Original Research Article

AMELIORATIVE EFFECT OF GRAPE SEED OIL ON ALUMINIUM CHLORIDE INDUCED NEUROTOXICITY ON RATS

ABSTRACT

Aims: Neurodegenerative disorder is characterized by progressive loss of structure and function of neurons. Exposure to aluminium causes neurodegenerative disorder like dementia, Alzheimer's disease (AD). The present study was designed to examine the ameliorative effect of grape seed oil on aluminium chloride induced neurotoxicity in rat.

Methodology: $AlCl_3$ was given orally (175 mg/kg, p.o.) to rats from 29th January to 27th February 2022 which markedly increased the level of acetylcholinesterase (AChE) activity and reduced the levels of antioxidant enzymes in the brain. Two doses of grape seed oil (3.7/kg, p.o.) were selected based on previous safety/toxicity studies and administrated from 17th to 27th February 2022. Behavioral and locomotor activity were determined using passive avoidance test, open field test, actophotometer and rota rod test. Biochemical parameter content and histology of brain tissue were assessed on the final day of the experiment.

Results: Administration of GSO along with $AlCl_3$ caused an improvement in the memory and locomotor activity. GSO inhibited the acetylcholinesterase enzyme and regulated the levels of antioxidant parameters (superoxide dismutase, glutathione reductase, catalase) in brain. Histopathological studies in the hippocampus and cortex of the rat brain also supported that the GSO markedly reduced the toxicity of $AlCl_3$.

Conclusion: The results implied that supplementation of rats with GSO caused a significant augmentation in memory and locomotor activity. It also increased the levels of antioxidant as well as acetylcholine levels. Therefore, the grape seed oil can be used as a neuroprotectant during Al induced neurotoxicity.

Keywords: $AlCl_3$, neurotoxicity, antioxidant, AChE, GSO.

1. INTRODUCTION

Neurodegenerative disorder such as Dementia, Alzheimer's disease (AD) and Parkinson's disease (PD), are characterized by progressive loss (and even death) of structure and function of neurons and have created great burden to the individual and the society. The actual cause of various neurodegenerative diseases remains a mystery in healthcare. Some of the commonly studied environmental factors causes for neurodegenerative diseases are protein degradation, oxidative stress, inflammation, environmental factor, mitochondrial defects, familial history, and abnormal protein accumulation in neuron. However, ageing plays a very important role in neurodegenerative diseases. Exposure to aluminium causes neurodegenerative disorders [1]. In the earth's crust, aluminium is a widely distributed metallic element, and it acts as a neurotoxin. Aluminium contains high levels in diet led to increased risk of central nervous system and like Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinsonism dementia complex [2]. It promotes aggregation of synthetic β-amyloid protein. It activates neurotoxicity in central nervous, skeletal, hematopoietic systems [3]. The extensive damage of nervous system causes learning and memory impairment in animals. Aluminium-containing chemicals are widely used in medicine, food additives, and cosmetics and are added to tap water in some areas as a flocculating agent during the water purification process, it is important to study factors that might increase the absorption of aluminium across the gastrointestinal barrier [4]. Therapeutic plants are utilized to treat psychological and neurodegenerative diseases. Different western medications being

utilized in cognitive decline are taken from plants. Plant inferred alkaloids, for example, anticholinesterase have been utilized to treat AD.

The berries of Vitis vinifera L. ssp. sativa grapes have been of interest worldwide due to the nutritional properties of derivatives, such as peel and seed extracts [5]. For instance, grape seed extract (aqueous or alcoholic) has a high antioxidant potential; its beneficial effects include the modulation of antioxidant enzyme expression, protection against oxidative damage in cells, antiatherosclerotic and anti-inflammatory effects, and protection against some cancer types, in both humans and animal models [6]. Grape seed is a by-product of winemaking process [7], and its oil content is traditionally extracted using either an organic solvent or mechanical techniques [8]. Cold pressing is a method of oil extraction that involves no heat or chemical treatment and hence may retain more health beneficial components [9].

2. MATERIALS AND METHODS

Collection and Authentication:

The fresh whole fruit of black grapes (Vitis vinifera) was collected from Madhavaram, Chennai district of Tamil Nadu in the month of January 2022.

Grape seed extraction using cold pressing method:

The fresh whole fruit of black grapes (Vitis vinifera) was collected and washed with running water. Seeds of the grapes were carefully removed using strainer/ hands, washed and dried for approximately 24 hours. Cold pressing is adopted to collect the extract from the dried seeds using pester and mortar (~100 g of dried seeds) until ground as fine powder with dropwise addition of methanol. Pressing the extracts until the oil shows the visible separation from the grape seed powder. Approximately after 16-18 hours, the cold pressed oil was separated and stored in closed glass bottles (~50 ml).

2.1 EXPERIMENTAL ANIMALS

The male wistar Albino rats weighing 180-200g of around 2-3 months old were used for the study. All experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethical Committee (IAEC).

IAEC Approval no: 01/321/PO/Re/S/01/CPSEA/dated 17/11/2021 valid up to 18/11/2021.

EXPERIMENT DESIGN:

On the first day of experiment the animals had been divided randomly into 5 groups of 4 animals each. Neurotoxicity is induced using Aluminium chloride (175mg/kg, p.o.) for the II, III, IV, V groups have been carried out from 29th January to 27th February 2022 and GSO (3.7g/kg) treatment was carried out from 17th to 27th February 2022. Control animals were given (0.9% NaCl 5ml/kg, p.o.) by means of using oral gavage. The final dose was given 60 min prior to behavioural checking and on 27th Feb 2022, scarification of animal has been done for ex vivo studies.

Group I- Normal saline (0.9% NaCl 5mg/kg) p.o.

Group II- Aluminium chloride (175mg/kg) p.o.

Group III- Aluminium chloride (175mg/kg) p.o. + Donepezil HCl (1mg/kg) p.o.

Group IV- Aluminium chloride (175mg/kg) p.o. + 2ml Grape seed oil (3.7g/kg) p.o.

Group V- Aluminium chloride (175mg/kg) p.o. + 4ml Grape seed oil (3.7g/kg) p.o.

2.2 ASSESSMENTS

PASSIVE AVOIDANCE TEST: [10]

This test is used to assess short term memory. Pole climbing apparatus chamber is used for passive avoidance response where pole is replaced by a wooden platform fixed on electrified grid floor. When rats stepped off the platform, they receive a continuous foot shock from grid floor. The normal reaction of rat was to jump back to the wooden platform. After about 4–5 trials, the animals acquired the passive avoidance response and they refrained from stepping down. The criterion was reached when the animal remained on the platform for at least 60 s.

2.3 OPEN FIELD TEST:[11]

Exploratory behavior was evaluated in an open discipline paradigm. The open are a turned into made of plywood and accommodates of 40 x 50 x 60 cm dimension. The entire apparatus become painted black and divided into 16 squares with white strains at the floor. Each animal was located on the nook of the equipment and for next 5 mins they have been discovered for ambulation which includes line crossings and head dipping.

2.4 MEASUREMENT OF LOCOMOTOR ACTIVITY: [12]

The spontaneous locomotor activity of each rat was recorded individually for 10 min using actophotometer. The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light

falling on the photocell is cut off by the animal, a count is recorded.

2.5 ROTA-ROD TEST: [12]
The effect of aluminium as well as grape seed oil treatment on muscle performance was evaluated using Rota-rod (Techno) test. All the rats were given two initial training trials of 300 s, approximately 10 min apart, to maintain posture on the Rota-rod (3 cm in diameter and rotating at a constant 20 rev/min). After the initial training trials, a baseline trial of 120 s was conducted. The time each animal remained on the Rota-rod was recorded. The animals that did not fall off the Rota-rod were given a maximum score of 120 s.

2.6 ASSESSMENT OF ANTIOXIDANT AND NEUROTRANSMITTER:
The animals were anaesthetized using chloroform and sacrificed. The whole brain was carefully removed from the skull. For preparation of the homogenate, the fresh whole brain was weighed and transferred to a glass homogenizer and a 10% (w/v) tissue homogenate was prepared in 0.1M phosphate buffer (pH 7.4, stored at −2 to–8°C). The homogenate was centrifuged at 3000 rpm for 10 min, and the resultant cloudy supernatant liquid was used for antioxidant and neurotransmitter assessments.

2.7 HISTOPATHOLOGICAL STUDIES
The animals were anaesthetized using chloroform and sacrificed. The whole brain was carefully removed from the skull. The brain sample from each group was selected and stored in 10% buffered formalin
solution sand further embedded in paraffin with wax. The blocks were processed for sectioning; the sections were then stained with haematoxylin and eosin as nuclear and cytoplasmic stains, respectively to assess the ameliorative effect of grape seed oil. Pathological changes, if any, were viewed under light microscope and recorded.

2.8 STATISTICAL ANALYSIS
The statistical analysis was carried out with the aid of one-way ANOVA followed through Dunnett's-t test. P values <0.05 (95%confidence restrict) was considered to be significant, by the use of software graph pad 9.

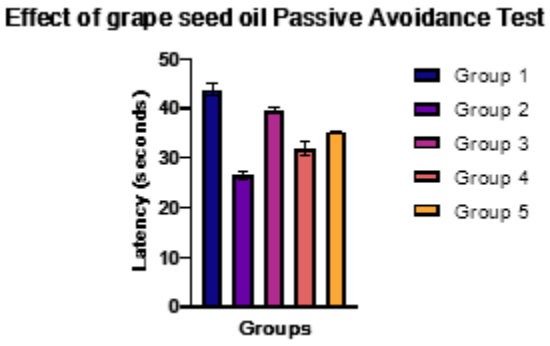
3. RESULTS

PASSIVE AVOIDANCE TEST:
The Step-Down Latency (SDL) of Group II animals shows significant decrease ($p<0.0001$) when compared with Group I animals. On treatment with Grape seed oil (2ml), the Step-Down Latency (SDL) of Group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the Step-Down Latency (SDL) of Group V animals shows significant increase ($p<0.05$) when compared with Group III animals. Results are given Table 1 and plotted in Graph 1.

TABLE: 1 List of groups and their latency

S.NO	GROUPS	LATENCY (SECS)
1.	Group I	43.83±1.24
2.	Group II	26.50±0.76a****
3.	Group III	26.50±0.76a****
4.	Group IV	32.00±1.39b***
5.	Group V	35.33±1.30 b*

GRAPH 1:



OPEN FIELD TEST:
The Group II animals shows significant decrease in head dipping and line crossing when compared with Group I animals ($p<0.0001$). The Group IV animals shows significant decrease in head dipping and line crossing behavior statistically when compared with Group III animals ($p<0.001$ and $p<0.01$). The Group V animals shows significant increase in head dipping and significant decrease in line crossing when compared with Group III animals ($p<0.05$ and $p<0.05$). Results are given in Table 2a, 2b and plotted in Graph 2a and 2b.

TABLE 2a: Headdipping among groups

S.NO.	GROUPS	NO OF HEADDIPPING
1.	Group I	47.00±1.41
2.	Group II	25.80±1.74a****
3.	Group III	39.09±0.09
4.	Group IV	30.80±1.41b***

5.	Group V	43.67±0.08b*

GRAPH 2a:

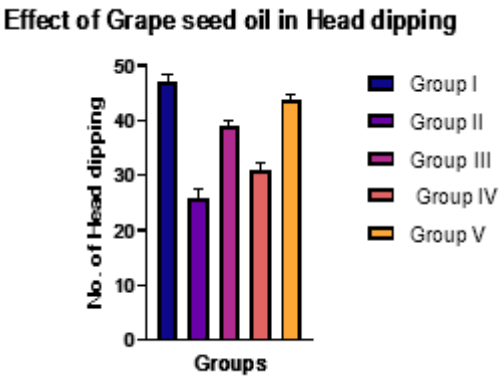
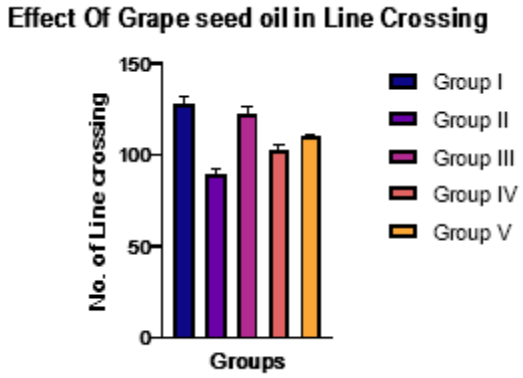


TABLE 2b: Linecrossing among groups

S.NO.	GROUPS	NO OF LINECROSSING
1.	Group I	128.34±3.42
2.	Group II	89.60±3.07a****
3.	Group III	122.25±4.49
4.	Group IV	102.80±2.85b**
5.	Group V	110.22±1.11b*

GRAPH 2b:



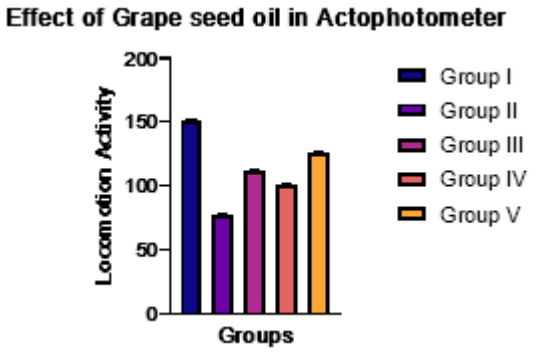
LOCOMOTOR ACTIVITY:

The locomotion activity of Group II animals shows significant decrease ($p<0.0001$) when compared with Group I animals. On treatment with Grape seed oil (2ml), the locomotion activity of Group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the locomotion activity of Group V animals shows significant increase ($p<0.001$) when compared with Group III animals. Results are given Table 3 and plotted in Graph 3.

TABLE 3: Locomotion activity among groups

S.NO	GROUPS	LOCOMOTION ACTIVITY
1.	Group I	151.22±1.68
2.	Group II	76.82±1.85a****
3.	Group III	112.15±1.15
4.	Group IV	100.49±1.93b***
5.	Group V	125.69±1.18b***

GRAPH 3:



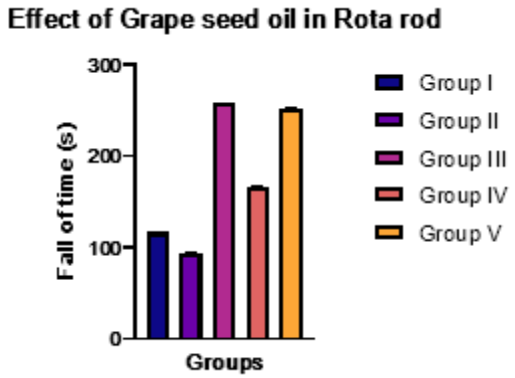
ROTA ROD TEST:

The fall of time of Group II animals shows significant decrease ($p<0.0001$) when compared with Group I animals. On treatment with Grape seed oil (2ml), the fall of time of Group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the fall of time of Group V animals shows significant increase ($p<0.001$) when compared with Group III animals. Results are given Table 4 and plotted in Graph 4.

TABLE 4: **Fall of time among groups**

S.NO	GROUPS	FALL OF TIME (s)
1.	Group I	116.5±1.24
2.	Group II	93.8±0.76a****
3.	Group III	257.5±0.53
4.	Group IV	165.0±1.39b****
5.	Group V	251.2±1.30b**

GRAPH 4:



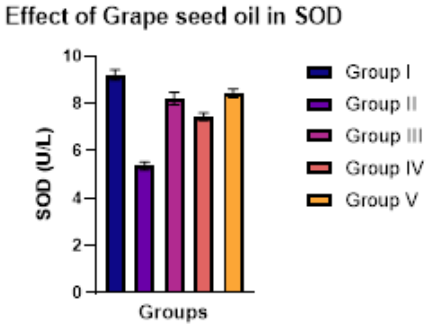
ASSESSMENT OF ANTIOXIDANT

The SOD level of Group II animals shows significant ($p<0.0001$) decrease when compared with Group I animals. On treatment with Grape seed oil (2ml), the SOD level of group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the SOD level of Group V animals shows
significant ($p<0.05$) increase when compared with Group III animals. The results are given in Table 5 and plotted in Graph 5.

TABLE 5: SOD level among groups

S.NO	GROUPS	SOD (U/L)
1.	Group I	9.21±022
2.	Group II	5.36±0.17a****
3.	Group III	8.22±0.15
4.	Group IV	7.44±0.07b***
5.	Group V	8.59±0.03b*

GRAPH 5:

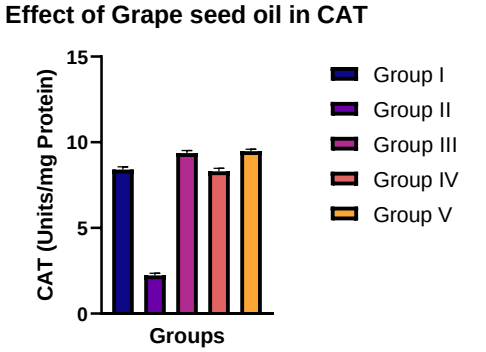


The CAT level of Group II animals shows significant ($p<0.0001$) decrease when compared with Group I animals. On treatment with Grape seed oil (2ml), the CAT level of group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the CAT level of Group V animals shows significant ($p<0.05$) increase when compared with Group III animals. The results are given in Table 6 and plotted in Graph 6.

TABLE: 6 CAT level among groups

S.NO	GROUPS	CAT (Units/mg Protein)
1.	Group I	8.40±0.17
2.	Group II	2.23±0.12a****
3.	Group III	9.37±0.15
4.	Group IV	8.31±0.17b***
5.	Group V	9.95±0.05b*

GRAPH 6:

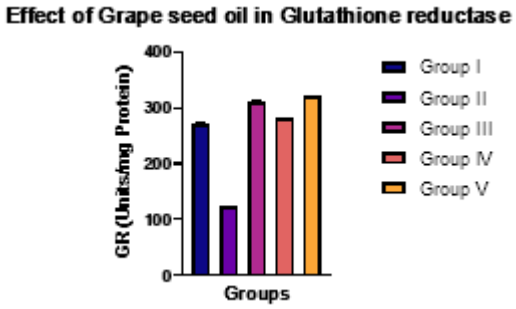


The GRD level of Group II animals shows significant ($p<0.0001$) decrease when compared with Group I animals. On treatment with Grape seed oil (2ml), the GRD level of group IV animals shows significant decrease ($p<0.0001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the GRD level of Group V animals shows significant ($p<0.001$) increase when compared with Group III animals. The results are given in Table 7 and plotted in Graph 7.

TABLE: 7 GRD level among groups

S.NO	GROUPS	GLUTATHIONE REDUCTASE (Units/mg Protein)
1.	Group I	272.51±0.19
2.	Group II	123.45±0.22a****
3.	Group III	312.15±1.15
4.	Group IV	281.12±0.17b****
5.	Group V	321.12±1.18b***

GRAPH 7:



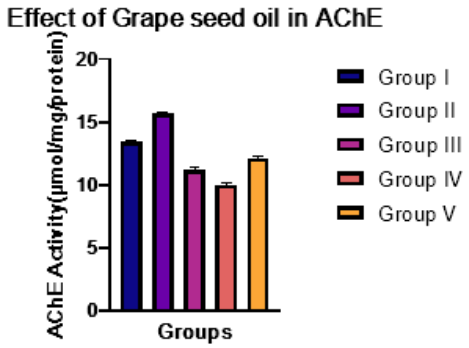
ASSESSMENT OF NEUROTRANSMITTER

The AChE activity of Group II animals shows significant ($p<0.0001$) increase when compared with Group I animals. On treatment with Grape seed oil (2ml), the AChE of group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment
with Grape seed oil (4ml), the AChE of Group V animals shows significant ($p<0.001$) decrease when compared with Group III animals. The results are given in Table 8 and plotted in Graph 8.

TABLE 8: AChE activity among groups

S.NO	GROUPS	AChE ACTIVITY (µmol/mg/protein)
1.	Group I	13.42±0.09
2.	Group II	15.69±0.15a****
3.	Group III	11.21±0.15
4.	Group IV	10.03±0.07b***
5.	Group V	12.15±0.15b**

GRAPH 8:



ASSESSMENT OF HISTOPATHOLOGY

On observation, it was found that there was decrease in density of neuronal cells and disruption in the normal arrangement of neuronal cells in hippocampal region of Group II animals when compared to Group I animals. Treatment groups (Group IV and V) revealed improved neuronal configuration than Group III. Group IV, V shows significant increase in the density of neuronal cells and hippocampal regions of brain when compared with neuronal loss in negative control group (Group II). Histopathological pictures are shown in FIG 1 to 16.

FIG 1: BRAIN HISTOPATHOLOGY OF CONTROL GROUP

CORTEX HIPPOCAMPUS

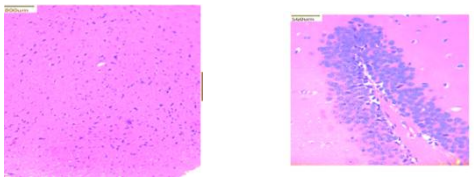


FIG 2: BRAIN HISTOPATHOLOGY OF NEGATIVE CONTROL GROUP

CORTEX HIPPOCAMPUS

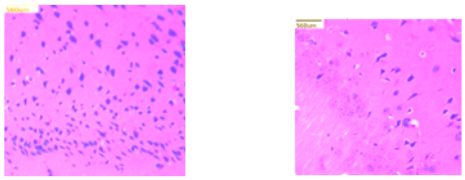


FIG 3: BRAIN HISTOPATHOLOGY OF STANDARD CONTROL GROUP

CORTEX HIPPOCAMPUS

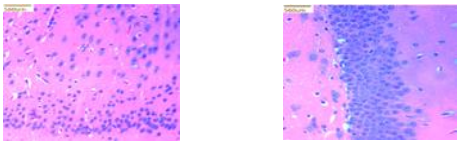


FIG 4: BRAIN HISTOPATHOLOGY OF GRAPE SEED OIL (2ml)

CORTEX HIPPOCAMPUS

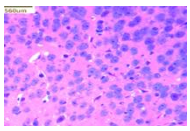
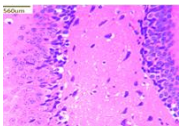
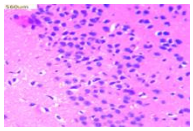
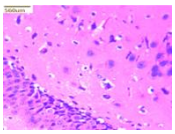



FIG 5: BRAIN HISTOPATHOLOGY OF GRAPE SEED OIL (4ml)

CORTEX HIPPOCAMPUS

DISCUSSION:

- Aluminium chloride causes a class of neurodegenerative disorder. The incidence of neurodegenerative disorder increases with age. Damage of short-term memory is the characteristic feature. When the condition proceeds, extra cognitive capabilities are impaired, like ability to calculate, and use objects and equipment. Acetylcholine esterase inhibitors are the best marketers authorized by means of the Food and Drug Administration (FDA) for the treatment of AD. All different retailers prescribed for the remedy of AD are used on an off- label foundation.
- As per preceding studies *grape seed oil* has superb antioxidant belongings, consequently it's far believed to have actions on CNS problems and neurodegeneration illnesses.
- The current study has revealed the ameliorative effect of *grape seed oil* on Aluminium chloride induced neurotoxicity in rats. Aluminium chloride induced impairment of reminiscence became assessed by using the behavioural parameters like Passive avoidance task, open field test and locomotor activity using actophotometer, rota rod. It became discovered that remedy with *grape seed oil* shields cognitive deficits in Aluminium chloride induced neurotoxicity.
- Spatial studying inside the open area habituation was used to access mastering and memory. The decline in response to regular surroundings after repeated exposures to the acquainted surroundings is cited to spatial learning. Recurrent publicity produces a lower in the exploratory tasks, that's implicative of memory referring to a selected feature of that surroundings. Exploratory activities may be reduced on subsequent touch with open subject. In the end of the study the animals which are treated with *grape seed oil* indicated elevated spatial routine and sleep deprivation decreased spatial routine learning.
- Passive avoidance behavior is based on poor reinforcement became used to observe the level of memory. An electro-shock is given during training period for about 15 sec inside the Step-Down Latency (SDL) which is then recorded. SDL was determined by the time taken via the rat to step down from the wooden platform to grid ground with its whole paw on the grid floor. SDL is enhanced as an indication of long-term memory in which Aluminium chloride treated animals showed reduced SDL. Treatment with *grape seed oil* showed development in long time reminiscence as index of elevation in SDL.
- Muscular and locomotion activities using Actophotometer, and Rota rod were found to be significantly decreased after Al treatment. Therefore, high levels of Al not only interfere with the memory but also affect the motor functions and leads to decreased motor activities and grip strength. On treatment with *grape seed oil*, animals enhanced the locomotor activity.
- Antioxidants SOD, CAT, GRD and neurotransmitter AChE activity was evaluated where there is a significant decrease in antioxidant level and increased AChE after Al treatment. On treatment with *Grape seed oil,* there was increase in antioxidant level and decrease in AChE activity.
- The histopathology studies of the brain shows that on treatment with *Grape seed oil,* there is a significant increase in the density of neuronal cells and hippocampal regions of brain while compared with neuronal loss in Aluminium chloride treated group.

CONCLUSION:

The *Grape seed oil* possessed significant recovery in memory and locomotor activity processes but higher dose 4ml showed better activity than lower dose 2ml.

The current study reveals the ameliorative effect of *Grape seed oil* on Aluminium chloride induced neurotoxicity in rat. From the effects it can be concluded that *Grape seed oil* has extraordinary impact in behavioural & locomotor activity enhancement and oxidative stress. Further studies are required for the identification of molecular level activity and individual phytochemical constituent which

UNDER PEER REVIEW

could be responsible for neuroprotective action.

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