protect the brain from impairment. In order to, a cerebral grievance of AChE enzyme is used in indisputable evaluation for its exploration due to its function as a neurotransmitter in transporting of neural synapse and brain reliability. Commonly, neurotransmitters are endogenous chemical messengers of the neural cells that can communicate with the neural signal crossways between synapse and neuromuscular connections [41].

Likewise, LDH enzyme is the fundamental biomarker used in the estimation of cytotoxicity through cerebral mutilation and hypoxia induction. The underlying pathophysiologic mechanisms of the greatest neurotoxins undergo not entirely understood or may be due to numerous concepts. They affect the structure of nucleic acids, the activity of confident enzymes, disturbance of the uptake of catecholamine, and levels of abundant neurotransmitters, leading to filibustering of the synaptic transmission at peripheral cholinergic and adrenergic synapses. Thus, the greatest neurotoxicants flow to the vascular system, generating a humiliation of neuronal survival and synaptic plasticity [42].

From the initial echo, the promotion in LDH activity can attribute to a hindrance in AChE activity, which causes constant stimulus in the nervous tissue, leading to lack in the source of the energy (ATP) and phosphocreatine. Hence, it can drive the increased glycolysis associated with a diminution in pyruvic acid concentration inside brain tissue. It is primarily results in neurological signs, including diminishing balance, coordination, and mutilation in the sensory nerves, muscle weakness, and grievance to the retina of the eye [43].

Some investigators revealed that neurotoxins could argue alterations in the integrity and permeability of BBB due to the assertion of LDH. It stimulates a discharge of the pro-inflammatory cytokines in the brain cells, causing inflammation. Pro-inflammatory cytokines may be incited complaints in the learning and memory functions [44].

An increase in neurotransmitters secretions plays an indispensable role in both physiological and pathological variations that cause an elevation in the intracellular calcium secretion. Therefore, it coaxes depression, instabilities in the neural hemostasis balance, and an anticipation of the cell death hitches, such as neuron shrinkage, swelling of astrocytes, and extra-vascular lymphocytes. Besides, neural cell death prompts a surge in sodium permeability, which surpasses a capacity of the pump to extrude the sodium ions. Accretion of the sodium ion in the intracellular tissues leads to rising in the water content, and producing cellular gliosis. Additionally, neural vacuolization might be attributed to shrinkage of the neural cells, leaving pericellular spaces. They are symptomatic of the neuronal necrosis [45].

Our records are reliable with our previous elucidations, Teleanu et al.[46] who showed that NPs management convinced neuron deficiency, an impairment of the spatial recognition memory, and a disorder in the knowledge abilities due to prevalence of the dysfunction of BBB and a neural atrophy incidence. Moreover, our results were in the like line with academics, Marzban et al.[47] who presented that NPs incited necrosis, hyperemia, gliosis, and spongy vagaries in the brain tissue after the appearance of oxidative damage instigated an alternation in the behavior of rats.

Still, our findings of the plant extract were in contract with preceding studies, Panziera et al.[48], García et al.[49] who presented that the treatment with *Senecio* plants instigated variations in the levels of dopamine and serotonin, maternal behavior complaint, panic, ataxia, tight walking, and blindness due to an accretion of their phytochemical amalgamations in the brain cells.

In distinction, our documents revealed that the administration of GA with either alone or dual treatments of ZnO NPs and SP created an augmentation in the atypical neural cell structure that stimulated the neural cell integrity, encompassing from their synthetic and secretive activities. Hence, GA acts as a neuroprotective agent that inhibits neurotoxicity deed induced by ZnO NPs and SP. Intriguingly, the neuroprotective role of GA against brainy mutilation due to its organic erection that can capture the free radicals and withdraw the mutilation to BBB integrity and neural grievance. It can recover the deterioration of synaptic plasticity, which persuades the hippocampal innervation and recognition memory [50].

Our outcomes were in contract with the aforementioned scientists, Sun et al. [51],Yadav et al.[52] who indicated that pretreatment of GA reversed BBB permeability and significantly decreased malondialdehyde (MDA) level due to its ability in an inequality of the mitochondrial potential, avoiding the cell death prevalence. Inclusive, GA expressively alleviates cholinergic dysfunction and inflammation in the brain because it repairs the disorders in synaptic transmission and tissue integrity for maintaining neural utilities. The histological outcomes demonstrated with the biochemical results, suggesting the beneficial effects of GA against neurotoxicity induced by ZnO NPs and SP in rats.

Moreover, our outcomes were similar to stripe with, Liu et al. [53], Chandrasekhar et al.[54] who found that GA persuaded a recovery of the brain defacements, such as inflammation and necrosis, leading to the regulation of LDH secretion. Thus, it can modify acetylcholine metabolism through a regulation of the secretion of the

AChE enzyme and its ability to protect neurons from the degeneration.

## 5. Conclusion:

Our results confirmed that either single or dual treatments of ZnO NPs and SP induced neural toxicity through the biochemical and histological analyses. Further, GA can reverse some the deleterious effects of them in the neural tissue.

## 6. Recommendations:

Unimaginative of noticing is that of single or dual treatments of ZnO NPs and SP can use as atypical insect repellents; yet, the hopeful GA is used as anti-neurotoxic agent in the future.

## 7. Declaration of interest:

The authors declare that there are no competing conflicts of interest.

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## 9. References:

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