Original Article
DOI: 10.4274/tjps.galenos.2023.11823

Alpha Amyrin Nano-Emulsion Formulation from Stem Bark of *Ficus Benghalensis* and its Characterization for Neuro-Behavioral Studies

Short title: Neurobehavioral Studies of Alpha Amyrin

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10.11.2022
13.03.2023
02.05.2023

Abstract:
Objectives: Alpha amyrin is a pentacyclic triterpene that shows erratic GI absorption and poor BBB permeability. The aim of the study is to isolate from alpha amyrin stem bark of *Ficus benghalensis* (*Fb*), its purification, and formulation of nano-emulsion which may improve its bioavailability, its characterization, intra-nasal administration to Swiss albino mice to check its neuro-behavioral effects in aluminium induced neuro-toxicity.
Materials and methods: Alpha amyrin was isolated from stem bark of *Ficus benghalensis* by Soxhlet extraction, purified by analytical methods, prepared chitosan decorated nano-emulsion of the same and characterized it, and was administered through intra-nasal route to aluminium treated Swiss albino mice for 28 days to check its effect on neuro-behavioral parameters**.**
Results: Intra-nasal delivery of chitosan-decorated alpha amyrin nano-emulsion brought significant improvement in neuro-behavioral parameters. It reduced fall off period in the rotarod test, and reduced escape latency in the Morris water maze test, and animals showed improved learning and spatial memory in the elevated plus maze, the transfer latency of animals improved with treatment compared to aluminum-induced groups indicative of the neuroprotective role of the drug.
Conclusion: Intra-nasal administration of alpha amyrin nano-emulsion isolated from the stem bark of *Ficus benghalensis* improved neuro-behavioral parameters in aluminium-induced neuro-toxicity in Swiss albino mice.
Keywords: Aluminium, alpha amyrin, nano-emulsion, intra-nasal, neuro-behavioral.

Abbreviations:
BBB: Blood-brain barrier; ***Fb***: *Ficus benghalensis;* AA- alpha amyrin; NE- nano-emulsion

Introduction:
Alpha amyrin (AA) is a pentacyclic triterpene of the ursane group with an alluring pharmacological profile. The drug posses' bioavailability issues due to poor water solubility and whimsical GI absorption, and poor BBB permeability which restrains its usage as a drug. Plant-derived secondary metabolites are formidable moieties that are richly present in plant species from tropical rainforests.
*Ficus benghalensis (Fb)*or the Great Indian banyan, a good source of phytocompounds can be utilized to isolate triterpenes like AA. It is found allover in Indian rainforests. The root contains phytosterolin, the leaves contain triterpenes, fridelin, and beta-sitosterol and the bark is rich in bengalinoside, flavonoid glycoside, leucocyanidin, leucopelargonidin, AA, phenols, alkaloids, tannins. Heartwood consists of alpha tatraxasterol and liglic acid. The milky latex is used for wound healing, swelling, skin diseases, to treat vaginal diseases, and diabetes, as a uterine

tonic, in diarrhea, nausea, vomiting, ulcers, IBS, bleeding disorders, etc. Studies related to the antimicrobial, anti-arthritic, and wound-healing effects of *Fb* confirm the potential of a Banyan tree.[1,2,3.]

Studies reveal that amyrins possess GI-protective action[4], anti-inflammatory activity,hepatoprotective[5] and help to regulate blood glucose levels and are efficacious against various cancer cell lines including liver breast colorectal cancers, etc as they induce cell death by apoptosis[6,7].The drug AA has also been reported to possess anti-hyperglycemic, and hypolipidemic action[8], and it has a role in modulating enzymatic, hormonal, and inflammatory responses[9].Despite its potential, factors such as poor water solubility, extensive half-life, deplorable clearance, and wavering GI absorption evinced by AA impede it from being used as a drug.[10] The route through which a drug is administered plays a vital role in determining the bioavailability of a drug especially when the candidate exhibits poor absorption through the GI route. To overpower the predicament, various approaches may be adopted like the complexation of the drug moiety or conversion into salt form, preparing nano-formulations, etc.[11] When a drug is supposed to target complex systems like the CNS, the BBB acts as a major barrier as it restricts and hinders the entry of xenobiotics. Various parameters related to the drug such as PKA, Log P, lipophilicity, bioavailability, and first-pass metabolism are important to ensure the proper drug action on the system involved. The problems associated with drug solubility and poor oral bioavailability, poor GI absorption, etc may be solved through the formulation of nano-particles or any nano-preparations. The same, when given through an alternate route like an intra-nasal or intra-venous route, would help to surpass the issues associated with GI absorption and first-pass metabolism[12]. Such alternate routes improve the bioavailability of the drug and ensure full fledge utilization of the pharmacological potential of the drug moiety.

Nanotechnology is a promising Promethean science that helps to meet the hurdles associated with adrug ADME hitches and thereby attenuates the bioavailability issues.[12] Incorporation of a muco-adhesive polymer like chitosan would help to overcome mucociliary clearance[13] and it helps to carry the intra-nasally administered drug moiety across the tight junctions of the BBB.[14,15]The approach opted here is through the formulation of a nano-emulsion of the drug AA[16]to be administered intra-nasally which would be carried to the brain[17] through the olfactory and trigeminal nerve supply which links the nasal mucosa directly to the brain[18].The work aims at isolating AA from the stem bark of *Fb* and developing an AA-loaded chitosan nano-emulsion suitable for intra-nasal delivery, targeting the brain and studying its effect in altering neurobehavioral parameters in aluminum chloride-induced neuro-toxicity.

MATERIALS AND METHODS:

Chemicals and Reagents:

Extraction and Isolation: Alpha amyrin standard (Sigma Aldrich, 98% pure), Methanol, Chlorophorm, petroleum ether, toluene: ethyl acetate: formic acid.

TLC: n-Hexane: Ethyl acetate (analytical grade) and Iodine crystals.

Apparatus: Soxhlet apparatus, Column chromatography: performed on silica gel (60–120 mesh, Thermofisher scientific) and TLC plates, Iodine chamber, glass chamber (Twin-trough)

Isolation of AA from *Fb* stem bark.

Plant raw material collection handling and extraction:

Fb (F: Moraceae) stem bark gathered during January 2022 from its habitat in Bengaluru (India), authenticated by a taxonomist and was preserved as a herbarium.(KCP-PCOG/FB/330/2021-22). The stem barks were segregated, followed by air drying and drying in an oven at 45˚ C, and coarsely powdered. 850gm of the powdered stem bark was subjected to exhaustive Soxhlet extraction with methanol and water (1:4) at 70 ˚C for about 2 hours and for 3 washes. The final liquid extract was reduced using a rotary evaporator and was conserved in a glass container for further studies.[19]

Isolation of AA:

TLC was performed with the methanolic extract of *Fb*. A measure of the extract was mixed with little methanol and was stowed and adsorbed on silica gel (grade 60– 120 mm, 245 g). The extract was loaded in silica gel column. The column was packed with petroleum ether and the phytoconstituents were eluted first with petroleum ether (60–80˚ C), followed by petroleum ether–chloroform (9:1, 1:1, 1:3, v/v),and finally with chloroform, chloroform-methanol (99:1, 98:2, 95:5, 9:1, 3:1, 1:1, 1:3, v/v) and methanol. Eluting the column with petroleum ether: chloroform (1:1) yielded colorless crystals of alpha-amyrin acetate. AA was obtained by recrystallizing the same from acetone.[19,20,21]

TLC: The crystallized form of AA was then subjected to TLC (Mobile phase: n-Hexane: Ethyl acetate (9:1)), IR spectroscopy (ATR-IR), HPLC (Mobile phase A- Ammonium acetate 10mm unadjusted, Mobile phase B: Acetonitrile: Methanol (1:1), Isocratic method: Flow-1.2ml/min; A-25% and B-75%, Column temp: 35˚C, Sample temperature: 15˚C) (HPLC waters 2695) and LCMS MS(LCMSMS Condition :LC condition:Solution A: 0.1 % Formic acid in water, Solution B: Acetonitrile MS Grade, Flow Rate: 1.2 ml/min, Column: WATERS X Bridge 50

X 4.6mm 3.5µ, C18, Mode of Elution: Gradient, Diluent: Methanol, Detector: UV-Visible. LCMS MS-(MS Quadrupole:Capillary-3.45,cone-3.3, Extractor-3,Source temperature-110, desolvation temperature-500, gas flow-desolvation L/hr-800 Cone(L/hr)-50, Scan time-0.2sec). The spectroscopic analysis confirmed the presence of AA (purity: 98.37%) in *Fb* stem bark.

Preparation of AA-loaded chitosan-decorated nano-emulsion preparation

Pre-formulation studies:

The melting point of the drug AA was performed using the Thiele tube method. Drug solubility in different solvents was assessed by the saturation shake flask method by dissolving it in solvents like water, phosphate buffer, dmso and methanol. The λmax of AA was determined by preparing solutions of different concentrations and was then scanned from 200-400 nm using a UV spectrophotometer.

Calibration curve of AA in methanol

A standard calibration curve of AA was made by drawing and making serial dilutions with AA stock solution and the absorbance of the same was measured at 205nm by UV method, methanol being kept as the blank.

Compatibility Studies

Physicochemical Characterization of C-AA NE Formulation: (Drug Excipient Compatibility Study (ATR-IR Analysis))

The physicochemical interactions of AA with chitosan were studied using Attenuated total reflectance-Fourier transform infrared (Shimadzu IR spirit, Japan). Drug and other ingredients (1:1) were stored in hermetically sealed glass vials at 40°C, 75% RH for one week. IR spectra of AA and the physical mixture was recorded using an ATR-IR (Shimadzu) instrument performed at a wavelength range of 4400cm^{-1} and 400cm^{-1}. This was to check for the compatibility between the drug and other components to check for any interaction.

Formulation of chitosan decorated nano-emulsion:

Chemicals and reagents: AA, low molecular weight chitosan (ICAR Central institute of fishery technology (85% deacetylated)), sesame oil, Tween 80, PEG 400(Sigma-Aldrich), glacial acetic acid, de-ionized water.

Apparatus: Polytron high – speed homogenizer(KinematicaPolytron™(PT-2100)), magnetic bead, stirrer, 0.2micron syringe filter, Horiba scientific particle size and zeta potential analyzer(Horiba SZ-100,z-type,Ver2.00),digital pH meter, Brookfield viscometer, UV Spectrophotometer.

Procedure:

A spontaneous emulsification technique was utilized to prepare chitosan decorated nano-emulsion of AA.[22] The procedure was carried out at 25˚C. NE's was prepared by adding the organic phase (AA, sesame oil, and polyethylene glycol stirred continuously) to the aqueous phase (chitosan solution and tween 80)[23] followed by continuous stirring. 20mg of chitosan (low molecular weight, ~50 kDa) dissolved in 100ml of 1% glacial acetic acid and homogenized at 2000 rpm for 24 hrs. To 25ml of chitosan solution, 2.5ml of Tween 80 was added and was blended well for 20 min and homogenized at 2000rpm. The oil phase consisting of 160mg of AA mixed with 10ml sesame oil and 5% Poly ethylene glycol was stirred for 1 hour at high speed and was added drop wise to the mixture of chitosan and tween and the mixture was agitated for 60 min at room temperature with continuous stirring at 2000 rpm (Kinematica Polytron™ PT2100).[24,25] The mixture was homogenized for around 2 minutes at 4000 rpm to produce NE with homogeneity and passed through a 0.2micron syringe filter to reduce and homogenize the size of droplets. NE so formed was collected, centrifuged and the supernatant is collected to determine the percentage drug content.

Evaluation of AA Chitosan NE:

The optimized AA-NE was investigated for 28 days under vigorous conditions as per ICH guidelines to analyze its thermodynamic constancy followed by cycles of heating and cooling to observe the physical appearance, evidence of creaming or turbidity, etc, and was centrifuged for about 10mins at 4000 rpm for 10min to check for any signs of instability.

The pH of the prepared chitosan NE was determined using a digital pH meter at room temperature. The Globule size and size distribution and zeta potential of AA-NE were determined using Horiba scientific instrument.

The morphology and structural attributes of the prepared formulation was examined using a simple light microscope. The viscosity of the formulation was checked using a Brookfield viscometer at room temperature. All investigations were performed in triplicate.

Percentage drug content was assessed using UV spectroscopy. A measured volume of the nano-emulsion was centrifuged for 40 min at a speed of 15,000, rpm at 25^0 C to separate the drug which is now separated in the supernatant from the drug in the NE after dilution. The percentage drug content was calculated by the formula

Drug content= $\frac{\text{Absorbance}}{}$ × dilution factor

slope

Surface morphology:

TEM analysis otherwise known as transmission electron microscope was used to determine the morphological attributes of AA-NE formulation and images of the NE were taken at various resolutions.[25,30]

Neuro-behavioral studies:

Apparatus used: Rolex digital rotarod apparatus, Elevated plus maze, Morris water maze.

Swiss albino mice were procured from the animal house at Krupanidhi College of pharmacy (in-house) after the animal ethical clearance (KCP/IAEC/PCOL/60/2020). Animals were grouped into 4 groups with 6 animals in each group. Normal, Positive control ($AlCl_3$ 100mg/kg *p.o*), Treatment group1 (1mg/kg *IN* of AA-NE + $AlCl_3$(100mg/kg *p.o)*), and treatment group 2(2mg/kg *IN* of AANE+ $AlCl_3$(100mg/kg *p.o))* for 28 days. Neuro-behavioral tests like the rota-rod test, Morris water-maze test, and elevated plus-maze tests were performed on days 14 and 28 of the study.

RESULTS:

Isolation of AA from *Fb* stem bark:

The stem bark extract of *Fb* on concentration yielded a brownish mass (74 g, 11%) from which y 0.457 g of the pure compound was isolated (0.609% yield).

Confirmation of AA by the analytical method:

TLC: Thin layer chromatography performed shows the presence of AA in the sample compared to the standard (fig:1).

FTIR: FTIR of isolated AA compared to the standard confirms the purity of the isolated compound (fig:2).

LCMS: LCMS MS results (fig 3 and 4), confirm the presence of AA with evident peaks at 426.61 and 218.72. Ions with a mass-to-charge ratio of 218 and 426.6 were identified to be of AA. Isolated AA shows an m/z value of 426.61similar to standard AA of 426.7.

HPLC of AA: HPLC shows that isolated AA as well as standard, has retention at 5 confirming the purity of AA.(fig:5)

Preparation of AA chitosan NE:

Pre-formulation Studies:

The melting point of AA was 186°C. Solubility studies of AA in various solvents showed its solubility (Table:1) in methanol and DMSO.

Calibration curve of AA*:* AA showed λ_{max} at 205nm and this wavelength was chosen for analysis. Serial dilutions from a solution from a stock solution (10mg of AA dissolved in 50ml of methanol and sonicated) were prepared and were analyzed using UV spectrophotometer which gave the calibration curve.(fig: 6)

Compatibility studies:

FTIR Analysis:

The FTIR studies of the drug and mixture of drug, polymer, and other components to was performed to investigate the interaction at wavelength between 4400 and 400cm^{-1} (fig7&8).O-H Stretching between 3550-3200 cm^{-1}, C-H bending at 3550-3200 cm^{-1}.C-H Bending at 1465 cm^{-1} , C=C bending at 995-985 cm^{-1}.The mixture of AA with Chitosan, Tween 80, PEG, and sesame oil has all the characteristic peaks of AA which confirms that the components are compatible.

Evaluation of AA Chitosan Nano-emulsion:

The nano-emulsion was found to be thermodynamically stable. The physical appearance remained unaffected and the preparation did not show a creaming effect or turbidity when subjected to heating and cooling cycles upon centrifugation did not show phase separation and was stable.

Particle size (globule size) zeta potential and PDI determination:

The composition of formulations F1 and F2 are given in Table 3, making use of same components in both formulations in different ratios.The average size distribution of prepared nano-emulsion F2 was 75.9 ± 5.4nm with a PDI of 0.3 which suggests the formation of nano-sized formulations (table:1), whereas F1 exhibited greater particle size, higher poly-dispersity index, and unstable zeta-potential hence F1 was not studied further and F2 was selected for the rest of the analysis.

pH and viscosity of NE is depicted in table 4. The NE was found to have a percentage drug content of 79.65%

Surface morphology by TEM analysis

Transmission electron microscopy images indicate that the average particle size of the NE F2 is coming between range of 50-100nm and as per globule size analysis was found to be 75.9 ± 5.4nm. The particle size of chitosan nano-emulsion is in accordance with the literature (fig 10).

Neuro-behavioral studies:

Neuro-behavioral studies:

Rota-rod test: The fall-off time of animals using rota-rod apparatus was performed on day 14 and day 28. The Statistical significance of the results of rota-rod test was ascertained by collating treatment groups with the respective positive control group by applying One-way ANOVA ordinary measures and ensued by Dunnett's test. The data are expressed as mean ± SD (n = 6), and [a] $p<0.001$ when in contrast with the normal group. [b,c] $p<0.01$ & when in contrast with the positive control group (fig 11).

The positive control group (AlCl3) animals showed a significant reduction in fall-off period, motor coordination, and balance when compared to the normal group on days 14 and 28.The experimental animals under AlCl3(100mg/kg *p.o*) induced oxidative stress exhibited significant improvement in the fall period when treated with AA at a dose of 1mg/kg *IN* and at a dose of 2mg/kg *IN* compared to the positive control group. The results suggest that AA treatment caused betterment of aluminium chloride-induced impairment in balance, and coordination in animals at doses 1mg/kg *IN* and 2mg/kg *IN* respectively.

Morris water maze test:

The escape latency spatial memory and learning in animals were tested using Morris water maze.

The Statistical significance of the results of Morris water maze test was determined by collating treatment groups with the respective positive control group by applying One-way ANOVA ordinary measures and ensued by Dunnett's test. The data are expressed as mean ± SD (n = 6), and [a,b] $p<0.001$and significant in contrast to the positive control (AlCl3) group. The treatment with AA at a dose of 1mg/kg and 2mg/kg *IN*. significantly reduced escape latency indicates that AA at both doses helps to improve learning and spatial memory in animals despite of AlCl3 treatment(fig:12).

Elevated plus maze test:

The Statistical significance of the initial, first and second transfer latency of animals in elevated plus maze test was ascertained by collating treatment groups with the respective positive control group by applying One-way ANOVA ordinary measures and ensued by Dunnett's test. Here the initial transfer latency data is shown as mean ± SD (n = 6), [a] $p<0.001$ and significant with respect to the normal group, [b, c] $p<0.01$ were significant with respect to the positive control(AlCl3) group(table 5), the first transfer latency data are expressed as mean ± SD (n = 6), [c,d] $p<0.01$ and significant compared to the positive control(AlCl3) group and data related to second transfer latency are expressed as mean ± SD (n = 6), and [a] $p<0.001$ when compared to the normal group and [b, c] $p<0.01$ compared to positive control (AlCl3) group.

Discussion:

The investigation involved isolation of AA from *Fb* and devising chitosan decorated nano-emulsion of AA, characterization of the same and evaluation of its effect on neuro-behavioral parameters after its intra-nasal administration to aluminum chloride administered Swiss albino mice. AA was isolated from the methanolic extract of *Fb* stem bark by subjecting the extract to silica gel column chromatography. The colorless crystals of AA. So obtained was subjected to TLC, infrared (IR), HPLC and LCMS MS. Spectroscopic analysis confirmed the presence of AA (purity: 98.37%) in *Fb bark*.

Chitosan decorated NE of AA for *IN* administration was prepared and characterized and it showed desired size range zeta potential PDI, percentage drug content etc. Upon treatment, animals showed significant improvement in neuro-behavioral parameters in AA treatment groups compared to groups with aluminum chloride-induced neurotoxicity. The amount of chitosan has a role in the size of the globule as well as for the proper coating of the globule. An optimum amount of chitosan resulted in F2 with particles with an appropriate globule size below 100nm with zeta potential and PDI. Viscosity of the nasal preparation is very important as it should be having optimum viscosity and should stay in the nasal cavity resisting mucociliary clearance and increasing the residence time, This attribute was also confirmed by the incorporation of chitosan in the preparation.[25] The optimum pH for the intra-nasal formulation is 4.5- 6.5 and the formulation F2 abided the same.[26]

The $AlCl_3$ induced impairment in motor coordination and balance when compared to the normal group which reduced the fall-off time on both days 14 and 28. The experimental animals under AlCl3(100mg/kg *p.o*) induced

oxidative stress exhibited improvement in the fall period when treated with AA administered *IN* at a dose of 1mg/kg and 2mg/kg respectively with a better effect on day 28. The results suggest that AA treatment caused betterment of aluminum chloride-induced impairment in balance, and coordination in animals at doses 1mg/kg *IN* and 2mg/kg *IN* respectively. The results are contrary to the results of a study where Alpha and beta amyrin (30 mg/kg), given *i.p*. 30 min prior, couldn't alter the motor response of the animals[21]. The probable reason is thought to be due to the effect of the NE form of AA administered through an *IN* route that allowed direct action of the drug on centers for coordination and balance in the brain.

In the Morris water maze, normal animals showed shorter escape latency on days 14 and 28 in a dose-dependent manner and the results were quite good as they expressed good learning and spatial memory as they identified the cues fast and showed shorter escape latency with respect to the positive control group. AA at a dose of 1mg/kg and 2mg/kg *IN* significantly reduced the escape latency and the animals could find out the hidden platform very fast no matter from which quadrant they started. A supportive study involving administration of amyrin rich bombax ceiba extract showed good effect in animals as it increased the escape latency in animals.[28] Incase of elevated plus maze test, thetotal entries made by the animals to the open arm increased in AA-treated groups(results not given here) indicative of its anti anxiety and antidepressant potential[27] and its role in improvement of retention memory in treated animals compared to the induced group. This indicates that AA at both doses helps to improve learning and spatial memory in AA-treated animals despite AlCl3 treatment.

Conclusion:

The findings of the investigation conclude that AA nano-emulsion administered intra-nasally brought about significant improvement in learning, spatial memory, retention memory, motor coordination, balance, etc in aluminium chloride-induced oxidative stress.

Acknowledgment:

We would like to thank ICAR Central institute of fishery technology for providing sample of low molecular weight chitosan.

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Table 1 :Solubility of alpha amyrin in different solvents:

Solvent	**Type**
Phosphate buffer pH 6.4	Insoluble
Methanol	Very soluble
Water	Insoluble
DMSO	Soluble

Table 2: :Formulation of alpha amyrin chitosan nanoemulsion: Composition of Formulation

Code	Acetic acid(%v/v)	Chitosan (%w/v) (25ml	PEG-400 (%w/v)	Sesame oil(ml)	Tween 80	Drug(mg/ml)
F1	1	5	5	10ml	1.25ml	16mg
F2	1	20	5	10ml	2.5ml	16mg

Table:3 Particle size (globule size) zetapotential and PDI determination

Sl No:	Globule size	Zeta potential	PDI
F1	236.1±507 nm	14.7mV	1.6
F2	75.9 ± 5.4nm	25.6mV	0.39

Table:4 pH and viscosity of chitosan nanoemulsion

Sl.No	Ph	Viscosity(cP)(20rpm)
1. F2	4.9 ± 0.1	Spindle 1- 48cP Spindle 2- 61cP

Table: 5 Transfer latency of animals (initial, first, and second transfer latency) of animals in the elevated plus maze

Sl No	Group	Initial transfer latency	First transfer latency	Second transfer latency
1	Normal	11.5±1.51	10.66±1.63	5.83 ± 1.47
2	AlCl3 group (positive control)	26.16± 1.72[a]	27±1.78	29.16±2.04[a]
3	Alpha amyrin 1mg/kg	18±1.89 [b]	15.16±1.60[c]	11.33±1.75[b]
4	Alpha amyrin 2mg/kg	19.5±1.87 [c]	14.66±1.75[d]	9.5+ 1.87[c]

One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean ± SD (n = 6), [a]p<0.001 and significant compared to the normal group, [b, c]p<0.01 were significant compared to the positive control(AlCl3) group.

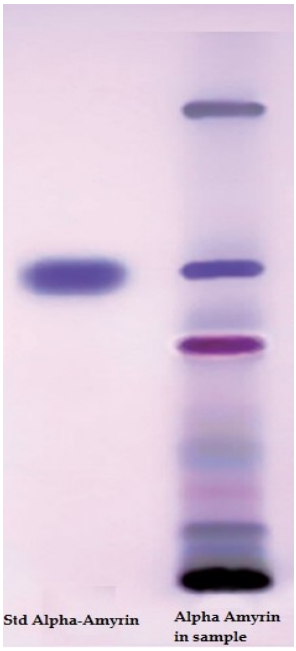


Figure:1 - TLC plate of Isolated Alpha amyrin
Mobile phase: n-Hexane: Ethyl acetate (9:1)Rf: 0.43.

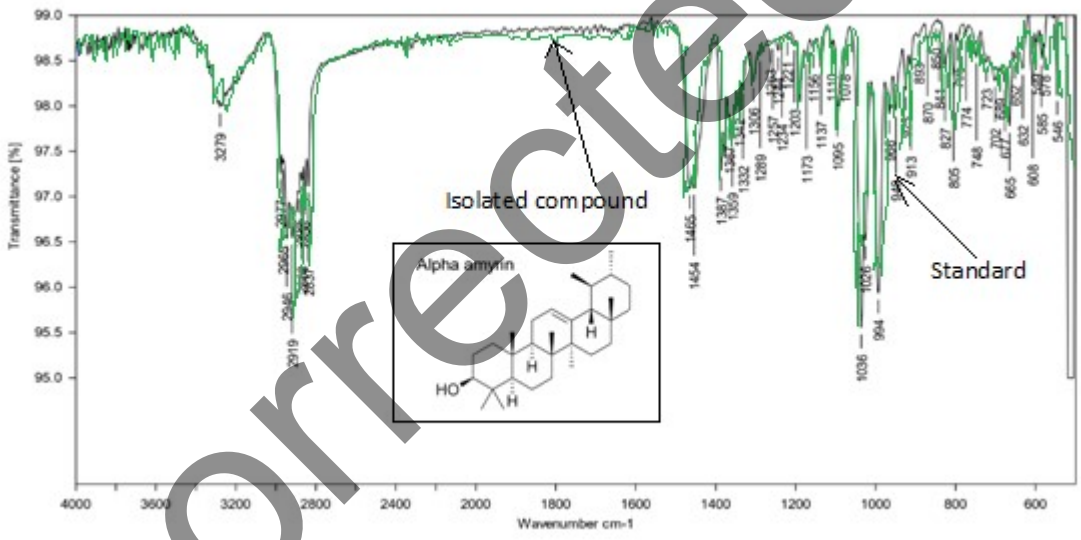


Fig:2 FTIR Confirmatory study of isolated alpha amyrin with standard
IR with green lines= Isolated alpha amyrin; IR with black lines= Standard alpha amyrin

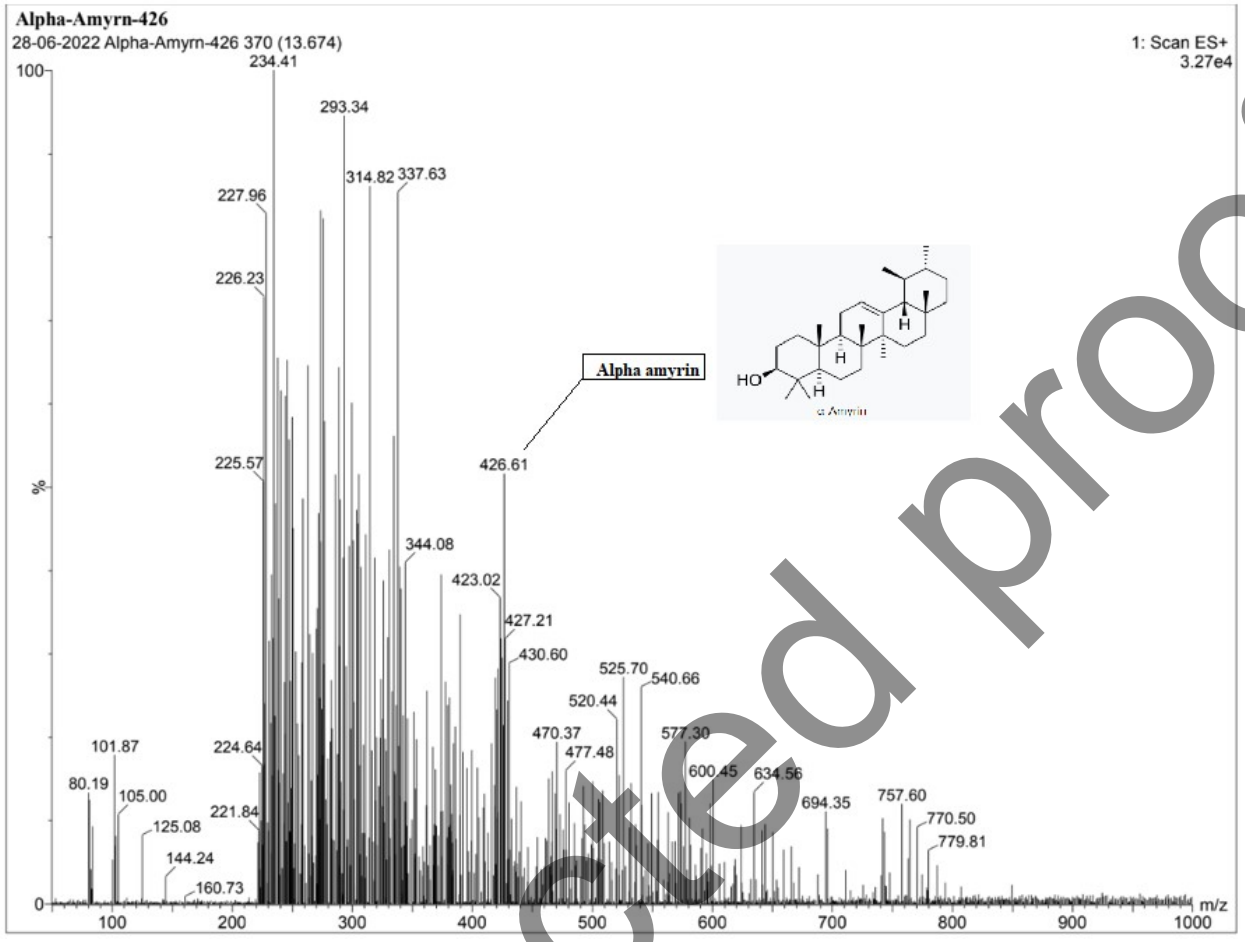


Fig:3 LCMS/MS analysis of alpha amyrin

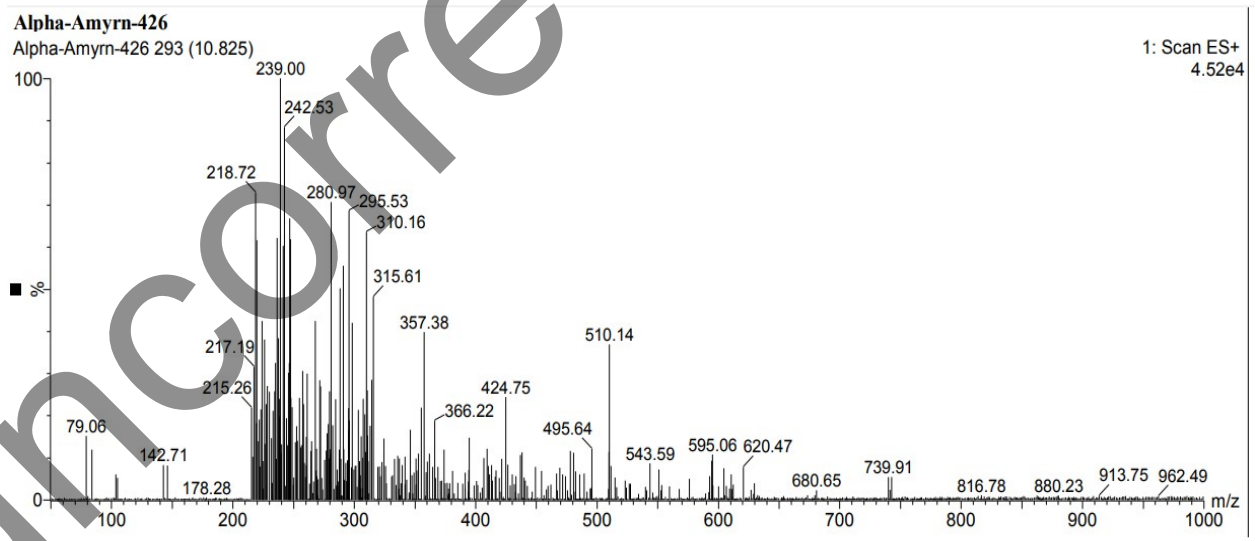


Fig:4 LCMS/MS analysis of isolated alpha amyrin

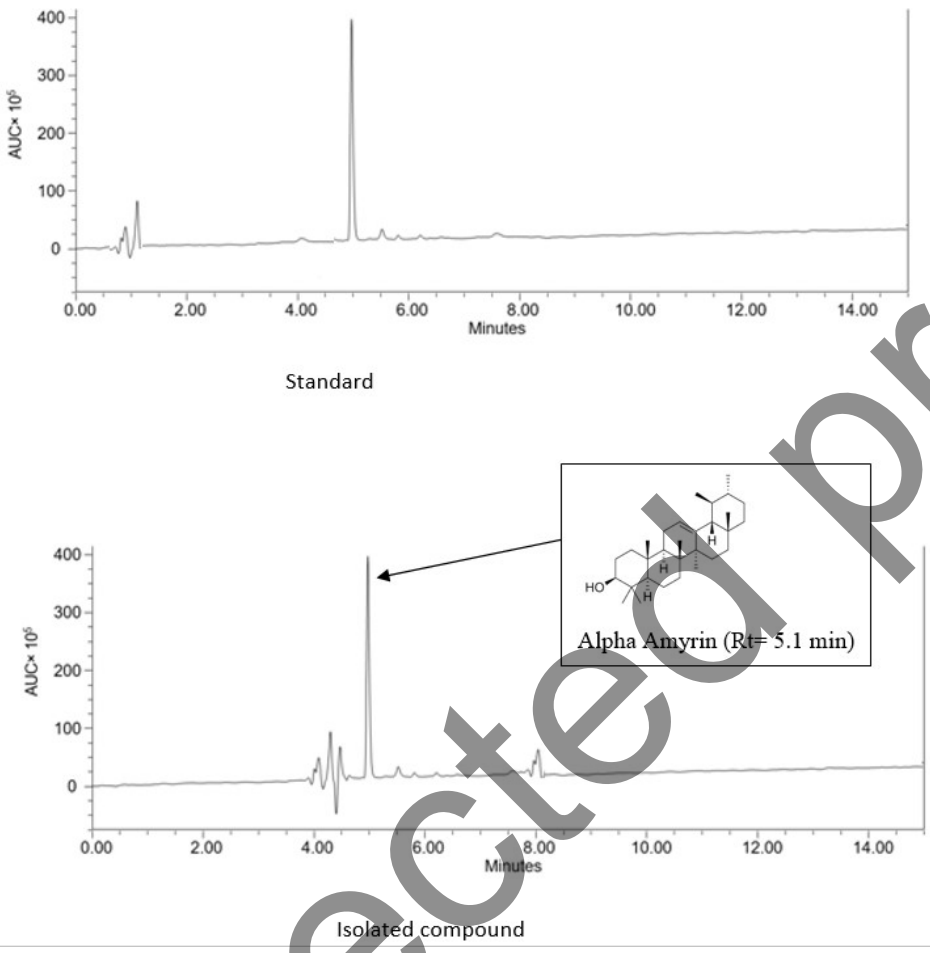


Fig:5 HPLC of standard and isolated alpha amyrin

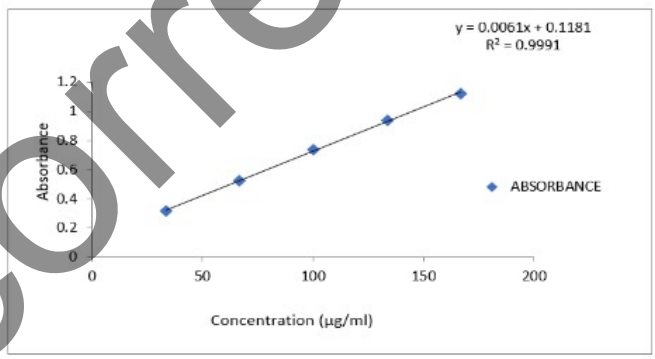


Figure: 6 Caliberation curve of alpha amyrin

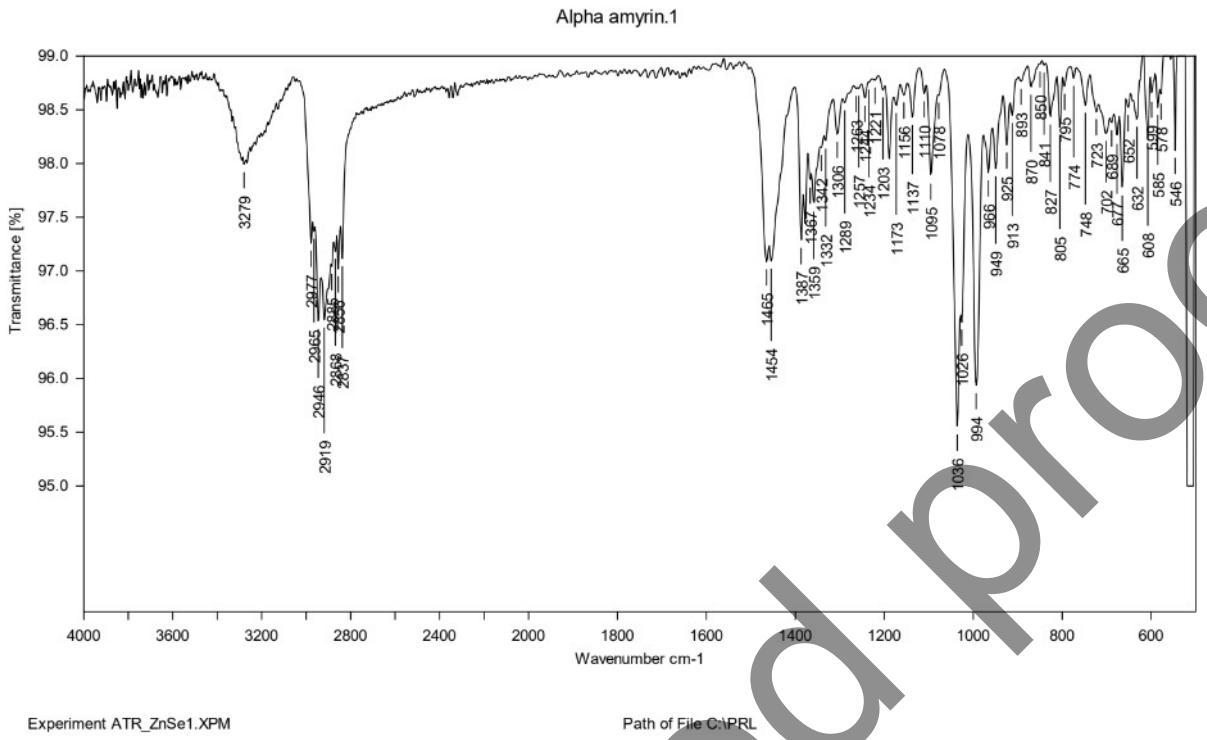


Fig:7 FTIR of Alpha amyirin

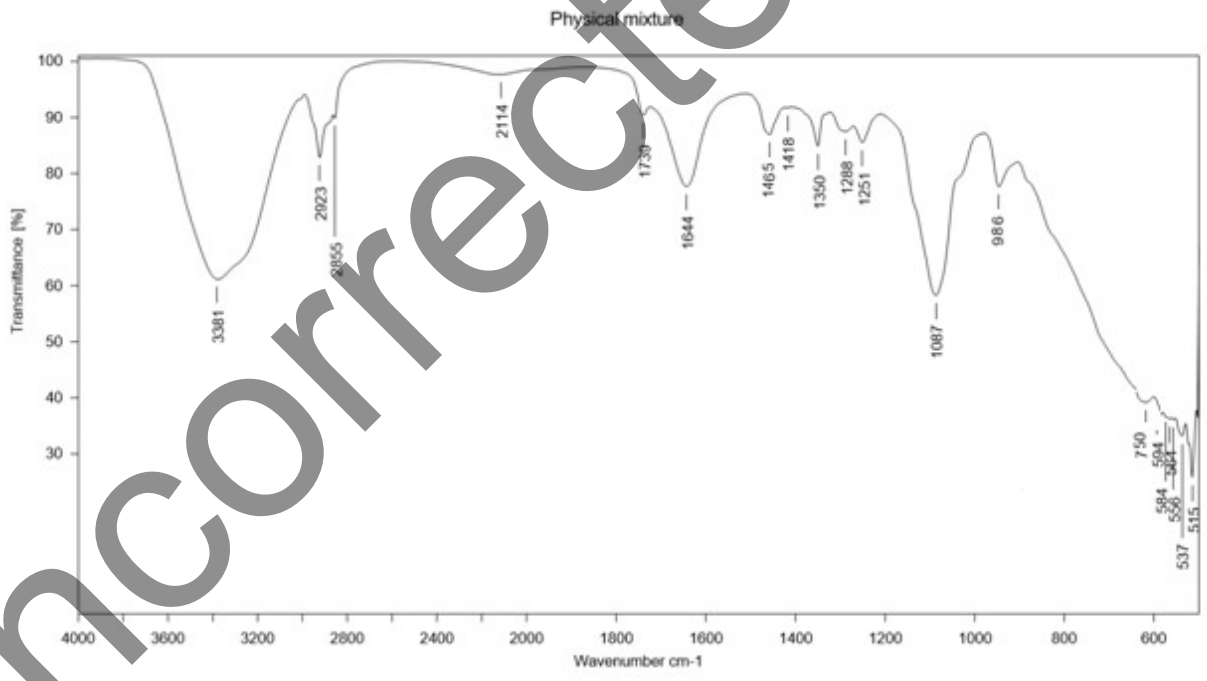


Figure 8: FTIR of Physical mixture

Fig:9 View of nanoemulsion F2 through an electron microscope

TEM analysis images:

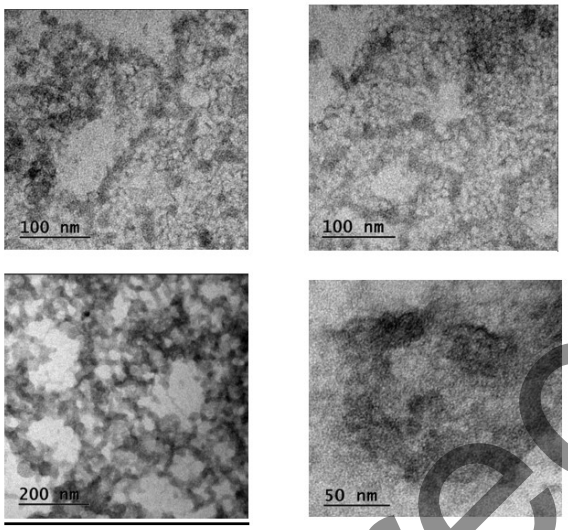
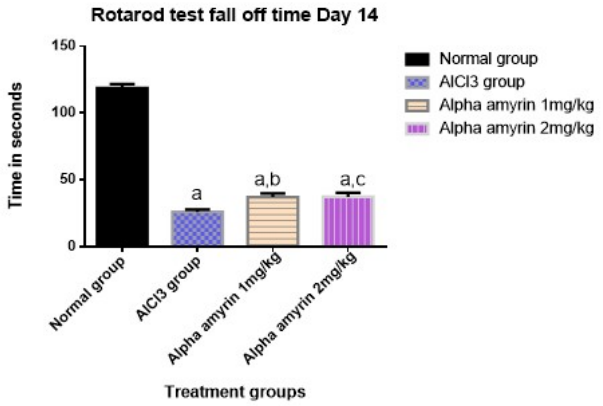


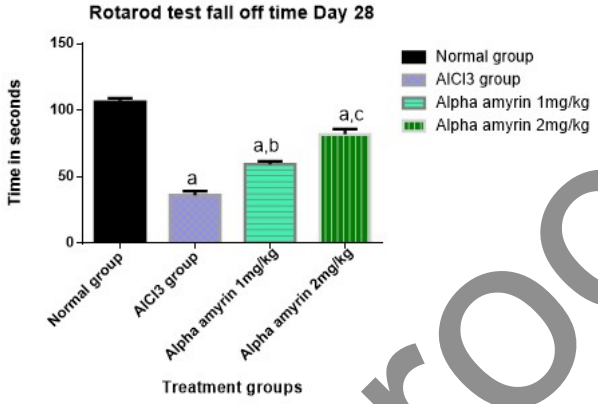
Fig:10 TEM images

Neurobehavioral studies:

Rotarod test:

Fig:11 depicts graphical representation of effect of alpha amyrin on fall off time of animals in rotarod apparatus in AlCl3-induced oxidative stress : Day 14 and day 28

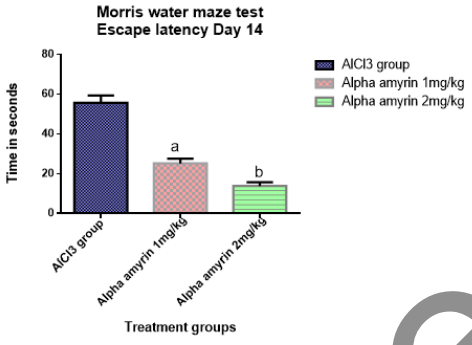


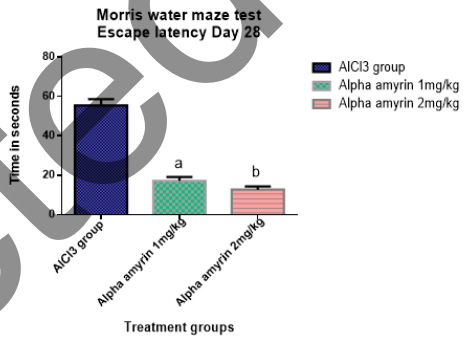


One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean ± SD (n = 6), and [a] $p<0.001$ when compared to the normal group. [b,c]$p<0.01$ & when compared to the positive control group.

Morris watermaze test:

Figure: 12 Effects of isolated alpha amyrin (1mg/kg and 2mg/kg) against $AlCl_3$ induced oxidative stress on escape latency - Day 14 and day 28.





One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean ± SD (n = 6), and [a,b]$p<0.001$and significant when compared to the positive control (AlCl3) group.