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Synthesis and characterization of Gum Acacia encapsulated Ursolic acid nanoparticles enhancing bioavailability and Acetylcholinesterase inhibition for therapeutic approach of Alzheimer's disease

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ABSTRACT

The management of Alzheimer's disease involves a variety of pharmacological and non-pharmacological therapeutic methods. Given this rationale, several plants and their bioactive chemicals have been analyzed for their capacity to combat Alzheimer's disease. These naturally occurring chemicals frequently demonstrate neuroprotective, anti-inflammatory, and antioxidant characteristics, which are essential in the management of Alzheimer's disease. Previous studies have shown Ursolic Acid (UA) to be a novel anti-Alzheimer's medicinal compound. For targeted drug administration, enhanced bioavailability, and reduced adverse effects, UA loaded Gum arabic nanoparticles (UGNPs) were synthesized in this study using the oil in oil emulsion solvent evaporation method. The size range of the produced nanoparticles varied from 126 nm to 253 nm. Zeta potential value of +27 mV for UGNPs indicates the relative stability of the synthesized nanoformulations. Encapsulation with Gum arabic may have offered protection to UA from oxidation. The drug loading and percentage encapsulation efficiency values were determined to be 2.82 mg and 69.98% respectively for sustained release from the UGNPs, during 1 hour after delivery (20% UA). The size range of the synthesized UGNPs was verified by transmission electron microscopy, therefore revealing a range of 43-61nm. UGNPs showed a high Acetylcholinesterase (AChE) inhibition percentage, close to that of Rivastigmine. UA followed with approximately 60% inhibition, and Gum Arabica has the lowest inhibition, near 50%. Hence, it is of great value to investigate the therapeutic effectiveness of UGNPs.

KEYWORDS: Ursolic Acid, Alzheimer's, Dammar, Nanoparticles

INTRODUCTION

Alzheimer's disease is an advancing neurological condition marked by a deterioration in cognitive function, loss of memory, and alterations in behavior. The primary etiology of dementia in elderly individuals is the buildup of amyloid plaques and neurofibrillary tangles in the brain, resulting in neuronal impairment and diminished brain volume. The condition generally advances through many phases, starting with modest memory difficulties and worsening to significant cognitive and functional deficits, finally leading to a total loss of autonomy[1]. While the precise etiology of Alzheimer's disease remains incompletely elucidated, it is postulated to encompass a confluence of genetic, environmental, and behavioral determinants. Contemporary therapy approaches largely concentrate on symptom management and enhancing quality of life, given the absence of a cure for the condition[2]. Continual research is being conducted to gain a deeper understanding of the fundamental processes of Alzheimer's disease and to refine more efficient therapies targeted at decelerating or stopping its advancement[3].

Apples, rosemary, thyme, and holy basil contain ursolic acid, a triterpenoid[4]. Due to its anti-inflammatory, antioxidant, anti-cancer, and neuroprotective qualities, biomedical research has focused on it[5]. Preclinical studies suggest ursolic acid may help treat neurodegenerative illnesses like Alzheimer's. Its neuroprotective effects may come from reducing brain oxidative stress and inflammation, which are linked to Alzheimer's. Ursolic acid also prevents Alzheimer's disease-causing amyloid-beta plaques[6]. Ursolic acid may prevent or reduce Alzheimer's progression by altering various degenerative mechanisms. While intriguing, further study, including clinical studies, is needed to fully understand ursolic acid's potential as an Alzheimer's disease treatment and confirm its safety and efficacy in humans[7].

Gum Arabic comes from Acacia senegal and seyal sap. Food, pharmaceuticals, cosmetics, inks, and paints use its emulsifying, stabilizing, and film-forming capabilities. Biocompatibility, non-toxicity, and biodegradability make Gum Arabic important in pharmaceutical and biological research[8]. Its features make it a good natural drug delivery polymer. Gum Arabic controls nanoparticle or microparticle release, improving medicinal stability and bioavailability. Protecting proteins, peptides, and small-molecule drugs before delivery is essential. Tablets are coated with gum Arabic to mask taste, prevent moisture, and extend shelf life. Gum Arabic maintains nanomedicine size and avoids aggregation. Its tissue compatibility and natural nature make it ideal for bio adhesive films and wound treatments[9]. Gum Arabic is

a promising natural polymer for pharmaceutical and biological applications that improve therapeutic agent dispersion, stability, and efficacy due to its safety and versatility[10].

By facilitating precise and effective medication administration across the blood-brain barrier, a major obstacle in conventional treatments, nanoparticles provide a promising strategy for treating Alzheimer's disease[11]. The capacity of different categories of nanoparticles, such as lipid-based, polymeric, metallic, dendrimers, and magnetic nanoparticles, to directly transport therapeutic substances to the brain is now under investigation[12]. These nanoparticles can be designed to transport medications that hinder the development of amyloid-beta plaque, decrease inflammation in the brain, and shield neurons from oxidative insult[13]. For instance, polymeric nanoparticles such as PLGA can yield prolonged medication release, whereas gold nanoparticles can be modified to selectively target proteins associated to Alzheimer's disease. Through the enhancement of drug bioavailability and targeting, nanoparticles have the potential to augment the effectiveness of Alzheimer's therapies. However, the issues of safety and accurate targeting continue to be subjects of ongoing study[14].

MATERIALS AND METHODS

Materials: Gum Arabic was purchased from MP Biomedicals, LLC, France. Ursolic acid was obtained from Sigma Aldrich India. The materials utilized for present research work was of analytical/reagent grade.

Preparation of ursolic acid loaded Gum arabic nanoparticles (UGNPs)

UGNPs were made by oil-in-oil emulsion solvent evaporation. UGNPs nanoparticles were made by dissolving 1.20 mg Gum arabic and 0.30mg Ursolic Acid in 13 ml ethanol and adding magnesium stearate (8% gum w/w)[15]. The mixture was agitated for 2 hours on a magnetic stirrer and slowly poured into 75 cc liquid paraffin at 43˚ C at 920 rpm. The mixture was stirred for 16 hours. After suspending UGNPs in cryopreservative (5% D mannitol aqueous solution), they were lyophilized at -94 °C and 0.0010 mbar pressure for 24 h. Lyophilization collected nanoparticles. The gum: drug ratio's effect on nanoparticle particle size, encapsulation effectiveness (%), and in vitro drug release profile was studied.

Characterization of synthesized UGNPs.

A comprehensive investigation was conducted on the morphology, entrapment efficiency, zeta potential, particle size, Fourier transform infrared spectroscopy, and in vitro drug release of

UGNPs. A Particle Size Analysis was performed to ascertain the size distribution (polydispersity index) and average nanoparticle size (Dynamic Light Scattering- DLS) of the nanoformulations that were successfully adjusted[16]. The mean particle size of the UGNPs was measured at 25 ℃ using the Zetasizer Nano ZS device manufactured by Malvern Instruments in Malvern, UK. Pharmacological loading and drug entrapment efficiency were determined by analyzing the drug concentration in a collected supernatant using UV-visible spectroscopy. An investigation of the morphology of the improved batch was conducted using TEM[17]. One droplet of nanosuspension was applied onto a copper grid, allowed to dry naturally, and then visually examined. The transmission electron microscopy (TEM) image was obtained using an accelerating voltage of 80 kV and a magnification factor of 60,000. Powdered samples (UA, Gum arabic, and UGNPs) were analyzed using FTIR spectroscopy in a Fourier transform infrared spectrophotometer within the range of 4500–500 cm−1 as a KBr pellet[18].

In vitro release profile of UGNPs: Release of UA from the nanoparticles under in vitro conditions was quantified using high-performance liquid chromatography (HPLC). This work utilized the dialysis sac technique to investigate the release kinetics. 5 mg of UA-loaded Gum arabic nanoparticles were dissolved in 5 ml of a 0.1 M solution with a pH of 7.4. The resulting solution was then added to a dialysis sac, which was thoroughly submerged in 125 ml of phosphate buffer saline[19]. The release media was repeatedly agitated at 80 revolutions per minute in a thermostat set at a constant temperature of 37 °C. Samples of one milliliter were collected at constant time intervals of 0, 2, 4, 6, 8, 12, 18, 24, 30, 36, 42, and 48 hours. In order to investigate the release of UA loaded Gum arabic nanoparticles in the buffer, the samples were analyzed using standard curves at 270 nm for ursolic acid. The drug released at time's' was determined by the formula[20].

Cumulative release (%) = (released amount of UGNPs/ Total amount of UGNPs) * 100

Antioxidant activity analysis of encapsulated NPs: The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was suspended in methanol at a concentration of 4.0 mg/100 ml. Under dark conditions, the pure UA and UGNPs were incubated with DPPH for 32 minutes. Absorbance was measured using a UV spectrophotometer at a wavelength of 517 nm. Three copies of each DPPH inhibitor were utilized to assess its inhibitory efficacy. Blank Gum arabicnanoparticles served as the negative control, while pure chemicals (Ursolic acid) were employed as the positive control[21]. The following equation was used to determine the % inhibition of DPPH by pure UA and UA-loaded Gum arabic nanoparticles.

$$\text{Percentage antioxidant activity} = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}} * 100$$

Evaluation of AChE inhibitory activity: A modified Ellman-based 96-well microplate test assessed AChE activity. Thiocholine reacts with Ellman's reagent (DTNB) to create 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate at 412 nm when the enzyme hydrolyzes acetylthiocholine. 50 mM Tris–HCl pH 8.0 buffered the experiment unless otherwise stated[22]. Electric eel AChE was utilized in the experiment (type VI-S lyophilized powder, 518 U/mg solid, 844 U/mg protein). We stored the enzyme stock solution (518 U/ml) at −80°C. Further enzyme dilution was in buffer with 0.1% BSA. DTNB was dissolved in 0.1 M NaCl/0.02 M MgCl2. Deionized water dissolved ATCI. To the 96-well plates, 100 µl of 3 mM DTNB, 20 µl of 0.26 U/ml AChE, 40 µl of buffer (50 mM tris pH 8.0), and 20 µl of each extract at varied doses (25, 50, 100, 250, and 500 µg/ml) were added. After mixing, the plate was incubated for 15 min (25°C) and measured at 412 nm in a 200-microplate reader as a blank. To begin the enzymatic reaction, 20 µl of 15 mM ATCI was added. The hydrolysis of acetylthiocholine was monitored by reading absorbance every 5 min for 20 min. Positive control: Rivastigmine[23]. All reactions were tripled. The percentage inhibition was calculated by following formula:

$$\% \text{ Inhibition} = (ES)/E \times 100$$

Where E represents enzyme activity without extract and S is enzyme activity with extract. The percentage inhibition

RESULT and DISCUSSION

Particle size and Zeta Potential

The UGNPs were analyzed for particle size and zeta potential. The prepared nanoparticles size was found to be 126 nm (Fig 1). The UGNPs had a zeta potential value of +27 mV (Fig 2), signifying relative stability of prepared nanoformulations.

Drug loading and percentage encapsulation efficiency: Drug loading is the quantification of the drug content inside a 100 mg quantity of nanoparticles. The efficacy of encapsulation is contingent upon the specific technique, character of the molecule, properties of the encapsulating materials, and the media used for nanoparticle formation[24]. The drug loading and percentage encapsulation efficiency metrics for UA were determined to be 2.82 mg. The

present work provides evidence that a quantity of 2.82 mg of UA is contained within 100 mg of nanoparticles (UGNPs) with percent encapsulation efficiency of 69.98 (Fig 3). Ursolic acids (UA) are hydrophobic compounds. They have extremely high solubility in a gum solution formulated in ethanol. The hydrophobic characteristic of Gum acacia resulted in a profound affinity between gum and UA. The affinity shown led to a higher encapsulation efficiency of the medicines within Gum acacia.

Morphological characterization using TEM: UGNPs were found to be segregated and uniformly spherical in shape when observed using TEM. The size range of prepared UGNPs was confirmed using TEM, which was found to be 43 - 61 nm. Variations in the results of particle size of nanoparticles from PSA and TEM studies can be attributed to the reason that dynamic light scattering measurements consider the ionic environment of the particle, while TEM calculates the size of the particle in isolated atmosphere[25]. Size of nanoparticles affects release rate, solubility and dissolution rate of a molecule/drug. The targeting, stability, biocompatibility, and infiltration of the nanoparticles inside the cellular tissues are also governed by the morphology and size of nanomaterials. Smaller sized nanoparticles are maintained in systemic circulation for extended period as compared to large sized nanoparticles. NPs undergo distribution to different organs of the body in size and shape dependent manner.

FTIR Analysis of Drug Samples: The FTIR spectroscopy is not only used for the interaction studies but also utilized to evaluate the encapsulation of bioactive molecules on the NPs FTIR spectroscopy. The FTIR spectra of pure drug UA, Gum acacia and UGNPs has been presented and compared in the present study. The image displays a Fourier Transform Infrared (FTIR) spectroscopy graph, which shows the transmittance spectra for three different substances: "UGNPs," "UA," and "Gum Arabic." The x-axis represents the wavenumber (in cm^{-1}), ranging from approximately 500 to 4000 cm^{-1}, and the y-axis represents transmittance in percentage. Three distinct lines on the graph correspond to the different materials tested. The "UGNPs" spectrum appears at the top, showing sharp peaks in the higher wavenumber region, particularly above 3000 cm^{-1}. The "UA" spectrum is in the middle and has a more consistent pattern with minor peaks, while the "Gum Arabic" spectrum at the bottom exhibits prominent peaks between 1000 and 1500 cm^{-1}, as well as some sharper peaks near 3000 cm^{-1}. These spectra highlight differences in molecular vibrations and chemical bonds for each substance, with

UGNPs showing more complex structures at higher wavenumbers, and Gum Arabic displaying distinctive absorbance patterns at lower wavenumbers (Fig 4).

***In-vitro* drug release:** The continuous release of the antibiotic from the Nanoparticle shields the medicine from its fast metabolism and breakdown. The in-vitro drug release experiments demonstrated that the nanoformulations exhibited regulated drug release as a result of the effective emulsification of the drug in dammar gum[26]. A controlled and sustained release was detected from the UGNPs one hour after the administration of 20% UA. The drug release profile of ultra-fine nanoparticles (UGNPs) exhibits a continuous and delayed release of uric acid (UA) over time (Fig 5). The regulated release may be attributed to the reduced release rate resulting from the hydrophobic property of UA. Gum acacia additionally generates a robust and compact matrix around the UA particles, therefore guaranteeing its continuous release.

Anti-oxidant activity: DPPH assay has been used earlier to evaluate the antioxidant activity of encapsulated molecules. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical, that contain spare electrons delocalized over the entire molecule, giving a deep violet coloration. It is characterized by an absorption band near about 517 nm. The loss of this violet color is observed, when a solution of DPPH is mixed with a molecule that can donate a hydrogen atom. The UA is well known antioxidant molecules. The solution of antioxidant molecules UA was incubated with DPPH (a hydrogen atom donor) and a stable non-radical form of DPPH was obtained, shifting the deep violet color to pale yellow. Thus, a decrease in absorbance band was observed (Fig 6). The UA contain labile hydrogen atoms, which on liberation inhibit DPPH. The lower inhibition of DPPH by the UGNPs compared to their unencapsulated analogue was observed. This might be due to delayed release of loaded drug particles during the incubation period in dark. Encapsulation might have slowed down the hydrogen radical's availability and provided protection to UA from being oxidized.

Evaluation of AChE inhibitory activity

The image represented bar graph comparing the percentage of inhibition for four different substances: Rivastigmine, UA, Gum Arabica, and UGNPs. The Y-axis represented"% Inhibition," ranging from 0 to 100, while the X-axis lists the four substances. Rivastigmine had the tallest bar, indicating the highest inhibition at around 90%. UGNPs also showed a high inhibition percentage, close to that of Rivastigmine. UA followed with approximately 60% inhibition, and Gum Arabica has the lowest inhibition, near 50%. Each bar is accompanied by an error bar, reflecting variability in the data, with Rivastigmine and UGNPs showed smaller error margins compared to UA and Gum Arabica (Fig 7). This graph illustrated a comparison

of the inhibitory effects of these substances on a specific biological or chemical process, with Rivastigmine and UGNPs emerging as the most effective inhibitors.

CONCUSIONS

When it comes to the search for effective treatments for Alzheimer's disease, the creation of nanoparticles made of gum arabic that are loaded with ursolic acid is a feasible approach. Through the usage of the neuroprotective and anti-inflammatory features of ursolic acid, in conjunction with the biocompatibility and stabilizing capabilities of gum Arabic, these nanoparticles enable a medication delivery mechanism that is both targeted and efficacious. This formulation not only enhances the bioavailability and stability of ursolic acid, but it also makes it simpler for the acid to be controllably released across the blood-brain barrier. This may result in a reduction in the rate at which Alzheimer's disease progresses. The inclusion of these natural chemicals into a nanoparticulate system represents a big step forward in the development of medicines for neurodegenerative illnesses that are both safer and more effective. The development of this new information lays the way for further research and practical use in the treatment of Alzheimer's disease.

References

1. Wang, S., Meng, X., & Dong, Y. (2017). Ursolic acid nanoparticles inhibit cervical cancer growth in vitro and in vivo via apoptosis induction. *International journal of oncology*, *50*(4), 1330-1340.
2. Jin, H., Pi, J., Yang, F., Wu, C., Cheng, X., Bai, H., ... & Chen, Z. W. (2016). Ursolic acid-loaded chitosan nanoparticles induce potent anti-angiogenesis in tumor. *Applied microbiology and biotechnology*, *100*, 6643-6652.
3. Antônio, E., Junior, O. D. R. A., de Araújo, I. S., Khalil, N. M., & Mainardes, R. M. (2017). Poly (lactic acid) nanoparticles loaded with ursolic acid: Characterization and in vitro evaluation of radical scavenging activity and cytotoxicity. *Materials Science and Engineering: C*, *71*, 156-166.
4. Antonio, E., Junior, O. D. R. A., Marcano, R. G. D. J. V., Diedrich, C., da Silva Santos, J., Machado, C. S., ... & Mainardes, R. M. (2021). Chitosan modified poly (lactic acid) nanoparticles increased the ursolic acid oral bioavailability. *International Journal of Biological Macromolecules*, *172*, 133-142.

5. Jin, H., Pi, J., Yang, F., Jiang, J., Wang, X., Bai, H., ... & Chen, Z. W. (2016). Folate-chitosan nanoparticles loaded with ursolic acid confer anti-breast cancer activities in vitro and in vivo. *Scientific reports*, *6*(1), 30782.

6. Yang, L., Sun, Z., Zu, Y., Zhao, C., Sun, X., Zhang, Z., & Zhang, L. (2012). Physicochemical properties and oral bioavailability of ursolic acid nanoparticles using supercritical anti-solvent (SAS) process. *Food chemistry*, *132*(1), 319-325.

7. Li, Y., Zhao, L., Zhao, Q., Zhou, Y., Zhou, L., Song, P., ... & Deng, G. (2023). Ursolic acid nanoparticles for glioblastoma therapy. *Nanomedicine: Nanotechnology, Biology and Medicine*, *50*, 102684.

8. Jaafar, N. S. (2019). Clinical effects of Arabic gum (Acacia): A mini review. *Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512)*, *28*(2), 9-16.

9. Sanchez, C., Nigen, M., Tamayo, V. M., Doco, T., Williams, P., Amine, C., & Renard, D. (2018). Acacia gum: History of the future. *Food Hydrocolloids*, *78*, 140-160.

10. Daoub, R. M., Elmubarak, A. H., Misran, M., Hassan, E. A., & Osman, M. E. (2018). Characterization and functional properties of some natural Acacia gums. *Journal of the Saudi Society of Agricultural Sciences*, *17*(3), 241-249.

11. Zhu, S., Qiu, Z., Qiao, X., Waterhouse, G. I., Zhu, W., Zhao, W., & Zheng, Z. (2023). Creating burdock polysaccharide-oleanolic acid-ursolic acid nanoparticles to deliver enhanced anti-inflammatory effects: Fabrication, structural characterization and property evaluation. *Food Science and Human Wellness*, *12*(2), 454-466.

12. Maphanao, P., Phothikul, Y., Choodet, C., Puangmali, T., Katewongsa, K., Pinlaor, S., ... & Sakonsinsiri, C. (2024). Development and in vitro evaluation of ursolic acid-loaded poly (lactic-co-glycolic acid) nanoparticles in cholangiocarcinoma. *RSC advances*, *14*(34), 24828-24837.

13. Payomhom, P., Panyain, N., Sakonsinsiri, C., Wongtrakoongate, P., Lertsuwan, K., Pissuwan, D., & Katewongsa, K. P. (2024). Chitosan-Coated Poly (lactic-co-glycolic acid) Nanoparticles Loaded with Ursolic Acid for Breast Cancer Therapy. *ACS Applied Nano Materials*, *7*(5), 5383-5395.

14. Sharma, R., Yadav, V., Jha, S., Dighe, S., & Jain, S. (2024). Unveiling the potential of ursolic acid modified hyaluronate nanoparticles for combination drug therapy in triple negative breast cancer. *Carbohydrate Polymers*, *338*, 122196.

15. Chauhan, A., Pathak, V. M., Yadav, M., Chauhan, R., Babu, N., Chowdhary, M., ... & Yadav, V. (2024). Role of ursolic acid in preventing gastrointestinal cancer: recent trends and future perspectives. *Frontiers in Pharmacology*, *15*, 1405497.

16. Yuan, C., Fan, W., Zhou, T., Sun, D., Liu, H., He, Z., & Wang, Y. (2023). Ligand-free high loading capacity ursolic acid self-carried nanovesicles enable hepatocyte targeting via absorbing apolipoproteins. *International Journal of Pharmaceutics*, *638*, 122931.

17. Nie, Y., Wang, L., Liu, S., Dai, C., Cui, T., Lei, Y., ... & Zheng, Z. (2023). Natural ursolic acid based self-therapeutic polymer as nanocarrier to deliver natural resveratrol for natural therapy of acute kidney injury. *Journal of Nanobiotechnology*, *21*(1), 484.

18. Ding, J., Tan, J., Peng, X., Cheng, L., Huang, W., & Luo, B. (2024). Ursolic acid loaded tri-block copolymer nanoparticles based on triphenylphosphine for mitochondria-targeted cancer therapy. *Biomedical Materials*, *19*(3), 035013.

19. Zou, J., Lin, J., Li, C., Zhao, R., Fan, L., Yu, J., & Shao, J. (2019). Ursolic acid in cancer treatment and metastatic chemoprevention: From synthesized derivatives to nanoformulations in preclinical studies. *Current Cancer Drug Targets*, *19*(4), 245-256.

20. Ren, J., Lin, H., Zhang, Y., Li, X., Zhang, Z., You, C., & Wang, F. (2024). The chitosan/cellulose nanocrystal cross-linked carriers effectively encapsulate ursolic acid to enhance the delivery of bioactive natural products. *Journal of Drug Delivery Science and Technology*, *97*, 105687.

21. Qiu, L., Zhao, X., Zu, Y., Zhang, Y., Liu, Y., Wu, W., & Li, Y. (2019). Ursolic acid nanoparticles for oral delivery prepared by emulsion solvent evaporation method: characterization, in vitro evaluation of radical scavenging activity and bioavailability. *Artificial cells, nanomedicine and biotechnology*, *47*(1), 609-620.

22. Bielecka, E., Zubrzycka, N., Marzec, K., Maksylewicz, A., Sochalska, M., Kulawik-Pióro, A., ... & Kantyka, T. (2024). Ursolic acid formulations effectively induce apoptosis and limit inflammation in the psoriasis models in vitro. *Biomedicines*, *12*(4), 732.

23. Singh, A. K., Pandey, H., Ramteke, P. W., & Mishra, S. B. (2019). Nano-suspension of ursolic acid for improving oral bioavailability and attenuation of type II diabetes: A histopathological investigation. *Biocatalysis and Agricultural Biotechnology*, *22*, 101433.

24. Lan, J. S., Qin, Y. H., Liu, L., Zeng, R. F., Yang, Y., Wang, K., ... & Ho, R. J. (2021). A carrier-free folate receptor-targeted ursolic acid/methotrexate nanodelivery system for synergetic anticancer therapy. *International Journal of Nanomedicine*, 1775-1787.

25. Jin, H., Pi, J., Yang, F., Jiang, J., Wang, X., Bai, H., ... & Chen, Z. W. (2016). Folate-chitosan nanoparticles loaded with ursolic acid confer anti-breast cancer activities in vitro and in vivo. *Scientific reports*, *6*(1), 30782.

26. Xu, F., Li, M., Que, Z., Su, M., Yao, W., Zhang, Y., ... & Tian, J. (2023). Combined chemo-immuno-photothermal therapy based on ursolic acid/astragaloside IV-loaded hyaluronic acid-modified polydopamine nanomedicine inhibiting the growth and metastasis of non-small cell lung cancer. *Journal of Materials Chemistry B*, *11*(15), 3453-3472.

27. Ma, T., Zhou, J., Li, J., & Chen, Q. (2023). Hyaluronic Acid-modified Liposomes for Ursolic Acid-targeted Delivery Treat Lung Cancer Based on p53/ARTS-mediated Mitochondrial Apoptosis. *Iranian Journal of Pharmaceutical Research: IJPR*, *22*(1), 131758.

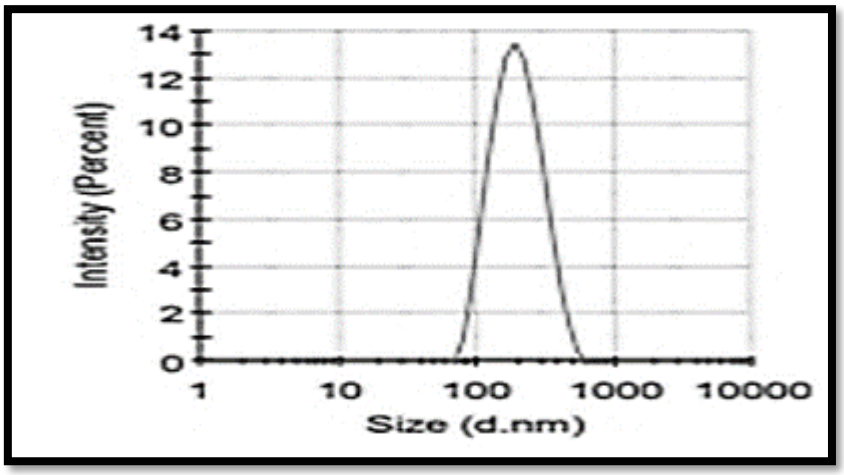

Figure 1: Particle Size

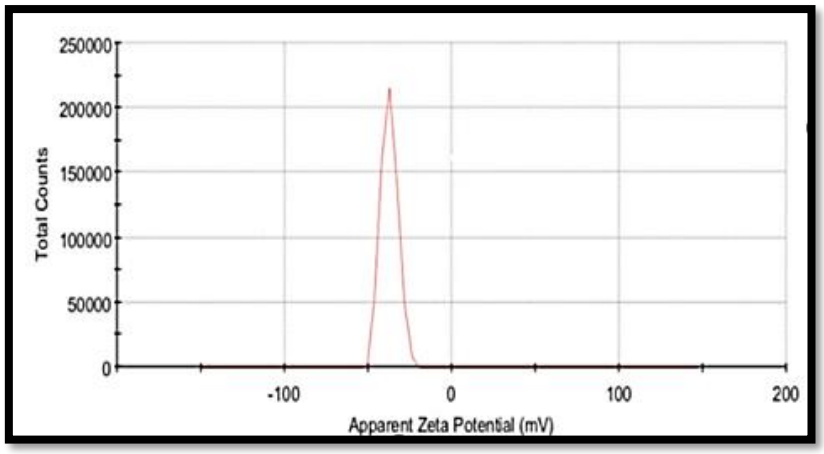

Figure 2: Zeta Potential

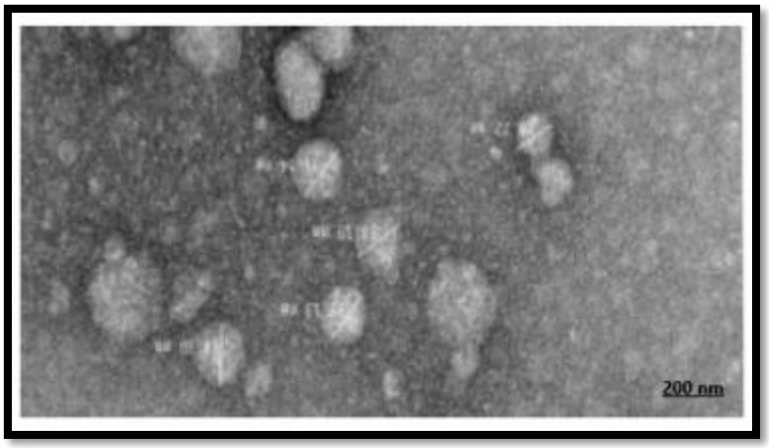

Figure 3: TEM image

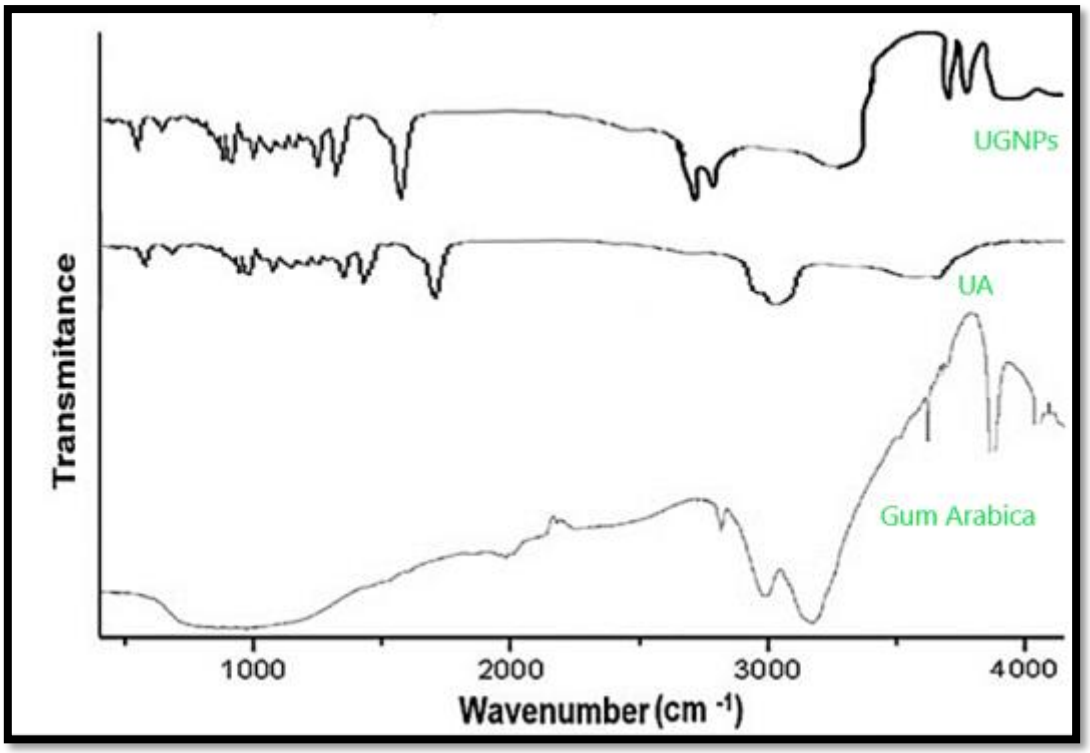

Figure 4: FTIR Spectra

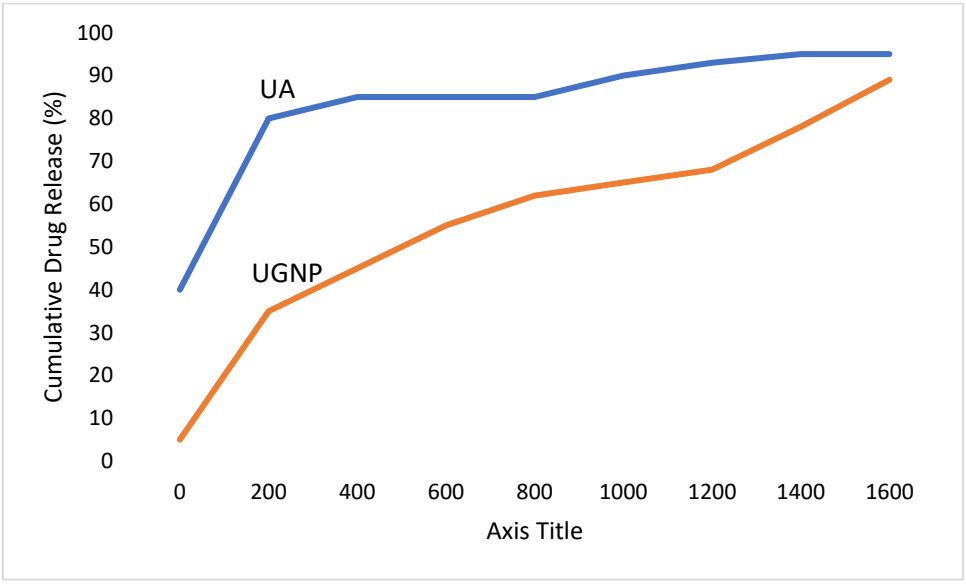

Figure 5: in-vitro Release Profile

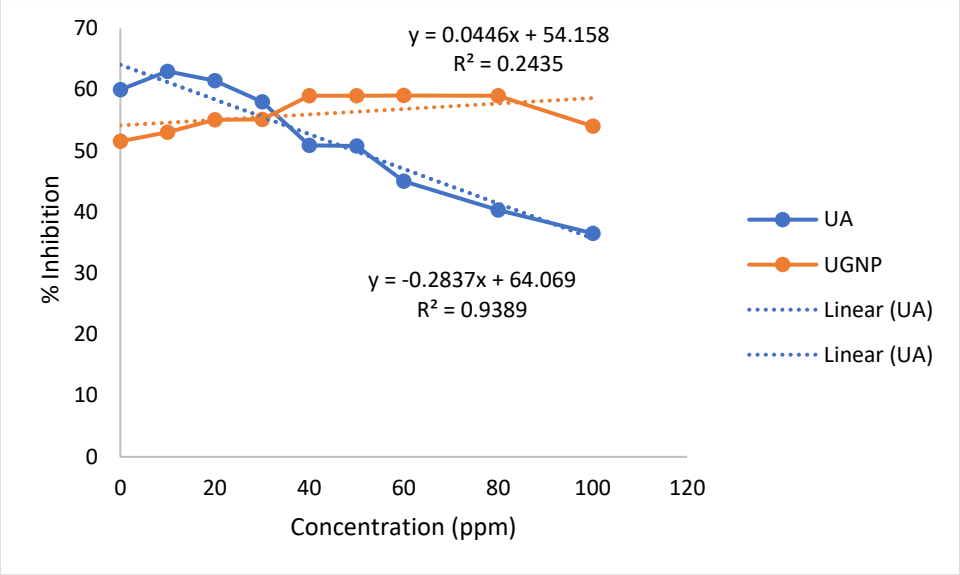

Figure 6: Evaluation of antioxidant activity

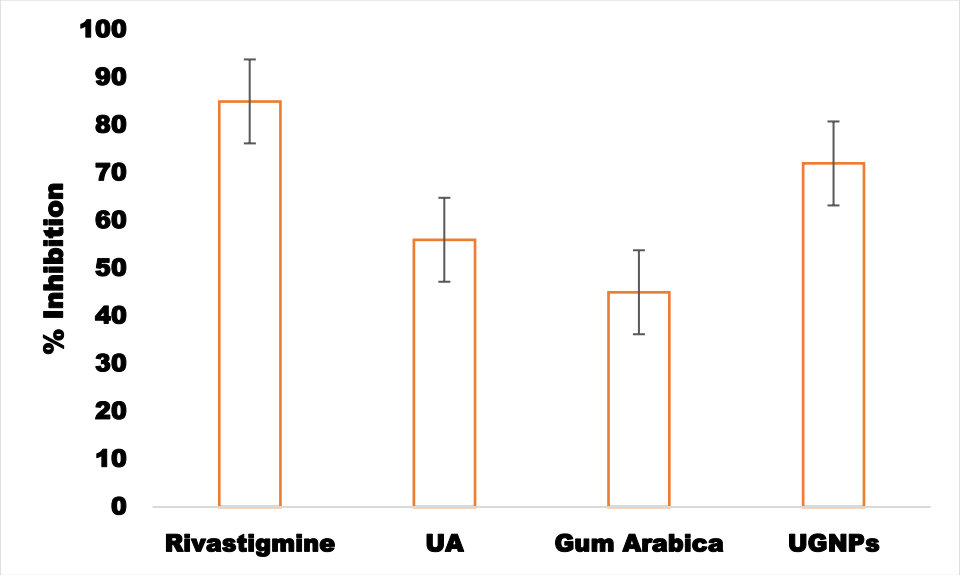

Figure 7: Evaluation of AChE inhibitory activity of UGNPs