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Summary and Conclusion

Neurodegenerative diseases have become a menace in the 21st century and currently there is no effective treatment to cure these types of diseases. Neurodegenerative diseases could be the second most common cause of death among elderly by the 2040's (Bachari *et al.,* 2024). Neuroprotection refers to the strategies and relative mechanisms to prevent neuronal damage caused by various neuropsychiatric and neurodegenerative disorders. Among the strategies for neuroprotection, phytochemicals represent a valuable remedy. Generally, herbal products contain a variety of bioactive phytochemicals including alkaloids, steroids, terpenoids, saponins, phenolics and flavonoids. Phytochemicals are naturally found in plants and are shown to have protective action against oxidative stress and neuroinflammation. Numerous natural products, primarily plant extracts, have been reported to be used in traditional medicine for neuroprotection. Most of the flavonoids in green tea are present as catechins, which possess many beneficial effects to human health. Nanotechnology today plays an important role in the pharmaceutical industry. The rapidly developing field of nanoscience has raised the possibility of using therapeutic nanomaterials in the diagnosis and treatment of neurological disorders.

Work has already been carried out with green tea on the role of phytochemicals in neuroprotection, neurorescueing and neurodegenerative disease. Also, the role of zinc oxide nanoparticles in various types of neuronal cell lines have been well researched. But still there is no proper treatment available for several neurodegenerative diseases. Several studies are available on the role of zinc oxide nanoparticles in neuroprotection. However, there is no information available on synthesized zinc oxide nanoparticle-capped catechin in *Camellia sinensis*. Studies have shown that polyphenols help in several neurodegenerative diseases and hence it was decided to exploit green tea rich in polyphenols to study its neuroprotective activity and employ zinc oxide nanoparticle-capped catechin from green tea to study neuroprotection.

Hence, the present study entitled '**Neuroprotective effect of synthesized zinc oxide nanoparticle-capped catechin**' was aimed at synthesizing zinc oxide nanoparticle-capped

catechin using *Camellia sinensis* (green tea) and studying its neuroprotective effect on neuro 2a cells.

The study was designed into 5 phases.

Phase I involved the evaluation of active compounds against neuroprotective target proteins using molecular docking studies.

Fifteen natural phytoconstituents such as Cyanidin, Catechin, Kaemopferol, Genistein, Epicatechin, Quarcetin 3-o-glucoside, Epicatechingallate, Rutin, Hesperetin, Thea flavin-3-o-gallate, Apigenin, Zinc, Herperetin, Kemopferol-3-o-rutinoside, Quercetin, Thea flavin-3'-gallate, Theaflavin, Galic acid, Caffine, Kaempferide 3-glucoside and five commercial drugs (Galanthamine, Rivastigmine, Tacrine, Memantine, and Donepezil) were analysed for their interaction with neuroprotective targets through docking studies. Protein ligand interaction study has been performed through Schrodinger's software (Maestro V: 11.8 Schrodinger_suite-2019). The three dimensional structures of acetylcholinesterase (AChE) (PDB ID: 5EIE 5DTI), butyrylcholinesterase (BChE) (PDB ID: 6QAA 6QAE), β-secretase (BACE) (PDB ID: 5HOX, 6UVP) and β amyloid (PDB ID: 4B5E, 4B41) were retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org/pdb). The natural compounds and commercial drugs were docked against the drug targets acetylcholinesterase, beta-secreatase, BACE and β amyloid proteins. The result, clearly showed that the catechin and rivastigmine have been identified to have good docking with the acetylcholinesterase protein as determined by the docking score, number of hydrogen bonds formed and the number of good contacts.

Compared to the other commercially available drugs and natural compounds, rivastigmine and catechin respectively were found to be the most effective in binding to the acetylcholinesterase active site because of their significantly low binding affinities as a result of high docking score values.

Based on the above results, a catechin-rich plant source (*Camellia sinensis*) was selected for further studies.

In **Phase II,** characterization of *Camellia sinensis* and identification of its bioactive compound was carried out.

The *Camellia sinensis* (L.) Kuntze leaves were collected from Aruvankadu in the Distirct of Niligris and the taxonomic identification of the plant was confirmed by Botanical

Survey of India, Coimbatore (Authentication No: BSI/SRC/5/23/2019/Tech./185). The *Camellia sinensis* leaves were used for the preliminary phytochemical analysis, antioxidant and free radical scavenging activities, identification of bioactive compound by spectral and chromatographical methods. The results revealed that the hydroethanolic extract of *Camellia sinensis* had more phytochemicals compared to the hexane, ethyl acetate, methanol and ethanol extracts. *Camellia sinensis* leaves had higher amounts of enzymic and non-enzymic antioxidant activities. Hence, it was evident that the hydroethanolic extract of *Camellia sinensis* can be separated and identified by column and thin layer chromatographic techniques and that the 3rd chromatographic fraction of the sample contains catechin an active constituent in *Camellia sinensis*.

Since the active component was identified in the 3rd column chromatographic fraction of the hydroethanolic extract of *Camellia sinensis*, this fraction was further subjected to HPTLC, UV, FT-IR, GC-MS, NMR and LC-MS to identify the component.

The results confirmed that the bioactive component isolated from the hydroethanolic extract of *Camellia sinensis* was catechin.

From the overall results of Phase II it can be concluded that *Camellia sinensis* leaves contain a good amount of antioxidants and radical scavenging activity. The main active component was identified to be Catechin.

Phase III included synthesis of zinc oxide nanoparticles from *Camellia sinensis* and their characterization.

The zinc oxide nanoparticles were synthesized from *Camellia sinensis* and characterized by UV, FTIR, SEM, XRD and zeta potential. Additionally antioxidant activity and acetylcholinesterase inhibitory activity of the synthesized zinc oxide nanoparticles were carried out. The results of the UV spectra of the synthesized zinc oxide nanoparticles showed characteristic peak at 280 nm. During the synthesis of zinc oxide nanoparticles, with increase in the time of exposure, the colour changed from brown to white. The XRD peaks were broad in nature which could be attributed to the nano crystalline nature of the synthesized zinc oxide nanoparticles. The SEM images of the synthesized zinc oxide nanoparticles were rod shaped and found to be in aggregates at 5 μm nanoscale. The FT-IR results confirmed the formation of synthesized zinc oxide nanoparticles by showing prominent absorption peaks. The Zeta potential spectrum showed the stability of the synthesized zinc oxide

nanoparticles. These nanoparticles were found to have an IC50 value of 70.37 %. A concentration of 1.25 µg/ml of synthesized zinc oxide nanoparticles had the most potent effect in inhibiting acetylcholinesterase activity.

From the overall finding of Phase III, it can be concluded that the synthesized zinc oxide nanoparticles from *Camellia sinensis* makes it a valuable source of antioxidants with acetylcholinesterase inhibitory activity.

Phase IV involved capping of synthesized zinc oxide nanoparticles with catechin, their characterization and study of *in vitro* neuroprotective activity.

The synthesized zinc oxide nanoparticles were capped with the isolated bioactive compound catechin through nanoprecipitation method and their stability and characteristics including topography, morphology, composition and crystalline structure, drug loading capacity and entrapment efficacy, *in vitro* release analysis and acetylcholinestrease activities studied. The UV and visible range spectra of the synthesized zinc oxide nanoparticle-capped catechin showed a characteristic peak at 318 nm indicating that synthesis of synthesized zinc oxide nanoparticle-capped catechin increased with increase in time of exposure, thus confirming that the immediate colour change was due to the synthesis of nanoparticles. The broad nature of the XRD peaks could be attributed to the nano crystalline nature of the synthesized zinc oxide nanoparticle-capped catechin. SEM results proved that the synthesized zinc oxide nanoparticle-capped catechin were completely capped on the catechin with uniform the distribution in the solution and they appeared to be rod-shaped . The FT-IR results also confirmed the formation of synthesized zinc oxide nanoparticle-capped catechin by showing prominent absorption peaks. The results revealed that the IC_{50} value of the synthesized zinc oxide nanoparticle-capped catechin was 52.3 %. *In vitro* release study and acetylcholinestrease inhibitory activity proved maximum inhibitory activity of acetylcholinestrease. The possibility of the synthesized zinc oxide nanoparticle-capped catechin having a therapeutic effect may be evidenced from their moderate acetylcholinestrease inhibitory activity.

From the overall findings of Phase IV, it can be concluded that there is a remarkable potential for the synthesized zinc oxide nanoparticle-capped catechin to be used for neuroprotection since it is a valuable source of antioxidants with acetylcholinesterase inhibitory activity and good encapsulation efficiency.

Phase V involved the study of the *in vitro* neuroprotective activity of the synthesized zinc oxide nanoparticle-capped catechin on neuro 2a cells

The *in vitro* neuroprotective effect of synthesized zinc oxide nanoparticle-capped catechin was evaluated against neuro 2a cells by MTT cell proliferation assay, acetylcholinesterase inhibitory assay and also apoptotic effect of neuro 2a cells by Flow Cytometry. The results of the MTT assay showed that synthesized zinc oxide nanoparticle-capped catechin mediated a concentration dependent increase in toxicity towards neuro 2a cells with the concentration of 20 µg/ ml of synthesized zinc oxide nanoparticle-capped catechin with no significant changes in the percentage of cell viability. The *in vitro* cell line culture study reported that the synthesized zinc oxide nanoparticle-capped catechin molecules exhibited neuroprotective activity towards the neuro 2a cell lines in a sustained manner. The *in vitro* cytotoxic effect of synthesized zinc oxide nanoparticle-capped catechin on neuro 2a cells showed less toxicity. Synthesized zinc oxide nanoparticle-capped catechin also increased the production of intracellular reactive oxygen species in a dose-dependent manner in the neuro 2a cells. The most potent inhibitor for acetylcholinestrease was the zinc oxide nanoparticle-capped catechin because it had the lowest acetylcholinestrease inhibitory activity. The results of the cell cycle analysis showed that cell cycle dysregulation has been involved in the quiescent state. Most of the cells in the G0/G1 phase showed the arrest of cell proliferation, mature senescence and differentiated cells in G0 phase.

From the overall outcome of Phase V, it can be concluded that exposure of neuro 2a cell lines to synthesized zinc oxide nanoparticle-capped catechin, reduced the neurotoxic effects including changes in cell morphology, cell viability, reactive oxygen species and protection of on neuro 2a cells.

It can thus be concluded from the overall study that the synthesized zinc oxide nanoparticle-capped catechin can be proposed as a novel therapeutic strategy for neuroprotective effect.

Recommendations for further studies

The findings of the current work have unfolded some prospects for future studies. A few of them are:

- The neuroprotective activity of synthesized zinc oxide-capped catechin can be further validated by conducting in the study in animals.
- Validation by gene expression can be studied *in vivo* for the development of novel drugs from *Camellia sinensis* with enhanced medicinal value through technological interventions.
- The proteomic and genomic profile of the synthesized zinc oxide-capped catechin can be further characterized.