NEUROPROTECTIVE EFFECT OF NANOEMULSION OF CUCUMIS SEED OIL IN RODENT MODELS

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ABSTRACT

Plant extract products are becoming more and more popular, and pharmaceutical corporations are starting to support this trend as natural medicinal methods gain popularity. Comprising multiple phytoconstituents that are essential for treating a range of ailments, Cucumis melo, sometimes referred to as musk melon, is a member of the family Cucurbitaceae. The safety of Oil and NE's up to a dose of 2000 milli grammes per kilogramme of body weight was validated by an acute toxicity evaluation. By employing hydroxyl radical scavenger and reducing power experiments to assess in vitro antioxidant activity, it was discovered that the oil efficiently scavenges unstable radical in a dose-dependent way. The length of the extension and flexion phases in maximal electroshock-induced seizures was dramatically shortened by oil, F2, and F3 at a dose of 400 mg/kg. In the Rotarod test, there was no evidence of motor incoordination. Because of the oil and formulae utilised, the treated groups buried a much smaller number of marbles than the control group. Triterpenoids and phytosterols may be the cause of the seed oil's anxiolytic and antiepileptic qualities. By influencing GABAergic neurotransmission or associated pathways, these substances may bolster the idea and account of their double anxiolytic and anticonvulsant properties. According to the study's findings, mice treated with pressed cold Cucumis melo seed oil and its O/W types F2 and F3 nano emulsions show anticonvulsant and anxiolytic properties.

Keywords: anticonvulsant, antianxiety, seed oil, nano emulsion, Cucumis melo

1. Introduction

A persistent neurological illness affecting people all over the world is epilepsy. Originally, Falco-Walter et al. defined epilepsy as a momentary manifestation of symptoms brought on by abnormally high or synchronised brain neuronal activity. The International League Against Epilepsy (ILAE) has revised this definition. One of the most prevalent neurological conditions, epilepsy affects about 50 million people globally. According to data, 80% or more of people with epilepsy live in low- and middle-income nations.

An estimated 230,000 individuals of all ages, genders, and ethnicities in Malaysia are reported to have been diagnosed with epilepsy; this amounts to 1% of the country's entire population. According to a survey research, Malaysia has a lifetime epilepsy prevalence of 7.8 per 1000 people. When treating epilepsy, antiepileptic medications (AEDs) like phenytoin & the drug are used to lessen seizure frequency and intensity without having negative side effects. Sadly, medication utilised to treat epilepsy has a low absorption and eventually develops drug resistance, making it ineffective. Because of their severe adverse effects, several of the more recent AEDs—like levetiracetam—are prescribed less frequently. AEDs are now solely given for safer and more tolerable patient outcomes. In comparison to newer AEDs, older medicines receive less prescriptions from doctors due to their major adverse effects, which include liver damage, encephalopathy, and gingival hyperplasia. These drugs also influence the central nervous system (CNS).

In addition, some older medications, such as phenytoin, have an adverse effect on bone metabolism by activating an enzyme. A low bone metabolism will lead to a decrease in bone mass and a higher chance of fracture. In general, there is little doubt about the drawbacks of using contemporary medications to treat epilepsy; as such, it is advisable to explore for other options, such as traditional medicine. The use of traditional herbal medicine is essential in the management of epilepsy.

Anxiety disorders are prevalent psychiatric conditions affecting people of all ages in society. Selective mutism has emerged as a key area of interest for psychopharmacology research, along with anxiety disorders such as Agoraphobia, Specific Anxiety, Anxiety Attack, Separation Anxiety Syndrome Disorder, and Social Phobia. Serotonin-reuptake inhibitors (SSRIs) and benzodiazepines are two important medication groups for the therapy of anxiety disorders. Hepatotoxicity, muscular relaxation, and sleeplessness are just a few of the negative effects that these substances have. A broader safety margin, fewer side effects, and a quick beginning of action are all factors that are relevant to the search for novel anxiolytic chemicals.

The Cucumis melo, or musk melon, is an annual plant that is a member of the Cucurbitaceae family. It has angular, scabrous stems, soft leaves the orbicular-reniform forms, and tendrils that help with training over trellises so it can climb or extend across the ground. In India, muskmelon is a popular crop, especially in the hot, dry northwest. Its principal byproducts are pulp, seeds, roots, and seed oil. It has cooling, emmenagogue, diuretic, and demulcent qualities. The fruit has long been used to treat conditions affecting the kidneys, including as kidneys and stones in the bladder, unpleasant and burning urination, infections of the urinary tract, retention of urine, inflammation of the liver, obstructions of the liver and bile ducts, dermatitis, and more. Lecithin, cephalin, cerebroside, and 60–70% linoleic acid are found in seeds oils, which are regarded as being extremely nutritious. Melon seeds include compounds such as 24-methylenecycloartenol, multiflorenol, and isomegaflorenol. Numerous positive effects, such as nephroprotective, cytotoxic, antioxidant, antimicrobial, anti-diabetic, anti-inflammatory, analgesic, diuretic, anti-helmintic, anti-hyperlipidemia, antiulcer, hepatoprotective, haemolytic, and antibacterial qualities, are associated with these compounds.

The medication utilised will have a particle with a smaller size, making it easier for it to cross the blood-brain barrier, making nano emulsions via parenteral delivery possible. If the droplet size of an emulsion is less than 200 nm, it is categorised as a nano-emulsion.

It is made up of oil, surfactants, and water. Because of their wide interfacial area, nano-emulsions make it easier to deliver drugs or active ingredients—like particular chemicals from crude extracts—to the intended location. The permeability of the oil molecule is increased when an active component is added through passive diffusion. This procedure can boost lipid transport into the brain by making them more soluble. Thus, stability and biodistribution may be enhanced by a nano-emulsion-based delivery method including Cucumis melo seed oil extract that is supported by comprehensive physicochemical characterisation for clinical application[1-25].

2. Materials and methods

2.1 Materials:

Solvents and acids used in this study are analytical grade and purchased from SD fine chemicals Ltd. and research lab fine chemical industries., Mumbai. The reference drugs phenytoin, Lorazepam were procured from research lab fine chem industries and Tarapur, M.I.D.C., Boiser, Mumbai. Materials were weighed using Shimadzu Corporation, Japan electronic weighing balance. The samples were centrifuged using REMI, India Centrifuge. The absorbance was measured using merk Prove 300 spectroquant® UV-spectrophotometer.

2.2. Seed collection

Cucumis melo seeds were collected from the fruits from a local market in Hyderabad, Telangana, and they were authenticated. After properly cleaning the seeds, they were leftto sun dry for around eight days. Oil was extractedby employing the cold press technique.

2.3 Extraction of oil by cold pressing

A screw press that runs under mechanical pressure is used in a cold press system to separate the solid-liquid phases. Depending on the specifics of the extraction process, oil viscosity can be decreased by adjusting the temperature during cold pressing. Cake and oil stream are the main outputs of cold press equipment [26]. The crushed particles' inner and exterior surfaces are firmly compressed together when pressure is applied. As a result, two different processes are used to make the cakes and the liquid components. Stated differently, the oil is removed from the cake's crushed interior [27].

2.4 GC-MS study for CMSO

Approximately 1 mL of CMSO was dissolved using 3 mL of acetone. Mix it well. To ascertain the phytochemical makeup, a GC-MS investigation was later carried out.

2.5 Characterization of CMSO

Determining the physicochemical characteristics of the oil derived from Cucumis melo seeds through cold pressing was done. The tests performed on the oil are described below.

Physical Characteristics of CMSO

Sensory analysis of the CMSO

Sensory analysis, which takes into account taste, smell, and sight, was used to determine the physical properties of oil.

Determination of solubility of the essential oil in water

Fill a test tube with a small amount of water and a few drops of oil. Use the glass rod to stir or shake the test tube. Oil is insoluble in water if two phases are separated [28].

Determination of Density:

The most reliable way to determine how much oil there is in relation to water is to measure its specific gravity. This aids in evaluating and comparing the oils. According to Pardeshi et al, a weighing balance was used to determine the mass for a dry, clean bottle. The bottle was weighed following the addition of distilled water to it. The identical bottle received an identical amount of oil applied in the same manner and weighed. To determine specific gravity, one might use the weight ratio of water to oil. Tsegaye Fekadu et al. used the following formula to determine the exact gravity of the crude extracted oils [28,29].

Determination of Viscosity:

A liquid's viscosity is determined by measuring how resistant it is to flow. The oil's viscosity was measured at room temperature using an Ostwald viscometer. Since earlier studies have demonstrated a negative correlation between oil viscosity and unsaturated fatty acids. The viscosity of the extracted oil was investigated as an additional surrogate for fat unsaturation. The amount of time that the liquid will need to travel due to gravity between marks A and B, respectively, in a capillary tube is calculated. A comparison is made between the time necessary for the oil being tested to flow and the time required for a liquid with a known viscosity to flow.

Determination of the Refractive index:

ABBE refractometer, it is used to measure the extracted oils' index of refraction, check whether the prism was dry and clean, and then modify the mirror. Apply a small amount of oil to the prism, close it, and allow it to set for a few minutes. To obtain the clearest reading possible, adjust the lighting and equipment. Then, compute the refractive index or ABBE-refractometer number[29].

Determination of PH:

The pH, or hydrogen ion concentration, is a measure of a sample's acidity or alkalinity. A digital PH meter was used to measure the PH of CMSO.

Chemical characteristics of CMSO

Assessment of saponification value of CMSO

How much KOH is required for saponification A single gramme of oil is measured using the saponification value. For this, 50 ml of 0.5M KOH was put to a 200 ml conical flask that had been weighed with 2g of oil. After adding two to one drop of phenolphthalein indicator, the oil sample and KOH mixture were titrated with 0.5M HCl until the pink colour that had been visible when the indicator was applied vanished. The procedure was carried out without the oil, and the titre value was computed using the blank value.

Assessment of acid value of CMSO

The acid value of 1grm oil is the number of bases required to neutralise its acidic constituents. Put two grammes of oil and fifty millilitres of ethanol into a conical flask, then add a couple of drops of the indicator phenolphthalein and give it a good shake. After titrating this mixture with 0.1M NaOH, a pink tint was observed. The volume that is used is noted.

Assessment of ester value of the CMSO

The value of ester is the quantity of potassium hydroxide required to neutralise one gramme of fatty acid esters. It was calculated as the acidity to saponification ratio of the oil [28].

Ester value = saponification value- acid value

2.6 Formulation of O/W CMSO nano-emulsion

CMSO NE was made up of 10% CMSO in various concentration ratios along with surfactant and co-surfactant. Different ratios of surfactants & surfactant concentrations were employed. Consequently, nine formulation combinations were produced and designated as F1 through F9. Using a motor and pestle, the traditional method was used to create these mixtures. where the oil is continuously triturated while the necessary amount of co-surfactant is added. Next, surfactant is added, and the mixture is stirred until a clicking sound is generated. Lastly, until the emulsion forms, water is gradually added into the mixture drop by drop while trituration is ongoing. Only one orientation should be used for trituration. After that, this emulsion is ultrasonically treated for 30 minutes. Using an ice bath helps prevent excessive heating during sonication[30].

Stability testing of the formulations

Centrifugation:

The emulsions' resistance to the procedure was evaluated by centrifuging each formulation for ninety minutes at a speed of one thousand rpm. Further testing was done on the formulations that did not exhibit phase separation.

Heating cooling cycle

The purpose of the experiment was to assess the thermodynamic dependability of the formulations. The formulations which passed were placed in glass ampoules and let to cool or heat between 4 and 45 degrees Celsius for a duration of 48 hours. After 48 hours, phase separation and creaming were investigated in these formulations. The best formulations were those that continued to function under those circumstances, and these were employed in later studies.

Characteristics of optimised nano emulsions

A density bottle was used to measure NE's density.

The Ostwald viscometer was used to determine the viscosity of NE's at room temperature. This study's main objective was to determine these formulations' rheological properties, and it was conducted using triplicate measurements.

The pH of the formulation is an important characteristic to offer reliability, and any difference in pH could affect the stability of the formulation. Digital pH meters were used to determine the pH of every CMSO NE. The average was calculated after each reading was recorded in triplicate.

The Abbe refractometer was used to determine the CMSO NE's refractive index.

Droplet size analysis and Zeta Potential

The Zeta sizer gadget found the optimum CMSO NE's polydispersity index, zeta potential, and droplet size. The dilution vehicle for the studies was deionised water, and they were conducted at 25°C.

FTIR Analysis

An infrared spectrophotometer with a Fourier transform is one of the greatest tools for identifying the types of functional groups found in substances. An FTIR analysis was conducted on CMSO, F2 & F3 CMSO to ascertain if the extracts were lipophilic or hydrophilic. A scan was conducted in the 4000-500 cm-1 spectral range after a little quantity of samples was put to the sensor.

2.7 Acute toxicity studies

After starving on water alone for all of the previous night, female rats were administered 2000 mg/kg bd. wt. of the oil, F2 and F3 NEs. After that, groups were monitored continuously for about a day in order to check for mortality as well as any alterations in behaviour linked to neurological and physiological issues. The animals' toxicological symptoms were assessed after 14 days. A dose is considered harmful if two or three animals die from it. The oil and NEs are regarded as non-toxic if no animals die; if an animal passes away, the exact same dosage is given again.

2.8 In-vitro antioxidant studies

Reducing power assay

CMSO was produced in different concentrations (10–50µL), and 1 mL of each solution was mixed with 2.5 mL of a buffered phosphate solution and 2.5 mL of 1% K3Fe(CN)6. Incubated at 50°C for 20 minutes was this combination. This solution was vortexed for ten minutes at 3000 revolutions per minute after 2.5 mL of 10% TCA was added. Together with 2.5 mL of water, 0.5 mL of ferric chloride (0.1%), and 2.5 mL of the supernatant were added. Calculations were done at 700 nm for the absorbance. As per Reddy et al. the experiment was conducted using ascorbic acid as the standard [31].

Hydroxyl free radical scavenging assay:

To the final 2 mL, different doses of CMSO, iron chloride, EDTA, H2O2, deoxyribose, and KH2PO4-NaOH buffer were added. 30 minutes at 37°C were spent incubating the mixture before adding 2 millilitres of trichloroacetic acid & thiobarbituric acid. After soaking in a hot water bath for half an hour, it became cold. At 532 nm, absorbance was measured using a UV-VIS spectrophotometer. According to Lahminghlui et al., ascorbic acid served as the standard of comparison [32].

2.9 Estimation of brain GABA

Preparation of a Sample: GABA (gamma amino butyric acid) was determined from whole brain of mice. PTZ (60mg/kg) was given to the animals after 60 mins of administration of CMSO and CMSO NE (400mg/kg). When the seizures started, animalswere sacrificed. The Brain was immediately isolated and subjected to homogenization with 5 mL of 0.01M HCl. The homogenate obtained was then put into a closed beaker with 8 mL of cold pure ethanol and left at 0°C for 1 hour. After centrifuging the mixturefor 10 mins at 16,000 rpm, the upper layer was then removed and placed on a petridish.Supernatant was coupled with washes, which were three times applied to the precipitatein 5 mL of 75% alcohol each. The samples were then dried by evaporating them at 70°C over a water bath. The dried bulk was then centrifuged at 2000 rpm after receiving 1 mLof H2O and 2 mL of CHCl3. The separated supernatant containing GABA was used to make a mark of 10µL on Whatman paper (No. 41).

Paper chromatography: Mobile phase was made up of water (7.5mL), acetic acid (1.5mL), and n-butanol (6.25mL). The paper chromatogram was made by using the ascending approach. Paper was smeared with ninhydrin suspension (0.5%) in ethanol solution 95% that has been heated air dried. The paper was dried in the air for 1hr at 90°C. A blue spot that had developed was separated out and kept for heating on a waterbath 5 minutes containing 2 mL of ninhydrin solution. After that, 5.0mL of water was added, and the solution was left for an hour. Spectrophotometry was used to assess absorbance at 570 nm after supernatant (2.0mL) was

decanted. The samples absorbanceswere extrapolated using the GABA standard [33].

Preparation of GABA solution: 1 mg/ml of standard GABA was produced as a stock solution in 0.01 N HCl. To obtain concentrations ranging from 1 ng/10µL to 1000 ng/10µL, serial dilutions were made. The similar method is utilised to produce a typicalGABA concentration curve, substituting GABA solutions for brain homogenate [34].

2.10 In-vivo anticonvulsant activity

Maximal Electro Shock Induced Seizures (MES)

Albino Five groups of six Wistar rats each were obtained, weighing between 180 and 250g. The rats might be of either sex. Group I received saline (0.9% NaCl); Group II received CMSO 400 mg/kg, bd wt., p.o.; Group III and IV received F2 and F3 CMSO NE 400 mg/kg, bd wt., p.o. Group V received phenytoin 25 mg/kg, bd wt, p.o. as a positive control. Following 45 minutes of medication, they underwent electroconvulsometer-assisted MES stimulation using transauricular electrodes and 150 mA of current for 0.2 seconds. There are different seizure phases, and the duration of each phase is recorded. There are several stages that convulsions might go through: flexion, extension, clonus, stupor, recovery, and death. It was believed that the flexion and extension time periods would be decreased to avoid MES-caused seizures [31].

Neurotoxicity test

Four groups of six Swiss Albino mice each were created after the mice weighting between 25 and 30 grammes were chosen. Animals are trained to walk on a rotarod at 15 revolutions per minute for two consecutive periods of 120 seconds prior to the test. Next, as a control, Group I was given saline (0.9%) NaCl; Group II was given CMSO 400 mg/kg bd wt, p.o.; Group III and IV were given F2 and F3 CMSO NE 400 mg/kg bd wt, p.o.; and the results were recorded using a rotarod. After administering the medication for 30 minutes, the animals were put on a rod at a speed of 15 rpm, and each animal's endurance was recorded [35].

2.11 In-vivo anxiolytic activity

Marble burying test

All male and female Swiss albino mice weighing between 25 and 30 grammes were selected and divided into four groups of six mice each. Typically, husk is piled up to a depth of roughly 5 cm in cages. To make the surface smooth and homogeneous, the bedding materials were gently pressed down. Ten glass marbles, spaced about 4 cm apart, are arranged in a grid pattern on the bedding within each cage. As a control, Group I received saline (0.9% NaCl), while Group II, F2, and F3 received CMSO 400 mg/kg bd weight, p.o. Group V received a dose of 0.05 mg/kg bd wt, p.o. of Lorazepam, whereas Group III and IV received a dose of CMSO NE 400 mg/kg bd wt, p.o. Mice were placed in their own separate cages after receiving medication for one hour. Mice are allowed to roam around their cage for thirty minutes. The mice were gently taken out of their cages after thirty minutes and put in their appropriate enclosures. The quantity of marbles buried by the mice is counted. If more than two-thirds of a marble is covered in bedding material, it is considered buried [31].

3. Result and discussion

The effects of cold-pressed CMSO and its formulations (F2 and F3) on anti-convulsant and anxiolytic properties were investigated using animal models. These are the findings of the research.

Calculation of oil yield

Oil from Cucumis melo seeds is extracted using a cold pressing process. To find the percentage of oil yield obtained, apply the following formula.

$$\text{Percentage yield} = \frac{\text{Weight of oil extracted}}{\text{Weight of seeds used}} \times 100$$

$$= \frac{490}{150} \times 100$$

$$= 32.66\%$$

The amount of *Cucumis melo* seed oil was obtained is 32.66%.

GC-MS analysis of CMSO

GC-MS was used to investigate CMSO Compounds. The GC-MS study produced a further list of bioactive compounds.

Figure 1. GC-MS analysis of CMSO

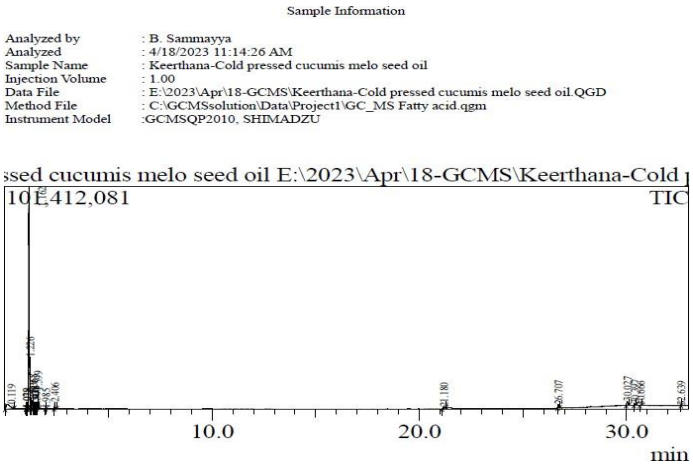


Table 1. List of Phytocompounds from GC-MS studies

Sr.No	RT	Compound name	Mol.wt (g/mol)	Peak area (%)
1	0.119	3-(Prop-2-enoyloxy) dodecane	240.38	7.68
2	1.078	heptane-5 [2.2.1] Bicyclo -(ethyl-1-amine)	124.2233	0.05
3	1.985	Ethyltrimethylsilane	100.2343	0.03

4	21.180	Oleyl Alcohol	268.47784	1.07
5	26.707	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	342.6	0.88
6	30.027	Cyclolanostane-3.beta.,25-diol	444.7	1.85
7	30.397	(3.beta.,5.alpha.) Stigmasta-7,25-dien-3-ol	412.6908	0.53
8	30.666	diethyl bis(trimethylsilyl) ester	296.58	0.06
9	32.639	1-(2-[(Trimethylsilyl)oxy] phenyl)-1-propanone	222.35	0.01

Characterization of the CMSO

CMSO was analyzed to figure out its physical and chemical characteristics.

Physical characterization CMSO

CMSO was analyzed to figure out its physical characteristics. The outcomes of the examination were given in the table 2.

Table 2. Physical characteristics of CMSO

Parameter	**Value**
Solubility	Insoluble in water
Color	Pale yellow
Smell	Musk melon seed smell
Taste	Bland
PH	6.04

Determination of Specific gravity:

The following formula is used to calculate density.

- Mass of empty specific gravity bottle (W1) = 21.15
- Mass of specific gravity bottle + water (W2) = 45.890Mass of specific gravity bottle + CMSO (W3) = 43.770

Specific gravity = $\frac{\text{Mass of CMSO}}{\text{Mass of equal volume of water}}$

$$= \frac{W3-W1}{W2-W1}$$

$$= \frac{43.770-21.150}{}$$

$$= \frac{45.890-21.150}{22.62}$$

$$= \frac{}{24.74}$$

= 0.9143g/ml

Assessment of Viscosity:

The following formula is used to calculate density.

$$\text{Viscosity of liquid } (\eta 2) = \frac{\rho 2\ t2}{\rho 1\ t2} \times \eta 1$$

- Viscosity of water at room temperature ($\eta 1$) = 0.9240 cP
- Density of oil ($\rho 2$) = 0.9143g/ml
- Density of water at room temperature ($\eta 2$) = 0.997g/ml

$$= \frac{0.9143 \times 449.33}{0.997 \times 14}$$

$$= \frac{410.822}{13.958} \times 0.9240$$

= 27.195cP

= 271mP

Assessment of the Refractive index:

The refractive index analysis for *Cucumis melo* seed oil had showed that the value of1.491.

Chemical characterization of CMSO

Determination of Saponification value of CMSO:

The saponification value was calculated using the following formula

$$\text{Saponification value} = \frac{V2-V1}{W} \times 28.1$$

$$= \frac{175-161}{2}$$

= 7 x 28.1

= 196.7

Determination of Acid value of the CMSO:

The following formula was used to determine the acid value.

$$\text{Acid value} = \frac{56.1.0 \times V \times N}{\text{Mass of oil}}$$

$$= \frac{56.1.0 \times 0.70 \times 0.10}{2}$$

= 1.963

Determination of Ester value of the CMSO:

It was obtained as the difference between the saponification value and the acid value of CMSO.

Ester value = Saponification value- Acid value
= 196.7-1.9635
= 194.73

Formulations OF O/W CMSO NE

F1 – F9 formulations of *Cucumis melo* seed oil were prepared by motor and pestle.

Figure 2. (F1 – F9) Formulations of CMSO Nanoemulsions

Over the course of 48 hours, the phase separation, breaking, creaming, and coalescence of various formulations (F1-F9) were monitored. F4 showed phase separation within 24 hours, whereas F5 showed it after 48. After 48 hours of observation, there were no changes to any of the other formulations.

Figure 3. Phase separation of F4 and F9

Stability testing of CMSO Nano emulsion

Centrifugation: Phase separation was evident in the F4, F5, F8, and F9 formulations after 90 minutes of centrifugation at 1000 rpm indicating that the stabilitytest for the F4, F5, F8, and F9 formulations was unsatisfactory.

Heating Cooling Cycle*:* Phase separation or creaming wasn't evident in the F1, F2, F3, F6, and F7 formulations after 48 hours at 4°C and 45°C. It proves the formulations' thermodynamic Stability

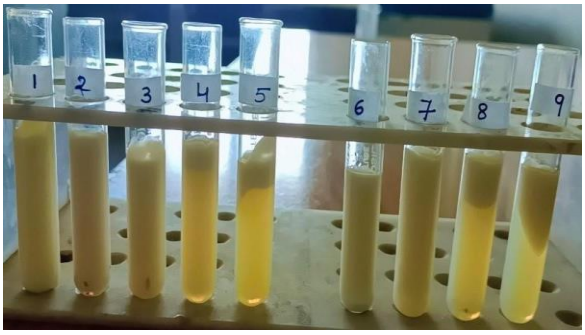


Figure 4. Phase separation of F4, F5, F8 and F9 after centrifugation after 24hrs

Figure 5. Formulations before H/C. No phase separation is seen

Figure 6. Formulations after H/Ccooling. No phase separation is seencooling.

Table 3. Stability tests of CMSO formulations

Formulation	Phase Separation		Inference
	Centrifugation	Heating Cooling Cycle	
F1	No	No	Pass
F2	No	No	Pass
F3	No	No	Pass
F4	Yes	-	Fail
F5	Yes	-	Fail
F6	No	No	Pass
F7	No	No	Pass
F8	Yes	-	Fail
F9	Yes	-	Fail

Characterization of CMSO Nano emulsion

Characteristics of CMSO Formulations was performed and tabulated

Table 4. Characteristics of CMSO formulations

Formulations	PH	Density	Viscosity (mP)	Refractive index
F1	7.49	1.013	20.059	1.365
F2	6.90	1.002	20.880	1.360
F3	6.78	1.007	30.219	1.343
F6	6.83	-	-	1.361
F7	6.90	1.006	52.166	1.379

Phase separation of F7 is visible after 10 days of storage and cloudy nature is noticed in F6 formulation. F6 and F7 consequently failed the stability test.

Figure 7. Formulations that are failed in the above tests

Droplet Size, Polydispersity index and measurement of Zeta potential

Zeta potential, PI and droplet size of the optimised CMSO NE'S were all determined using Zeta sizer device. The experiments were carried out at 25°C and the dilution vehicle was deionized water.

Standard range:

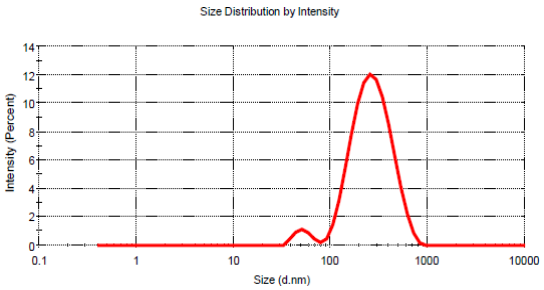
Nano emulsion droplet sizes typically range from 50 to 200 nm.

PI, which ranges from 0.0 to 1.0, indicates the particle homogeneity. Theparticles are more homogeneous if it is closer to zero.

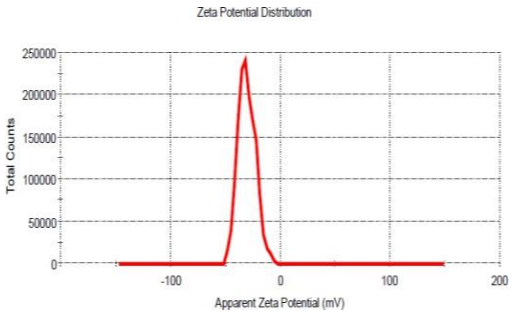
Stable particles have zeta potentials between +30mV and -30mV, which are morepositive and negative respectively.

Table 5. Droplet size, PI and Zeta potential of CMSO NE's

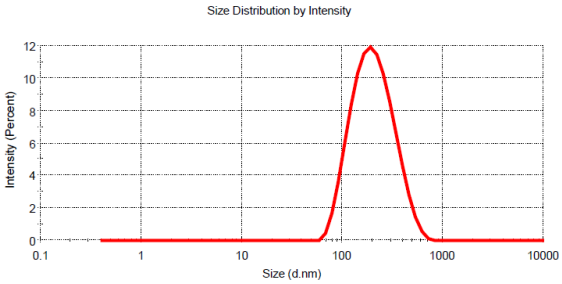
Formulations	Particle size (nm)	Polydispersity index	Zeta potentials (mV)
F1	224.3	0.238	-30.5
F2	178.5	0.182	-32.7
F3	180.5	0.203	-15.0



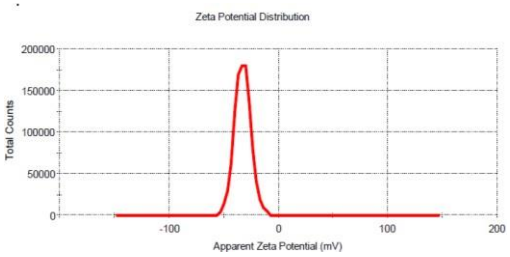
A. F1 droplet size



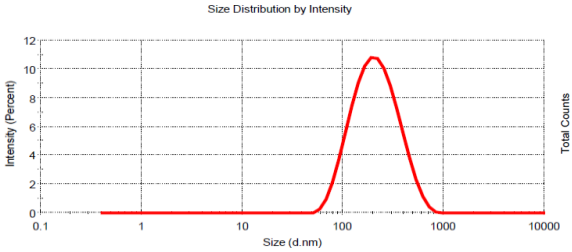
B. F1 Zeta potential



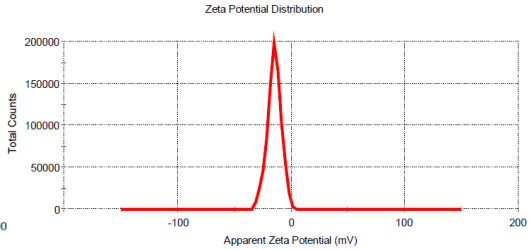
C. F2 droplet size



D. F2 Zeta potential



E. F3 droplet size



F. F3 Zeta potential

Figure 8. Droplet size and Zeta potential graphs of F2 and F3 CMSO NE's

Since F2 and F3 NEs succeeded the stability tests and had acceptable NE properties as well as typical ranges for particle size, PI, and Zeta potential, these were regarded as the most optimal formulations.

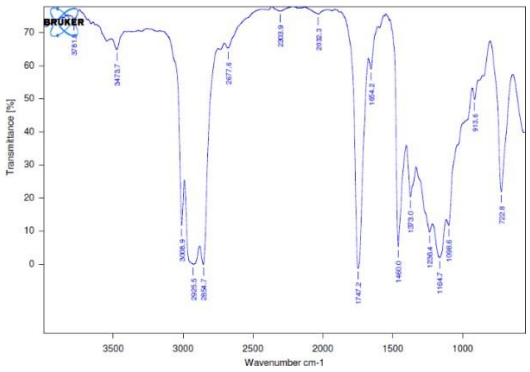
Figure 9. Optimised formulations (F2 and F3)

FTIR analysis

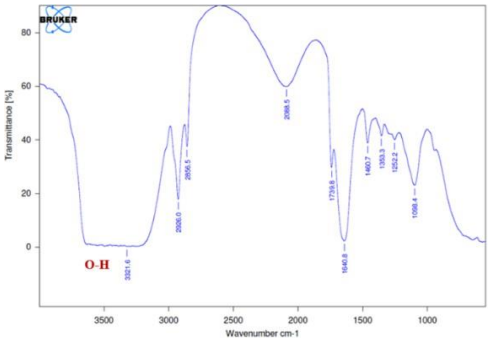
The functional groups found in the FTIR spectra of the CMSO, F2 and F3 CMSO NE'swere examined. The distinctive characteristics of the bands are listed in table number:

Table 6. IR Functional groups with range

Functional group	IR Stretching bands (cm^{-1})
O-H	3600-3300
C=C	1600-1500
C-H	2950-2850



(A)



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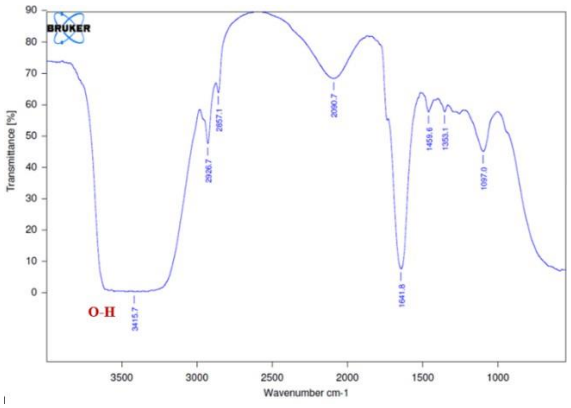

(C)

Figure 10. FTIR spectrum of (A) CMSO, (B) F2 CMSO NE and (C) F3 CMSO NE With comparison to CMSO, which displayed a narrow band at 3473.7, the enhancedband width of the OH functional group in F2 and F3 CMSO NE found at 3321.6 and3415.7 indicates that the NE's are hydrophilic.

ACUTE TOXICITY STUDIES OF CMSO AND CMSO NE'S F2 AND F3

CMSO was tested on female mice at a dose of 2000 mg/kg bd. wt., per oral. 2000 mg/kgbd. wt., of CMSO, F2 and F3 CMSO NE's exhibited no perilous or lethal effects. The14 days of observation did not put any of the animals in peril. The dose was thus deemedto be safe up to 2000mg/kg body wt.

Dose selection

Assessments on toxicity have determined that a dosage of 2000 mg/kg body weight is protective. Pharmacological evaluations for the present research were finished employing 400mg/kg bd. wt.

EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY

Reducing Power Assay

Table 7. Antioxidant activity of CMSO by Reducing power assay

Sr. No	Compound	Concentration (µg/mL)	Percentage Inhibition (Mean±SEM)	IC50 (µg/mL)
1.	Ascorbic acid	10.0 20.0 30.0 40.0 50.0	12.50±0.10 28.2±0.20 37.0±0.10 46.2±0.10 56.2±0.20	43.41
2.	CMSO	10.0 20.0 30.0 40.0 50.0	18.8±0.10 26.3±0.10 37.0±0.20 42.2±0.10 52.9±0.20	47.25

Study was run as a triplet and MEAN±SEM was used to represent the percentage of inhibition.

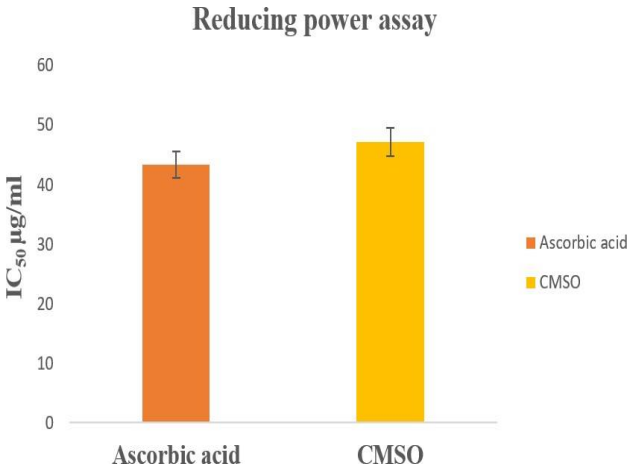

Figure 11. CMSO's comparable impact on the IC50 value in the reducing power test compared to standard ascorbic acid

Using the reducing power test, the antioxidant capacity of Cucumis melo seed oil was determined. CMSO's IC50 value of 47.25µg/mL was found, and its ability to block a greater number of free radicals as a percentage increased with dosage. The standard's IC50 value of 43.41µg/mL showed that CMSO's capability was almost identical to that of ascorbic acid in its traditional form.

Hydroxyl Scavenging Activity

Table 8. Antioxidant activity of CMSO by Hydroxyl radical scavenging assay

Sr. No	Compound	Concentration (µg/mL)	Percentage Inhibition (Mean±SEM)	IC50 (µg/mL)
01.	Ascorbic acid	10.0 20.0 30.0 40.0 50.0	23.5±0.20 37.0±0.10 48.6±0.20 65.1±0.30 71.3±0.30	31.96
02.	CMSO	10.0 20.0 30.0 40.0 50.0	13.3±0.20 29.0±0.10 46.5±0.10 57.6±0.20 66.08±0.30	35.16

The study was run in a triplet, and MEAN±SEM was used to represent the percentage of inhibition.

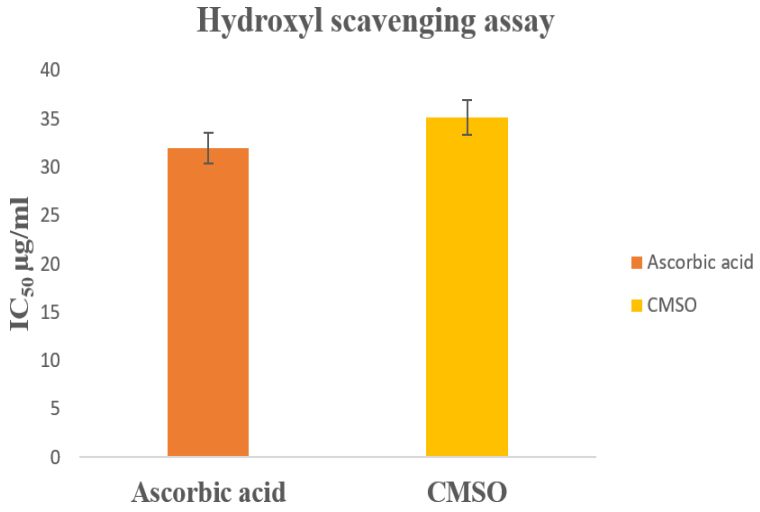

Figure 12. CMSO's comparable impact on the IC50 value in hydroxyl radicalscavenging test compared to standard ascorbic acid

The hydroxyl radical scavenging test has been used to investigate the antioxidant potential of Cucumis melo seed oil. The IC50 value of CMSO was found to be 35.16µg/mL, indicating that a higher dosage resulted in a greater percentage of hydroxyl radical inhibition. The extract exhibited the same predicted activity as the ascorbic acid standard, and its IC50 value was determined to be 31.96µg/mL

ESTIMATION OF GABA IN MICE BRAIN

PTZ induced sample was identified to have an absorbance and a concentration of 0.02 and 21 ng/ µl, respectively. The CMSO sample absorbance as well as concentration was determined to be 0.05 and 78ng/µl, respectively. The CMSO NE sample absorbance as well as

concentration was determined to be 0.06 and 97ng/µl, respectively. In the PTZ-induced model, animals that underwent PTZ administration after 1 hour of being administered with 400mg/kg of CMSO and CMSO NE dramatically raised the concentrations of brain GABA. Comparing the testconcentration to the disease control revealed higher GABA levels.

Table 9. GABA standard calibration curve along with CMSO and CMSO NEeffects on mice convulsions brought on by PTZ

Sr. No	Concentration of Standard GABA (ng/µL)	Absorbance
01.	200.0ng/µl	0.120
02.	400.0ng/µl	0.230
03.	600.0ng/µl	0.33
04.	800.0ng/µl	0.42
05.	1000.0ng/µl	0.54
06.	**CMSO**	**0.05**
07.	**CMSO NE**	**0.06**

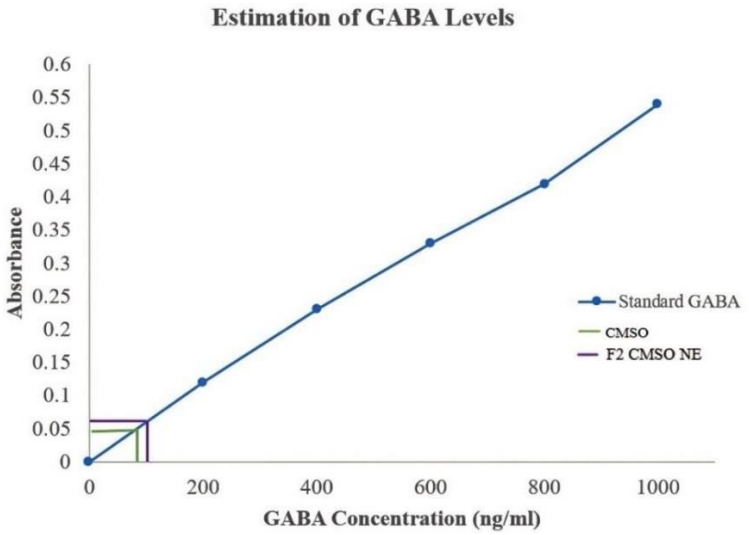

Figure 13. Standard calibration curve of GABA

IN VIVO METHODS OF ANTIEPILEPTIC ACTIVITY

MES induced seizures and the Neurotoxicity test were employed to assess the antiepileptic activity of CMSO, F2 and F3 CMSO NE's.

Maximal Electro Shock Induced Seizures (MES)

Table 10. Impact of CMSO and CMSO NE's on MES induced seizure

Groups	Phases of convulsions (Time in seconds)				
	Flexion	**Extension**	**Clonus**	**Stupor**	**Recovery/ Death**
Control	11.5±0.17	12.5±0.15	11.5±0.09	173.0±0.63	Recovery
CMSO 400mg/kg (bd.wt., *p.o*)	7.3±0.20*^{A}	6.1±0.11*^{A}	6.1±0.19*^{A}	60±0.21*^{A}	Recovery
F2 CMSO NE 400mg/kg (bd.wt., *p.o*)	3.3±0.13*^{ns}	2.5±0.08*^{ns}	2.3±0.13*^{ns}	38.6±0.32* ns	Recovery
F3 CMSO NE 400mg/kg (bd.wt., *p.o*)	4.5±0.13*^{C}	3.1±0.11*^{D}	3.3±0.13*^{B}	42±0.21*^{B}	Recovery
Phenytoin 25mg/kg (bd.wt., *p.o*)	2.3±0.08*	1.8±0 .06*	1.6±0.08*	37.6±0.17*	Recovery

The calculated values are presented as Mean±SEM (n=6). One-way ANOVA was used to analyse the data, followed by a Dunnett's multiple comparison test against the Control (*=p<0.0001) and Standard (^{A}=p<0.0001, ^{B}= p<0.005, ^{C}=p<0.001, ^{D}=0.01 and ^{ns}=non significant)

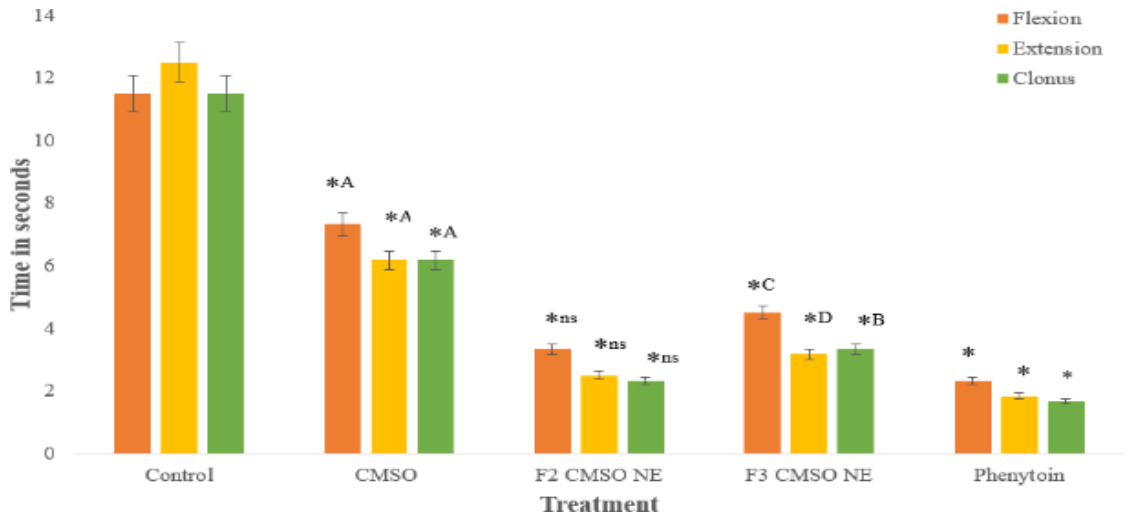

Figure 14. CMSO and CMSO NE's effect on flexion, extension, clonus in MEStest

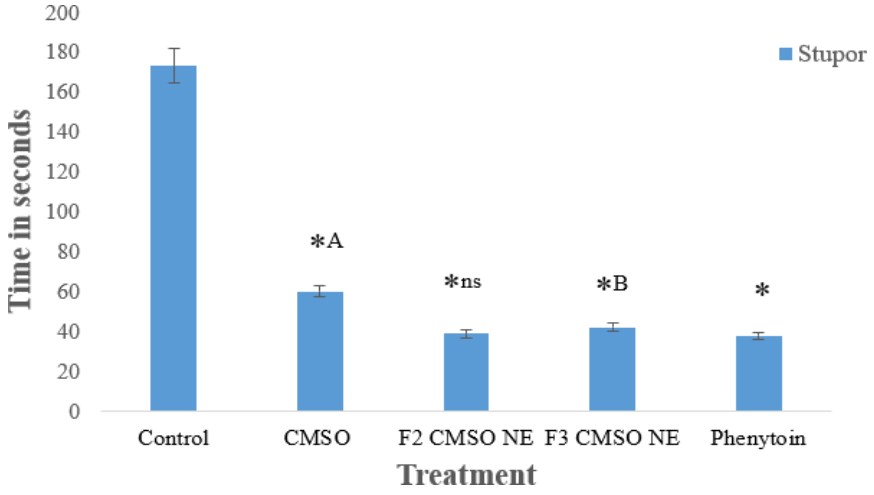

Figure 15. CMSO and CMSO NE's effect on stupor in MES test

E. Flexion D. Extension C. Clonus B. Stupor A. Recovery

Figure 16. Phases of convulsions

There was a considerable reduction in the duration of flexion and extension in rats treatedwith CMSO and CMSO NE's 400mg/kg in comparison with control. The rats received protection from seizures brought on by MES at a dose of 25mg/kg phenytoin.

Neurotoxicity test

Table 11. Impact of CMSO and CMSO NE's on Neurotoxicity test

Groups	**Before treatment**	**After Treatment**
Control	113.3±0.36	113.3±0.15
CMSO 400mg/kg (bd.wt., *p.o*)	115.1±0.51	115.3±0.45
F2 CMSO NE 400mg/kg (bd.wt., *p.o*)	116.1±0.59	116.3±0.47
F3 CMSO NE 400mg/kg (bd.wt., *p.o*)	114.8±0.53	115±0.51

Values are represented as Mean±SEM (n=6). The outcomes were analysed utilisingpaired t test

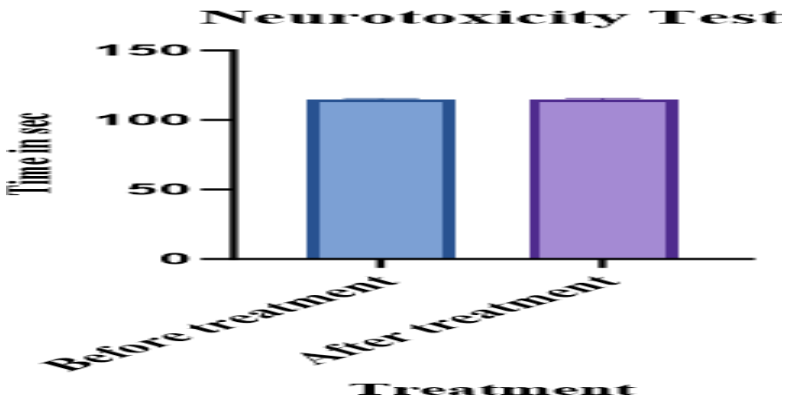

Figure 16. CMSO and CMSO NE's

Time spent on rotarod of mice administered with CMSO and CMSO NE's 400mg/kgdid not experience neurotoxicity. which indicates absence of motor incoordination.

***IN VIVO* ANXIOLYTIC ACTIVITY**

Marble Burry Test (MBT) were employed to assess the antiepileptic activity of CMSO, F2 and F3 CMSO NE's.

Marble Burry Test

Table 12. Impact of CMSO and CMSO NE's on MBT

Groups	**Number of marbles buried Mean±SEM**
Control	7.0±0.14
CMSO 400mg/kg (bd.wt., *p.o*)	$4.16\pm0.12^{*A}$
F2 CMSO NE 400mg/kg (bd.wt., *p.o*)	$2\pm0.10^{*ns}$
F3 CMSO NE 400mg/kg (bd.wt., *p.o*)	$2.5\pm0.09^{*ns}$
Lorazepam 0.05mg/kg (bd.wt., *p.o*)	1.83±0.16*

The calculated values are presented as Mean±SEM (n=6). One-way ANOVA was used to analyse the data, followed by a Dunnett's multiple comparison test against the Control (*=$p<0.0001$) and Standard (^{A}=$p<0.0001$ and ^{ns}=non-significant).

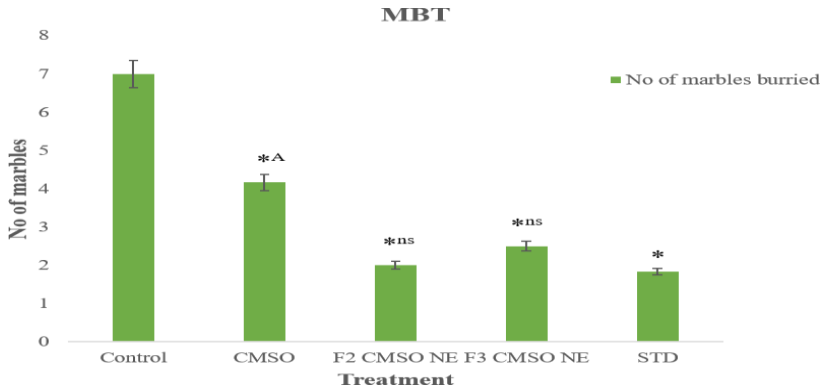

Figure 17. CMSO and CMSO NE's effect on the Marble Bury Test

Figure 18. Marblebury test on mice

To quantify its anxiolytic effects, the total number of marbles buried was counted. Marbles buried at a dose of 400 mg/kg were significantly fewer with CMSO and NEs than in the control group. Lorazepam demonstrated significant anti-anxiety effects at 0.05 mg/kg dose.

Conclusion:

A collection and verification of Cucumis melo seeds have been made. The oil yield of 32.66% was obtained by the use of the cold press method of oil extraction. CMSO's identification, quality, and purity have been established by its physical and chemical features. Phytosterol and triterpenoids such as cycloartenol and stigmasterol, which may have anxiolytic, anticonvulsant, and antioxidant properties, are confirmed to exist by CMSO's GC-MS analysis. Particle size, polydispersity index, zeta potential, stability tests, and other factors were taken into consideration when creating the O/W type CMSO NE Formulations (F1–F9). It was determined that F2 and F3 were the optimal formulations that passed all of the aforementioned tests. Comparing NEs to CMSO, FTIR research revealed that NEs are naturally hydrophilic. It was established that the acute toxicity of CMSO, F2 and F3 NEs was non-toxic and safe up to 2000 mg/kg bd.wt. CMSO has a significant impact on preventing free radicals generated due to oxidative stress, according to an assessment of its in-vitro anti-oxidant activity using a hydroxyl scavenging and reducing power assay. The biochemical estimation of GABA was elevated in mice. Rats maximal electroshock seizure induced model were used to assess the in vivo anticonvulsant efficacy of CMSO, F2, and F3 NEs. When given in 400 mg/kg doses to these mice, CMSO, F2 and F3 CMSO NE's demonstrated strong anticonvulsant action. Comparing CMSO and formulations to the control group, they decreased the duration of clonus, stupor, tonic flexion, and tonic extension. When F2 and F3 were administered at a dose of 400 mg/kg for the rotarod test, CMSO, they did not result in any motor incoordination and did not reduce the rod's duration. Utilising the Marble Buried Test, CMSO, F2 and F3, NE's in vivo antianxiety efficacy was assessed. Within the Marble Buried Test (MBT), F2 and F3 NEs lowered the marble count buried in comparison to the CMSO control group.

The anticonvulsant and anxiolytic activities of all in vivo experiments revealed that F2 CMSO NE out performed F3 CMSO NE and CMSO in terms of efficacy. In order to precisely implement these actions, more research is required to identify the phytochemicals and clarify the Cucumis melo seed oil's mechanism.

Acknowledgement

I would like to extend my heartfelt thanks to Dr. M. Ganga Raju, Principal of Gokaraju Ranga Raju College of Pharmacy for their indispensable support throughout the research project.

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