PRECLINICAL EVALUATION OF NEUROPROTECTIVE POTENTIALOFNUTRACEUTICAL FORMULATION NC20 IN SPRAGUEDAWLEYRATS

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Abstract

The term" nootropics" commonly referred to as "smart drugs", refers to a broad category of pharmaceuticals that enhance memory, learning, and thought processes in people, particularly when these processes are compromised. Nowadays, a lot of nutritional supplements are methodically examined and, if they show promise, added to therapies. In accordance with that, the goal of the current study was to assess the nootropic ability of a nutraceutical composition. The formulation was evaluated using an aluminium chloride-induced neurotoxicity model with parameters for locomotoractivity measured by an actophotometer, muscle strength measured by a rota rod device, the elevated plus maze test, and the Morris water maze Blood parameters, Acetylcholiesterase and histopathological studies test at doses of 200 mg/kg and 400 mg/kg bodyweight. As a result of the findings, a statistically significant and dose-dependent action was found. On the other hand, the outcomes fell short of expectations to the standard drug Rivastigmine.

1. Introduction:

Alzheimers disease (AD) is a progressive chronic neurological illness characterized by memory loss and the buildup of strange proteins. One in 85 people will be affected by dementia by the year 2050, which will have affected 36 million people worldwide, with an estimated 75% of those individuals having Alzheimers disease. Due to lifestyle factors, stress, despair, and frustration, AD is most prevalent in persons over 65 and can potentially start earlier in life. The most prevalent symptoms are increased mood swings, diminished memory, particularly with regard to short-term memory tasks like forgetting names, dates, or locations, as well as aggressiveness and irritability. Deterioration of cognition and learning processes, mathematical difficulty, loss of object-based memory, and emotional issues are all symptoms of the condition as it advances from mild to moderate. The neuronal loss in basal forebrain cholinergic neurons that project to the hippocampus and cortex, the accumulation of hyperphosphorylated tau protein in neurofibrillary tangles (NFT), also known as tau proteins, and the subsequent formation of A plaques by the proteolytic action of -secretases 1, 3,are among the pathologic changes in AD. The cholinergic system of the hippocampus and cortex underwent considerable modifications, which are directly associated to memory loss and other cognitive problems in AD. Although the actual cause of the illness is unknown, genetic and environmental factors have been suggested as potential contributors. Aluminum (Al) toxicity is one environmental component that has been connected to an increase in Alzheimers disease cases.It is the most prevalent non-essential element in our surroundings and, at around 8%, the third most prevalent element in the crust of the planet, behind silicon and oxygen. Human exposure is extremely high because it is found in food, water, dust, air, beverages, flavored drinks, energy drinks, and medications. The substance is also used in the production of paper, fire retardants, water, treatment, filters, food additives, paints, drug preparations, and the consumption of corn, shellfish, yellow cheese, dairy products, spices, salt, bread, pastries, toothpaste, cakes, sausages, sugary foods and beverages, tea herbs, and cosmetics.Another study found that tea, the most popular beverage in the world, is a significant dietary source of aluminum. The quantity of aluminum in tea infusions ranged from 0.035 to 16.82 mg/L, and the oral bioavailability of aluminum from the beverage was 0.37 percent4, according to the study.

Al is also employed in the pharmaceutical sector to prepare drugs like antacids, phosphate binders, buffer aspirin formulations, vaccinations, and allergy injections.Al exposure is more likely to affect people who live close to cement plants.In the hippocampus, a region involved in memory formation and synaptic plasticity during learning, aluminum-exposed animals have demonstrated the formation of neurofibrillary tangles, loss of cholinergic neuronal terminals in the hippocampus and cortex, amyloid protein aggregation (AB), development of oxidative stress, and neuronal apoptosis.

Nutraceuticals;

A meal or food product that offers health and medical benefits, including the prevention and treatment of disease, is referred to as a "nutritional pharmaceutical". For many years, dietary supplements have been viewed as an alternative kind of treatment. All food-derived products that offer additional health advantages above and beyond the basic nutritional content of foods are referred to as " nutraceuticals". These products typically make claims about their ability to prevent chronic diseases, improve health, slow the aging process, and lengthen life.

The role of nutraceuticals in AD:

The most prevalent age-related brain condition in the world, Alzheimers disease, has undesirable side effects with prolonged therapy. Food becomes more crucial in this struggle for both prevention and treatment. The advantages of dietary supplements have made it possible to adopt alternate methods to combat neurodegenerative illnesses like Alzheimers disease. Because they are naturally occurring substances with less adverse effects, The Role of Nutrients in Alzheimers Disease have been able to establish themselves as a safer and superior option. Therefore, the minerals provide defense against this age-related cognitive deterioration. Additionally, it highlights how vitamins, flavonoids,antioxidants,and other naturally occurring compounds can help prevent Alzheimers disease and maintain cognitive function.

2)Methodology: -

Procedure: Following Steps are involved in the formulation of NC20 nutraceutical formulation.

2.1 Procurement of high standard raw materials:

When sourcing raw material, the most important step in sourcing good products is to get safe and high-quality raw material.

2.2 Separation:

Raw material separation is a technical methods used to separate products from impurities or otherproducts.It include separation of dust from recycled materials. Removal of the foreign body, if present any.

2.3 Weighing:

Weighing Consider each material separately. Transfer to suitable containers such as steel, aluminium, pingani or plastic containers.

2.4 Soaking/Dipping:

This is the process of moistening and softening the seeds to facilitate the removal of the seeds. This is theintake of water to activate the germinal process in the nucleus. Soak the materials by adding a measured amountof solvent (water.) Overnight /12-24 h.

2.5 Drying:

Drying is a mass transfer process that involves the removal of water by evaporation. Drain the solvent usinga filter device such as a steel strainer / meat cloth / coconut cloth.

2.6 Spreading:

Spread the material evenly on a wide cloth to dry or air dry. Turn the material every 3-6 hours and let it dry.Check the humidity regularly. After complete drying, collect all materials in suitable containers.Heat the materials at the appropriate temperature. Weigh the given amount of material and transfer it to a heatingvessel with a low flame. Transfer to a suitable container and leave to cool.

2.7 Pulverising/grinding:

Weigh the material and grind/grind to a coarse powder. sift them together and collect and weigh the rawpowder. And transfer them to airtight container.

Determination of fundamental properties of powder:

1.True density: It is the density of the material itself .It is defined as :

True density ρp = weight of powder / true volume of powder

Where,

True volume = volume of the powder

The density is dependent on type of atoms in a molecule, arrangement of the atoms ina molecule and the

arrangement of molecules in the sample .

2. BULK DENSITY :-
3. Bulk density ρb = mass of a powder (w)/bulk volume (Vb)
Where, bulk volume = volume of the powder itself+ volume of intraparticle spaces + volume of inter particle spaces (voids).
3. Tapped density:
TAPPED density = mass of a powder / volume of the powder at zero tapping ortapped volume
4.Carr's consolidation index :
It is defined as :
Consolidation index = tapped density – fluff density / tapped density × 100
This property is also known as compressibility **.**
5.POROSITY :
Terminology related to porosity :
True volume = volume of the powder itself .
Granular volume = volume of the powder itself + volume of intra particle spaces .
Bulk volume = volume of the powder itself + volume of intra particle spaces + volume of inter particle spaces (voids).
Void volume = bulk volume – true volume
• The porosity or voids , ε, of the powder is defined as :
Porosity or voids[ε] = void volume / bulk volume
= Bulk volume – true volume / bulk volume
ε = Vb- Vb/ Vb
• Porosity is frequently expressed in per cent .
Per cent ,£=[1-Vb/Vb]×100.
Porosity influences the rate of disintegration and dissolution.
Phytochemical Evaluation:
The NC20 formulation was tested for phytoconstituents such as antioxidants, flavonoids, vitamins carbohydrates, proteins, alkaloids, tannins and glycosides using various standard phytochemical methods such asalkaline reagent test, lead acetate test, molish's test, Benedict's test, xanthoproteic test and ninhydrin test etc,

3)Experimental work: -

Experimental animals:

Adult male SD rats weighing 200-250 g was obtained from VAB BIOSCIENCES, Hyderabad.They were kept in polypropylene cages kept in the animal house at Teena Biolabs Pvt. Ltd., Hyderabad. Undercontrolled conditions in a temperature-controlled environment (25 ± 2 °C) with 50–70% relative humidity during12-h light-dark cycles and had ad libitum access to normal rat chow and water. They were acclimatized tolaboratory conditions for a week and then randomly divided into four experimental groups. All experimentalprocedures were performed in accordance with the guidelines of the Committee for the Control and Supervisionof Experiments on Animals (CPCSEA) (reg. No: 177/PO/RcBi/2000/CPCSEA), Govt. from India. The studywas reviewed and approved by Teena Biolabs Pvt. institutional animal ethics committee. Ltd., Hyderabad.

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines. Rats were selected randomly anddivided into five groups (n=4). The animals are marked to permit individual identification, and kept in theircages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions. As with othersequential test designs, care must be taken to ensure that animals are available in the appropriate size and agerange for the entire study.

Administration of doses

The formulation is administered in a single dose by gavage using a stomach tube or a suitable intubation cannula.In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over aperiod not exceeding 24 hours. Animals should be fasted prior to dosing (e.g., with the rat, food but not watershould be withheld overnight; with the mouse, food but not water should be withheld for 3-4 hours). Followingthe period of fasting, the animals should be weighed and the test substance administered. Nutraceuticalformulation diluted with normal saline and centrifuged for complete dissolution and was

administered orally at the doses5, 50, 300 and 2000 mg/kg body weight. The animals were observed continuously for any untoward symptomssuch as tremors, convulsions, exophthalmos, salivation, diarrhoea and lethargy followed by observation for further 14days. At the end of the experimental period, the animals were observed for any changes in behaviouralpattern and mortality.

Selection of dose

Based on the acute toxicity studies formulation was found to be safe at 2000mg/kg body weight, hence asper OECD 423 guidelines (HAEAL) at doses of 200mg/kg and 400mg/kg respectively.

Induction of Alzheimer's disease by Alcl3: -

Alzheimer's disease was induced in animals by oral administration of AlCl 3 dissolved in normal saline ata dose of 150 mg/kg b.wt for 20 days.

Experimental protocol: -

Animals were divided into five groups, each consisting of 4 rats, 20 sprague dawley rats were employed in this study and the treatment was carried out for 28 days. The protocol was designed as follows.

4)Results: -

Evaluation studies: -

1.Bulk density: - 0.416 gm/ml

2.Tapped density: - 0.576 gm/ml

Carr's consolidation index: - 27.7

Porosity: 0.58.3

% porosity:58.3%

According to the porosity value it will show high dissolution rate.

And carr's index says about about that the powder will have poor and passable flow property

The results of various parameters were expressed in table 4.1,4.2,4.3 and histopathological studies

5.Discussion of results: -

The goal of the current investigation was to determine whether the test chemical could protectagainst the neurotoxicity caused by aluminum chloride. Currently, in-vivo testing may be a crucialcomponent of safety evaluation and may be a legal prerequisite before a medicine enters clinicaltrials. We have been able to conduct in-vivo experiments thanks to this factor using SD rats. Thenutraceutical formulation (NC20) contains a variety of vitamins, fatty acids, carbs, proteins, flavonoids, andantioxidants.Their pharmacological effects may be brought on by the presence of flavonoids.In order to employ this formulation as an ingredient in foods and medications that promotehealth, it must first be recognized as promising in terms of its pharmacological properties andavailability in nature. Additionally, both models of the nutraceutical formulation had substantialantioxidant activity, which may be because this treatment successfully averted the histologicalalterations seen in the AlCl3-treated animals. These plants have compounds that are beneficialfor treating neurological illnesses. Therefore, these compounds may be to blame for thenutraceutical formulations neuroprotective effects in this study.So, it is possible to think of nutraceutical formulation as a viable potential treatment option for anumber of neurodegenerative illnesses.

Furthermore, chemical component isolation and investigation at the molecular level utilizingmore biochemical parameters are required in order to assess the potential mode of action andthe active ingredient responsible for the neuroprotective impact of nutraceutical formulation.

6.Conclusion and further scope of research: -

According to historical reports, specific plant species and plant products may containtherapeutically effective compounds that, when taken in adequate quantities, can have effectssimilar to those of drugs. The nutraceutical formulation provides positive neuroprotective andantioxidant effects, according to this study findings. To support Hippocrates (the father ofmedicine) assertion that "Let food be your medicine and medicine be your food" (400 BC),rigorous pharmacological, photochemical, and bioanalytical studies must be conducted, followedby observational studies in humans.

Future scope:Future research will focus on conducting extensive tests to see how well this potent nutraceuticalcomposition performs in a variety of capacities.

7.Acknowledgement: -

We are special thanks to JNTUA-OTPRI Anantapuram for doing the project work

8.References:-

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Table:3.1Treatmentschedule

S. no	GROUP	TREATMENT	PURPOSE
1	Normal	Standardpelletedch owand drinking water *ad libitum.*	Tostudythenormalrat liver physiology
2	Control	Daily dose ofAlcl3(150mg /kg b.wt. p.o).	To serve as disease control as well to study the protective effect of testsubstancesunder Study.
3	Test:200 mg/kg	200mg/kg of NC20 was given from the 20th day of inducing.	To know therapeutic effect of nutraceutical formulation against alcl3 neurotoxicity.
4	Test:(400 mg/kg)	400mg/kg of NC20 was given from the 20th day of inducing with alcl3	To know therapeutic effect of nutraceutical formulation against alcl3 neurotoxicity.
5	Standard: (Rivastig mine)	Rivastigmine (1mg/kg b.wt) was given form 20th day of inducing	To evaluate the preventive effect of nutraceutical formulation against alcl3 neurotoxicity.

Table no:4.1 Blood parameters: -

Contents	Normal	control (Alcl3)	Standard (Rivastigmine)	Lower dose of NC20	Higher dose of NC20
Hemoglobin (g/dl)	14.5	14.9	15.0	14.9	14.9
RBC ($\times10^6$/µl)	7.95	8.01	7.95	7.0	7.9
WBC ($\times10^3$/µl)	5.51	2.55	4.55	6.55	6.8
Platelets ($\times10^3$/µl)	710	650	912	721	725
PCV%	39.1	38.0	42.2	40.5	39.5
MCV(flum)	52.0	51.2	52.9	52.1	53.5
MCH(pg)	19.8	18.9	19.9	19.9	18.9
MCHC(g/dl)	34.5	35.0	34.5	35.0	35.0
Reticulocytes ($\times10^9$/l)	135.1	153.3	130.1	131.1	135.0
Neuteophills%	35	30	28	31	29
Lymphocytes%	60	65	68	64	66
Eosinophills%	03	03	02	03	02
Monocytes%	02	02	02	02	02
Basophills%	00	00	00	00	00

Table no:4.2 Effectof NC20 treatment on Acetylcholinesteraseactivity.

S.No.	Group	Acetylcholinesteraseactivity(µ moles of thiocholine iodide /min/mgofprotein)
1	Normal	21.43±0.14
2	Diseasecontrol	42.12±0.43
3	StandardRivastigmine	32.12±0.23***
4	NC20(200mg/kgbodyweight)	22.26±0.083 **
5	NC20(400mg/kgbody weight)	26.66±0.036**

Eachvalueisexpressedasmean±SEM(n=6),where,

Each value is expressed as mean ±SEM (n=6), where, NS represents on-significant;

***P<0.001–highly significant;
**P<0.01 very significant;
*P<0.05-significant, when compared to AlCl3 alone treated rats

Table no:4.3 Behavioral parameters: -

Treatment	Locomotor activity (Photoactometer)	Motor coordination (Rota Rod Test) (sec)	Elevated PlusMaze Test(% memory retention)	Morris water maze (Escape Latency time)(sec)
Normal	427.7 ± 5.536	55.4 ± 12.167	50.878 ±6.731	56.35±0.84
Disease control	99.4±2.501	13.4 ± 4.780	7.148 ±3.075	86.78±1.14
Lower dose ofC20(200 mg/kgbody weight)	166.5±0.274***	20.17±4.766*	30.71±5.796***	72.56±1.87*
Higher dose of NC20(400 mg/kgbody weight)	193.167±0.904***	36.17 ±4.498**	34.910±5.594***	64.76±0.45**
Standard Rivastigmine (1 mg/kg body weight	223.84±2.655***	39.7±2.824***	45.573±8.737***	68.53±0.94***

HISTOPATHOLOGICALSTUDIESINAlCl3INDUCEDNEUROTOXICITY

NORMAL GROUP

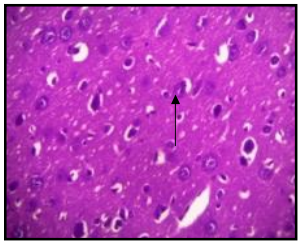


Fig.1[H&E,x400]

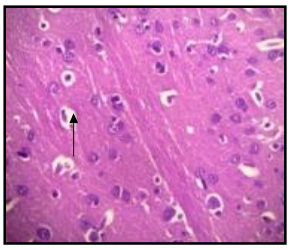
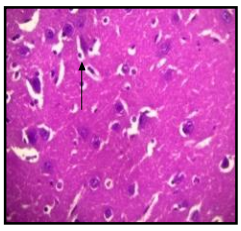


Fig.2[H&E,x400]

DISEASE CONTROLGROUP

Fig.1[H&E,x400]



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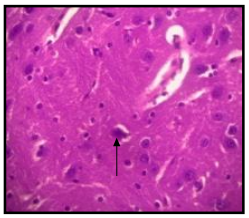


LOWER DOSE OF NUTRACEUTICAL FORMULATION(200MG/KG):

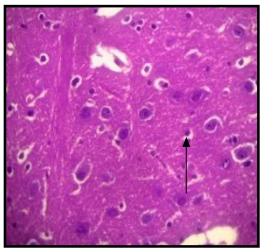


Fig.1[H&E,x400]

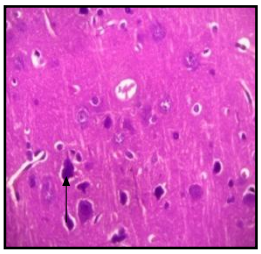
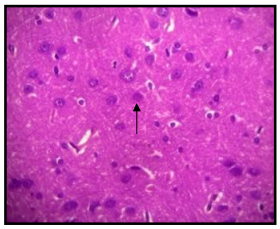


Fig.2[H&E,x400]

HIGHER DOSEOF NUTRACEUTICAL FORMULATION(400MG/KG):



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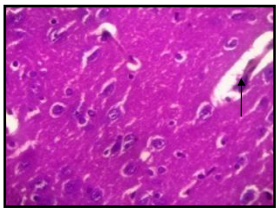
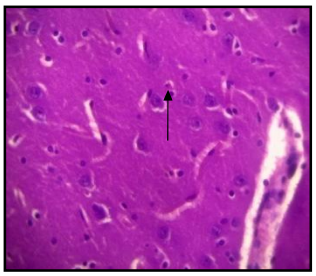


Fig.2[H&E,x400]

STANDARDRIVASTIMINEGROUP:



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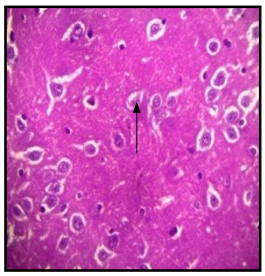

Fig.2[H&E,x400]