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Assessment of the Protective and Ameliorative Role of Quercetin Nanoparticles against Histopathological Changes Induced in the Cerebral Cortex of Rats by Acrolein

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ABSTRACT **: Alzheimer's disease is the most prevalent cause of dementia. The flavonoid quercetin has low bioavailability. Quercetin nanoparticles have been shown to have greater bioavailability. The purpose of the current investigation was to assess any potential histopathological damage caused by acrolein in the cerebral cortex. The potential effects of quercetin nanoparticles against the neurotoxicity caused by acrolein were also investigated. In the present investigation, rats were divided into six groups as follows: control group; acrolein-treated group in which rats were given acrolein (3 mg/kg) for 30 days; quercetin nanoparticles treated group in which rats were given quercetin nanoparticles (30 mg/kg) for 30 days; ameliorative group in which rats were given acrolein and quercetin nanoparticles at the same time daily for 30 days; protective group in which rats received first quercetin nanoparticles for 30 days, followed by acrolein for another 30 days; recovery group in which rats received acrolein for 30 days, and they left without any treatment for an additional 30 days. The results indicated that acrolein administration was linked to histopathological changes and significant damage in the cerebral cortex. These were visible in acute ischemia neuronal damage, which was manifested as nuclear pyknosis, neuronophagia, and focal areas of Malacia connected to gliosis. It has been resulted that the treatment of rats with quercetin nanoparticles protected and improved the cerebral cortex from the oxidative stress caused by acrolein. This result could pave the way for additional research in nanomedicine and a new line of therapeutic intervention in Alzheimer's disease using nanoparticles.**

KEYWORDS: ***Acrolein, Albino rats, Cerebral cortex, Histopathology, Quercetin nanoparticles.***

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I. INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in the elderly and is a deadly neurodegenerative brain illness [1, 2]. Neurons in different parts of the brain lose their ability to function, which is a hallmark of Alzheimer's disease. This disease is brought on by hyperphosphorylated Tau proteins and deposits of β-amyloid fragments (Aβ), which result in the formation of neurofibrillary tangles and amyloid plaques, respectively [3,4].

Acrolein is α, β-unsaturated, highly electrophilic aldehyde [5]. It's presence in the environment is primarily because of the incomplete combustion of organic molecules. In kitchens, it is produced during high-temperature roasting and when food is cooked or fried in solid or liquid oils [6]. Endogenous acrolein synthesis occurs during lipid peroxidation and polyunsaturated fatty acid degradation [7]. It is also found in a variety of meals, fruits, and drinks, as well as in volatiles created by the high-temperature thermal treatment of animal or vegetable fat [8]. It is a biocide that is commonly found in agricultural and industrial water supply systems [9]. It contributes to the advancement of oxidative damage and, as a result, the pathogenesis of certain disorders such as Alzheimer's disease [10,11].

Quercetin is a flavonoid which is present in many fruits and vegetables, such as white onion bulbs, fennel leaves, red onion, sweet potatoes, berries, citrus fruits, and tea [12]. Anti-inflammatory, antioxidant, and anti-cancer characteristics are just a few of the health advantages of quercetin [13]. In many neurodegenerative conditions, the combination of anti-inflammatory and antioxidant properties can increase neuron survival and help to decrease cell death, delaying the onset of disease [14]. It has numerous medicinal advantages, but it is

also water-insoluble and has a low oral bioavailability in the blood [15]. Additionally, this blood-brain barrier, a close relationship between blood capillaries and interstitial fluid, cannot be penetrated by this water-insoluble polyphenolic molecule, which poses a significant obstacle to central nervous system therapy [16]. It's bioavailability in rats is 17% and in humans, it is only 1%, which limits its absorption and bioactivity [17,18].

To circumvent this limitation, nanoparticles were used, with the goal of improving the movement of quercetin and other natural and synthetic chemicals [19]. Drug nanoparticle technologies have received a lot of attention in recent years since they are recognized as one of the most successful approaches to developing poorly soluble medications [20, 21]. Quercetin nanoparticles improve quercetin solubility and bioavailability [22]. Because of their unique physicochemical properties, they can cross multiple barriers, including the blood-brain barrier [23, 24].

Because of their remarkable stability, high bioavailability, and rapid ability to cross the blood-brain barrier, nanoparticles have sparked substantial interest in the treatment of Alzheimer's disease, particularly for hydrophobic compounds like quercetin. As a result of its low oral bioavailability, low brain permeability, and hydrophobic nature, researching the positive effects of quercetin remains a difficult challenge. As a result, the current work is thought to evaluate and assess the potential histological abnormalities which might result from any potential toxic consequences generated by acrolein, as well as the ameliorative and protective impact of quercetin nanoparticles.

II. MATERIALS AND METHODS

1. Experimental animals:

The current investigation employed 90 adult male albino rats (*Rattus norvegicus*) weighing 150-200 grams (aged 10 to 12 weeks). They were received from the National Research Center in Cairo, Egypt. Throughout the trial, the animals were housed in polypropylene cages with wire-bar covers and pine shaving for bedding, and they were kept under standard laboratory conditions of aeration, a room temperature of roughly 22-25˚c, and a 12-hour light/dark cycle. The current experimental design has been examined and authorized by the research ethical committee of Zagazig University, Egypt (approval number Zu-IACUC/1/F/306).

2. Chemicals:

Acrolein (99% pure) was obtained from Pharmachem Fine Chemicals for Research and Industry, Mumbai, India. Quercetin (more than 98% pure) was purchased from Fine-Chem Limited (SDFCL), Industrial Estate, 248, Worli Road, Mumbai, Maharashtra, India. All the chemicals utilized in this research were of the highest commercial grade.

3. Quercetin nanoparticle preparation:

The precipitation method described with some modifications by [25] was used to manufacture quercetin nanoparticles.

4. Experimental design:

The rats were divided into six groups of fifteen rats each after two weeks of acclimatization, as follows:

- Group 1 (Control group): rats were given 1 ml of distilled water orally for 30 consecutive days.
- Group 2 (Acrolein-treated group): rats were administered acrolein orally (3 mg/kg body weight) using a gastric tube [26] for 30 consecutive days.
- Group 3 (Quercetin nanoparticles treated group): rats received a daily dose of quercetin nanoparticles (30 mg/kg body weight) for 30 days [27] by using a gastric tube.
- Group 4 (Ameliorative group): rats were fed acrolein (3 mg/kg body weight) and quercetin nanoparticles (30 mg/kg body weight) orally at the same time every day for 30 days using a gastric tube.
- Group 5 (Protective group): With the aid of a gastric tube, rats were initially administered a dose of quercetin nanoparticles (30 mg/kg body weight) every day for 30 days followed by administration of acrolein (3 mg/kg body weight) every day for another 30 days.
- Group 6 (Recovery group): Using a gastric tube, rats were administered acrolein (3 mg/kg body weight) every day for 30 days and after that, the rats were left for 30 days without any treatment for recovery.

5. Necropsy schedule:

Following treatment, the animals underwent cervical decapitation after 24 hours and their skulls were opened. To expose the cerebral cortex, brains were removed and were split into two hemispheres. Both the control and treated rats' cerebral cortexes were sectioned at the same thickness and fixed in the same fixative.

6. Histological techniques:

After decapitation, materials from both the control and treated rats were immediately fixed in 10% neutral buffered formalin for 24 hours, then washed in tap water, transferred to 70% ethanol, dehydrated in an

ascending series of ethanol, cleared in xylene, and finally embedded in paraffin wax. A rotary microtome (Leica, Model Rm 2125, Germany) was used to cut serial sections around 5 µm in thickness, which were subsequently stained with Ehrlich's haematoxylin and aqueous eosin [28] to demonstrate the histological structure and the histopathological changes.

7. Morphometric analysis for the cerebral cortex:

The thickness of the cerebral cortex was measured from the haematoxylin and eosin-stained horizontal brain sections (five randomly chosen sections from five different animals from both control and treated groups) at 400X magnification [29]. The thickness of the cerebral cortex was determined by using the image analysis software Image J.

8. Semiquantitative scoring of neuronal histopathological lesions:

A semiquantitative scoring technique was used to assess the extent of brain tissue damage, in which five random fields were examined from each section. The severity of neuronal damage in the cerebral cortex was scored semiquantitatively. Cellular edema, ischemic injury, Malacia, gliosis, and the extent of the lesions were all examined in tissue sections.

The severity of lesions was scored and graded according to the percentage of affected tissue, as follows: none (-) = 0%, meaning no detectable lesions; mild lesions (+) = 5–25% of the examined field; moderate lesions (++) = 25–50% of the examined field; severe focal lesions (+++) = 50–80% of the examined field; and severe diffuse lesions (++++) = 80–100% of the examined field [30].

9. Statistical analysis:

The results were expressed as the mean ± SD of the different treated groups. The differences between the mean values were evaluated by ANOVA followed by the Student's t-test using the Minitab 12 computer program (Minitab Inc., State College, PA, USA), and a P-value of $P < 0.05$ was considered statistically significant.

III. RESULTS

1. **Histological studies**:

Investigation of sections from the cerebral cortex of the normal control group of rats revealed that the cerebral cortex is a dense layer of neurons that are classified into five distinct types with distinct morphological characteristics, which form distinct layers of the cortex. These cell types are horizontal cells, stellate cells, Martinotti cells, pyramidal cells, and fusiform cells. The most common cell types are the pyramidal and the stellate cells. The cerebral cortex is divided into six basic layers that are distinguished by cell type, size, density, and arrangement, with regional variation. The six layers from the pia surface to the deep part of the cortex are the molecular layer, external granular layer, pyramidal layer, internal granular layer, ganglionic layer, and multiform layer as shown in Fig. (1). The pyramidal layer of the cerebral cortex is made up of medium-sized pyramidal neurons in the upper part and a few larger neurons in the deeper part as presented in Fig. (2).

The pyramidal layer of the cerebral cortex sections of the acrolein-treated group demonstrated severe ischemic neuronal injury as shrunken cytoplasm, nuclear pyknosis, pericellular vacuolation, and marked neuronophagia, as well as focal areas of Malacia associated with marked gliosis, as seen in Figs. (3&4). The pyramidal layer of the cerebral cortex sections from rats of the quercetin nanoparticles treated group exhibited a normal histological structure. Pyramidal-shaped neuronal bodies appeared normal with obvious tangles of unmyelinated nerve fibers, as presented in Fig. (5). The ameliorative role of quercetin nanoparticles was apparent in rats from the ameliorative group. In this case, the pyramidal layer of the cerebral cortex showed a marked reduction in the ischemic neuronal degenerative alterations as indicated in Fig. (6). In specimens obtained from rats of the protective group, quercetin nanoparticles were found to have a marked protective effect. The pyramidal layer of the cerebral cortex demonstrated minimal ischemia neuronal damage. Neuronal bodies in the pyramidal layer appeared normal as present in the control group but they still contained mild neuronophagia as shown in Fig. (7). The histological structure of the cerebral cortex's pyramidal layer has been improved slightly in the recovery group. As seen in Fig. (8), the pyramidal layer of the cerebral cortex indicated a modest degree of ischemia neuronal damage.

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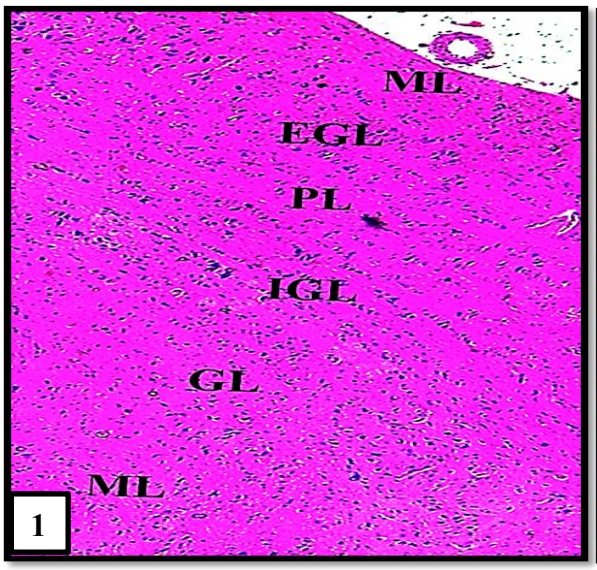


Fig.1: Photomicrograph of a section in the cerebral cortex of the control group showing the normal histological structure of the cerebral cortex, as it consisted of six fundamental layers: Molecular layer (ML), External granular layer (EGL), Pyramidal layer (PL), Internal granular layer (IGL), Ganglionic layer (GL) and Multiform layer (ML). (X100)

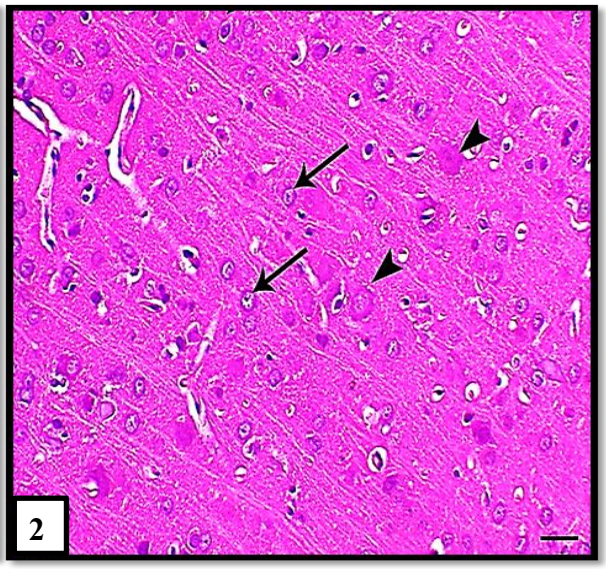


Fig.2: Photomicrograph of a section in the pyramidal layer of the cerebral cortex of the control group showing normal neuronal cells (arrowheads) and normal astroglia cells (arrows). (X400)

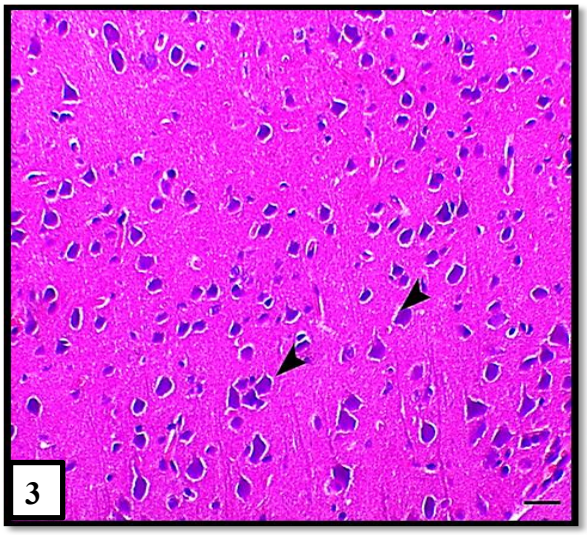


Fig.3: Photomicrograph of a section in the pyramidal layer of the cerebral cortex of the acrolein-treated group showing a severe degree of ischemic neuronal injury noticed as shrunk cytoplasm, nuclear pyknosis, pericellular vacuolation, and marked neuronophagia (arrowheads). (X400)

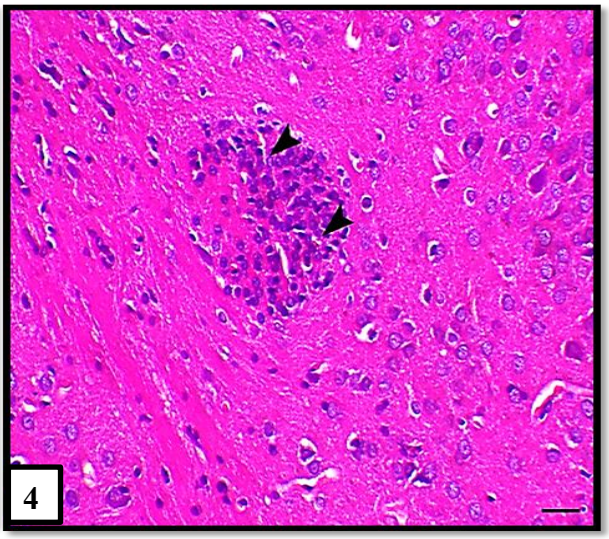


Fig.4: Photomicrograph of a section in the pyramidal layer of the cerebral cortex of the acrolein-treated group showing a focal area of Malacia associated with marked gliosis (arrowheads). (X400)

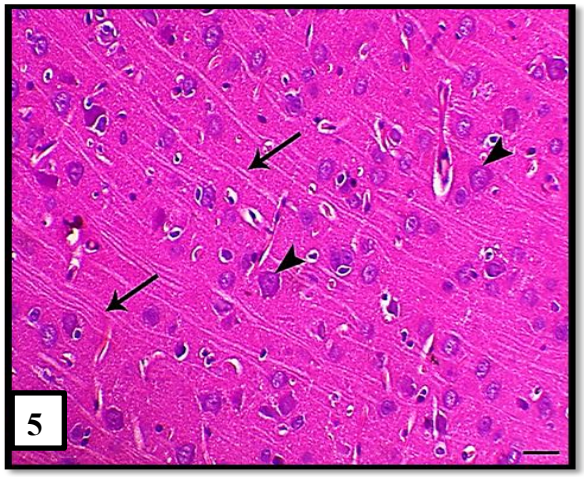


Fig.5: Photomicrograph of a section in the pyramidal layer of the cerebral cortex from the quercetin nanoparticles treated group showing normal pyramidal-shaped neuronal bodies (arrowheads) with apparent tangles of unmyelinated nerve fibers (arrows). (X400)

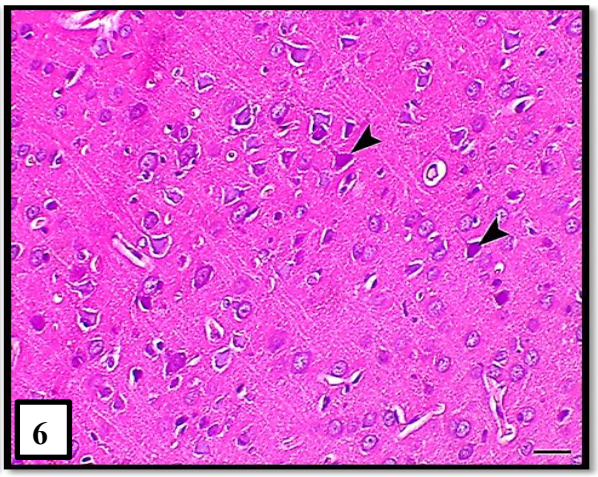


Fig.6: Photomicrograph of a section in the pyramidal layer of the cerebral cortex from the ameliorative group showing a marked decrease in the ischemic neuronal degenerative changes (arrowheads). (X400)

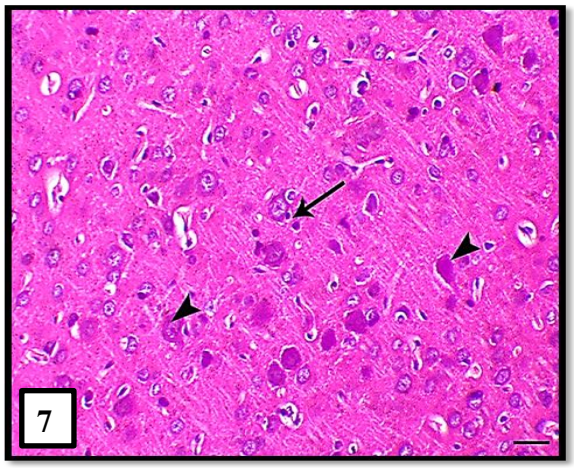


Fig.7: Photomicrograph of a section in the pyramidal layer of the cerebral cortex from the protective group showing a minimal degree of ischemic neuronal injury (arrowheads indicate normal neuronal bodies and arrow indicates mild neuronophagia). (X400)

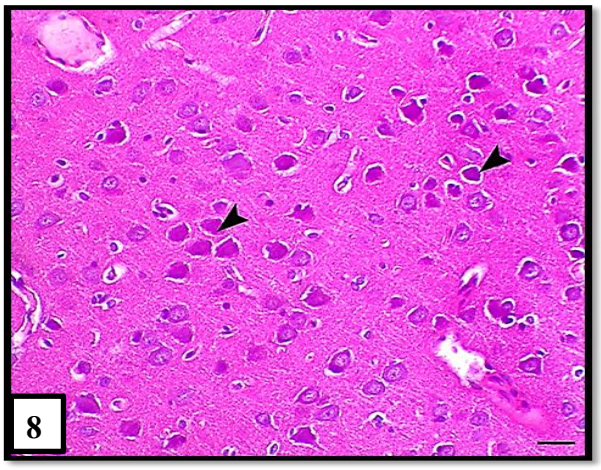
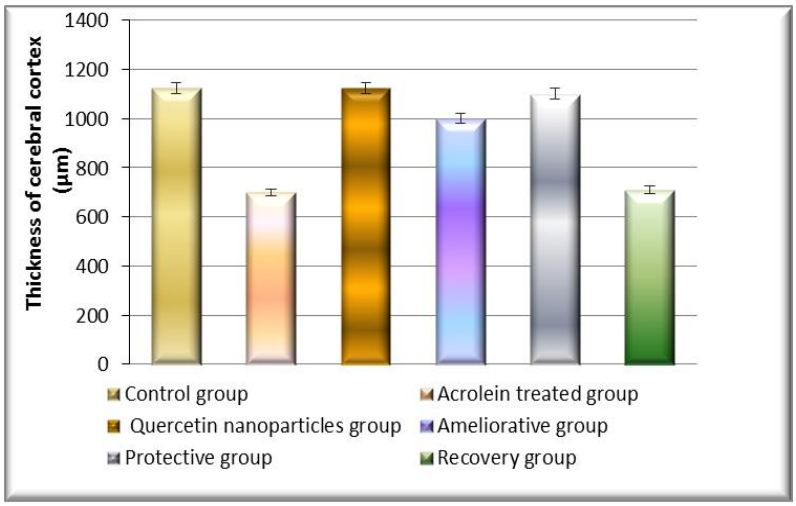


Fig.8: Photomicrograph of a section in the pyramidal layer of the cerebral cortex from the recovery group showing a moderate degree of ischemic neuronal injury (arrowheads indicate the degenerated neurons). (X400)

2. Morphometric analysis for the cerebral cortex:

- **The thickness of the cerebral cortex:**

The thickness of the cerebral cortex of the acrolein-treated rats was significantly lower than that of the control group. This thickness was improved dramatically after treatment with quercetin nanoparticles in both the ameliorative and protective groups. In the recovery group, the thickness of the cerebral cortex was somewhat improved compared to the acrolein-treated group (Histogram 1).



Histogram (1): The thickness of the cerebral cortex (µm) in the different experimental groups. Data are expressed as (mean±SD) in the different treated groups, ($P < 0.05$).

3. Semiquantitative scoring of neuronal histopathological lesions in the cerebral cortex:

According to the semiquantitative grading of the reported lesions, the acrolein-treated group exhibited considerably higher cerebral cortex damage than the control group. Table (1) shows a semiquantitative assessment of neuronal histopathological lesions in the cerebral cortex that demonstrated significant cellular edema, ischemic injury, Malacia, gliosis, and lesion extent in the acrolein treated group. Animals administered quercetin nanoparticles in both the ameliorative and protective groups had considerably less cerebral cortex damage compared to the acrolein-treated rats.

Semiquantitative scoring revealed moderate ischemic injury and mild Malacia, gliosis, cellular oedema, and the extent of lesions in the ameliorative group. According to semiquantitative scoring, the protective group exhibited no cellular edema, gliosis, or extent of lesions, moderate ischemic injury, and mild Malacia. The semiquantitative grading of the recovery group revealed the severe ischemic injury, cellular oedema, and the extent of lesions, as well as moderate Malacia and gliosis as presented in Table (1).

Table (1): Semiquantitative scoring of neuronal histopathological lesions in the cerebral cortex within the different treated groups.

Groups	**Cellular oedema**	**Ischemic injury**	**Malacia**	**Gliosis**	**Extent of lesions**
Control group	-	-	-	-	-
Acrolein treated group	+++	++++	+++	+++	+++
Quercetin nanoparticles treated group	-	-	-	-	-
Ameliorative group	+	++	+	+	+
Protective group	-	++	+	-	-
Recovery group	+++	+++	++	++	+++

(-) means no detectable lesions; (+) indicates mild lesions; (++) indicates moderate lesions; (+++) indicates severe focal lesions; (++++) indicates severe diffuse lesions.

IV. DISCUSSION

Alzheimer's disease is a neurological illness that destroys brain cells, causing memory loss and severe mental and behavioral problems [31]. This disease is caused by the accumulation and misfolding of the amyloid-b (Aβ) protein, such as Aβ 40 or Aβ 42, which is known to be harmful to neuronal cells [32]. Another crucial factor contributing to this disease is oxidative stress [33]. Alzheimer's disease currently has no viable treatment available [34].

The present study aimed to evaluate the possible histopathological alterations which might occur as a result of any possible toxic effects induced by acrolein and to assess the ameliorative and protective roles of quercetin nanoparticles in the cerebral cortex.

Quercetin has been identified to be a promising chemical with neuroprotective properties against Alzheimer's disease [35]. It has been demonstrated to prevent lipid peroxidation, protein oxidation, and neuronal cell death associated with Alzheimer's disease [36, 37]. Because of its low water solubility, it is currently challenging to employ it as a chemopreventive medication when taken orally [38]. The promise of quercetin hasn't been fully exploited due to a number of possible variables, including low brain permeability, low oral bioavailability (2%), significant first-pass metabolism, hydrophobic nature, physiological pH instability, and photodegradation [39, 40]. This difficulty can be solved using nanotechnology, which increases the chemical's bioavailability and makes it possible to target it to certain tissues and organs [41]. Drug-loaded structures that can pass the blood-brain barrier, targeted medication delivery, and increased drug bioavailability have all been made possible through nanotechnology [42, 43]. Nanoparticles have a higher surface area due to their tiny size, which improves their effectiveness and medication absorption [44]. Additionally, because of their small size, nanoparticles are particularly helpful for delivering chemicals that are insoluble in water [45]. Quercetin nanoparticles increased the relative oral bioavailability of quercetin by 523% [46].

In the present study, acrolein has been found to cause damage to the pyramidal layer of the cerebral cortex, as evidenced by the presence of severe ischemic neuronal injury as shrunken cytoplasm, nuclear pyknosis, pericellular vacuolation, and marked neuronophagia, as well as focal areas of Malacia associated with marked gliosis. Similar results have been reported by Selmanoğlu et al. [47] who found that acrolein caused many histological abnormalities in brain tissues, such as shrinkage of cell nucleus/pyknosis and loss of cytoplasm, as well as aberrant accumulation of neurofilament. Pyknosis, which is an irreversible condensation of the nucleus, has traditionally been utilized as a cell death marker [48]. Furthermore, acrolein-induced neurotoxicity has been previously linked to oxidative stress, protein aggregation, and cell death [49]. According to Tanel and Averill-Bates [50], Acrolein-induced neurotoxicity has been linked to a variety of cell death mechanisms, including necrosis and apoptosis. Acrolein is a lipid peroxidation byproduct and catalyst [51].

The mechanism by which acrolein produces oxidative damage and neurotoxicity indicated in acrolein primarily binds and depletes cellular nucleophiles, such as reduced glutathione (GSH), lipoic acid, and thioredoxin. Acrolein causes the loss of antioxidants such as GSH and Superoxide dismutase, as well as an increase in ROS generation [52]. Oxidative stress can limit dopamine production, as well as cause damage to neuronal cell nuclei and mitochondrial DNA, decrease antioxidant enzyme activity, and increase lipid peroxidation products [53]. Acrolein attacks the free thiol-treated group of cysteine residues, γ-amino treated group of lysine residues, and histidine residues, forming an acrolein-amino acid adduct that inhibits the function of selected proteins by adding a carbonyl-treated group of rats [54]. The toxicity of acrolein has been linked to its extremely electrophilic character, which makes it easier for it to react with cellular compartments [55].

Acrolein can cause oxidative stress by interacting with proteins [56] and it can potentially interact and alter the structure of some proteins such as albumin α-1-proteinase, human serum albumin, and axonal cytoskeletal proteins. As a result, it may damage neurons by attaching to a variety of proteins and causing oxidative stress, which can lead to neuronal dysfunction and death [57]. It has been proposed that acrolein's neurotoxic effect is mediated through protein conjugation and aggregation [58]. A pathogenic vicious cycle of acrolein-induced neurotoxicity appears to involve oxidative stress, protein aggregation, and cell death [59]. Acrolein is neurotoxic, inhibiting enzymes such as Na^+/K^+ ATPase, glucose, and glutamate transporters, which are essential for neuron survival [60].

In the current study, quercetin nanoparticles showed a significant improvement and were found to counteract the degenerative effects of acrolein administration. The protective role of quercetin nanoparticles was apparent in rats from the ameliorative and protective treated groups. The ischemia neuronal degenerative changes in the pyramidal layer of the cerebral cortex were significantly reduced. These results are in agreement with Ghosh et al. [61] who reported that the administration of quercetin nanoparticles protects brain cells from arsenic-induced damage. The mechanism of the quercetin nanoparticles transport across the blood-brain barrier appears to involve endocytotic absorption by brain capillary endothelial cells, followed either by quercetin release in these cells and diffusion into the brain or by transcytosis. Pinheiro et al. [62] demonstrated that

quercetin nanoparticles that target the blood-brain barrier while also protecting neurons from amyloid-beta fibrillation in a thioflavin T binding experiment showed to be an effective method of quercetin administration and a potential technique for future Alzheimer's disease treatments.

The use of quercetin nanoparticles, which prevent oxidative damage and restore normal cellular function, is an excellent way to improve the cellular antioxidant defense system [63]. Nanoantioxidants, such as quercetin nanoparticles, are nontoxic, biocompatible, and biodegradable. They also have intrinsic antioxidant capabilities that help to reduce oxidative damage caused by free radicals. Numerous studies have shown that using nanoparticles to deliver medicines can drastically alter their original physicochemical characteristics [61]. As a result, neuronal cell death caused by oxidative stress and progression of neurodegenerative diseases can be reduced by the supplementation of antioxidants and free radical scavengers [64].

According to Kowluru and Chan [65], antioxidants are essential because they aid in the suppression of reactive oxygen species production and the enhancement of antioxidant enzyme defense capabilities, as well as scavenging free radicals. The antioxidant enzyme capacity of oxidatively stressed brain tissue is especially important for the primary endogenous defense against free radical-induced injury, which involves the cooperative action of intracellular antioxidant enzymes like superoxide dismutase and catalase. By regenerating antioxidants and reducing hydroperoxide via the glutathione peroxidase cycle, reduced glutathione aids in antioxidative defense [66]. Palle and Neerati [67] stated that quercetin nanoparticles provide a preventative approach against the development of Alzheimer's disease. Quercetin nanoparticles outperformed the quercetin-treated group of rats in terms of efficacy, suggesting that the improved efficacy is attributable to a longer residence period in systemic circulation and greater bioavailability. Quercetin nanoparticles significantly reduced malondialdehyde (MDA) and acetyl cholinesterase (AchE) levels while increasing brain catalase and glutathione (GSH). Mohamed et al. [68] reported that quercetin nanoparticles improved brain oxidation by increasing antioxidant enzyme activity and reducing pro-oxidant effects. Quercetin nanoparticles reduced reactive oxygen species (ROS), protein carbonyl (PC) and myelopreoxidase (MPO). In addition, they increased the activity of glutathioneperoxidase (GPx) and acetylcholine esterase (AChE). Treatment with quercetin nanoparticles improved the gamma-aminobutyric acid (GABA) level in the brain. Ghaffari et al. [69] demonstrated that the quercetin nanoparticles increased superoxide dismutase activity, GSH levels, and reduced lipid peroxidation in the brain regions. This finding revealed that enhanced antioxidant enzyme activities can lead to decreased intracellular H_2O_2 generation, which, when combined with an increase in GSH levels, can reduce lipid peroxidation, suggesting that quercetin nanoparticles have significant antioxidant properties.

The morphometric analysis of the cerebral cortex showed a great decrease in its thickness in the acrolein-treated group of rats. The current study reported that in the case of treatment with quercetin nanoparticles in both the ameliorative and protective treated groups, there was a significant increase in cerebral cortex thickness following quercetin nanoparticle administration. These results confirm the results obtained from the histopathological studies.

In the current study, multiple pathological lesions were observed in the cerebral cortex of the acrolein-treated group. Semi-quantitative analysis of neuronal histopathological lesions in this case indicated severe cellular edema, ischemic injury, Malacia, gliosis, and extent of lesions. However, treatment with quercetin nanoparticles had reversed the previously mentioned morphological aberrations. This could be attributed to the antioxidant properties of quercetin nanoparticles or the combined antioxidant and anti-inflammatory role of quercetin nanoparticles in neurotoxicity and neurodegenerative diseases as was documented by previous studies [70].

V. CONCLUSIONS

It has been demonstrated that acrolein causes histopathological alterations in the rat cerebral cortex. Therefore, it's crucial for health to limit the amount of acrolein in the environment. It has been discovered that quercetin nanoparticles greatly lower the possibility of aberrant toxic lesions brought on by acrolein in the cerebral cortex, and they are advised to be used in nanomedicine to safeguard and enhance the nervous tissues. After oxidative stress caused by acrolein, quercetin nanoparticles offer insight into prospective treatments to ward off Alzheimer's disease.

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