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ANTIEPILEPTIC POTENTIAL OF *ARTEMISIA NILAGRICA* LINN.DURING ETHANOL EXPOSURE AND ITS WITHDRAWAL IN ALBINO RATS

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ABSTRACT

Epilepsy is the second most common neurologic disorder after stroke. Approximately 1% of the world's population has epilepsy, is a condition where the people suffer from recurrent seizure. The present study was aimed to evaluate the antiepileptic activity of aqueous extract of *Artemisia nilagrica* Linn. On ethanol induced seizures in albino rats. Experiments were carried out in wistar strains of albino rats and rats were divided in five groups. Group I rats was treated as normal, Group II rats was administrated with ethanol (38/ 1g bw/ day) for 30 days, Group III and Group IV rats was administrated with ethanol (38/1gbw/day) and followed by aqueous extract of *Artemisia nilagrica* Linn (100 and 200mg/1g bw/day) for 30 days. The effect of ethanol in the brain was estimating the physical behaviors like Immobility and swimming test, nociceptive function, locomotors activity, lipid peroxide antioxidants (SOD, LPO, and GSH) and neuro transmitters like acetylcholine esterase was determined. The histopathology of brain tissue of experimental animals was studied to support the effectiveness of the plant drug. Acute administration of ethanol, in a dose of (5g/kg bw/30 days) produced sedative which resulted in increased immobility and decreased swimming time. The ethanol intake also enhanced per oxidation of lipids in which produced oxidative stress followed by neurodegeneration. Oral administration of aqueous extracts of *Artemisia nilagrica* Linn in a dose 100 mg and 200mg/1gbw and ethanol for 30 days did not change either swimming and nociceptive behaviour or normal function of neural cells. Conclusion: The result concludes that the

aqueous extract of *Artemisia nilagirica* Linn has nociceptive, antiepileptic and antioxidant effects against ethanol.

KEYWORDS: *Artemisia nilagirica* Linn, Epilepsy, Ethanol, Antioxidant.

INTRODUCTION

Epilepsy may be defined as neuro physiological disorder, which occurs due to over discharge of neurotransmitter substance. [1] Epilepsy is a common chronic neurological condition that is characterized by recurrent unprovoked epileptic seizures. Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing country is 100 per 100,000. [2] There is number of drugs available for the treatment of epilepsy in modern therapy. But the major drawback is being faced is their chronic side effects. Medicinal herbs constitute the cornerstone of traditional medicinal practice worldwide. Plants may serve as the alternative sources for the development of new anti-consultant agents due to their biological activities.

Artemisia nilagrica Linn is an aromatic perennial herb, belonging to the family Asteraceae. The leaves and flower of the plant are useful for the treatment of skin diseases, ulcers, brain and inflammatory diseases. And also used as tonic, anti-helminthic and expectorant. Thus the present study was undertaken to validate the anti epileptic activity of *Artemisia nilagrica* Linn in ethanol induced models.

MATERIALS AND METHODS

Collection and Authentication of Plant

Plant source selected for the present study is *Artemisia nilagrica* Linn. Whole plants of the *Artemisia nilagrica* Linn were collected from in around Trichy, identified and authenticated with RAPINAT Herbarium, St.Joseph's college, Trichy, Tamilnadu.

Preparation of Plant Extract

Fresh plant material was shade dried and powdered coarsely using electric blender. 200gm of coarse powder of *Artemisia nilagriga* Linn. was taken and extracted with water. The plant material was mixed with and it was six parts of water was added, boiled and reduced to one third and filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to pre-clinical screening.

Parameters Studied

The physiochemical and preliminary phytochemical screening were determined by standard textual procedure. [3, 4]

Induction of Epilepsy by Ethanol

Epilepsy was induced in Wistar albino rats (starved for 16 hours) by administrating, 3g/kg body weight of a 30% ethanol orally**.**

Experimental Design

Wistar strains of albino rats of either sex weighing 150-200gm were used as the experimental models. The experimental animals were divided into five groups each consisted of six animals. In experimental design, Group I rats were served as normal control, Group II rats were served as disease control (Rats were induced with ethanol (3g/kgbw) for 29 days in order to induce neurotoxicity), Group III rats were induced with ethanol (3g/kgbw) and treated with aqueous extract of Artemisia nilagrica (AEAN, 100mg/kg of bw), Group IV rats were induced with ethanol (3g/kgbw) and treated with aqueous extract of Artemisia nilagrica (AEAN, 200mg/kg of bw), Group V rats were treated with plant extract alone (200mg/kg of bw) , Group VI rats were induced with ethanol (3g/kgbw) and treated with standard drug (Imipramine, 15mg/kg body weight) for 30 days orally.

After 30 days, animals were sacrificed by cervical dislocation. Brain tissue was collected and homogenized. The homogenate was used for the determination of lipid peroxide [5], antioxidants like superoxide dismutase [6] and reduced glutathione [7] and Acetyl choline esterase [8] The physical behaviors like immobility; swimming , nociceptive ,and motor activities [9,10] were also determined.

Statistical Analysis

All the results were expressed as mean ± S.E . The data were statistically analyzed by one way analysis of variance (ANOVA) and P value <0.05 was considered as significant.

RESULTS AND DISCUSSION

Table: 1. Physiochemical Constants of *Artemisia nilagirica* Linn.

S. No	Parameters	Value % W/W
1.	Foreign Matter	0.952
2.	Moisture	1.764
3.	Total ash content	11.5
4.	Water soluble ash	8.5
5.	Acid insoluble ash	2.9

Total Ash, Water Soluble Ash and Acid Insoluble Ash of *Artemisia Nilagrica* Linn.

The physiochemical constants of *Artemisia nilagirica* Linn is given in the Table 1. The foreign matter of *Artemisia nilagirica* Linn was found to be 0.952%. the moisture content was calculated as 1.764%. The total ash, water soluble ash and acid insoluble ash were found to be 11.5%, 8.5% and 3.8% respectively.

Table: 2. Preliminary Phytochemical Screening of dry leaf powder and aqueous leaf extract of *Artemisia nilagrica* Linn.

Phytochemicals	Powder	Aqueous extract
Coumarin	Present	Present
Sterol	Present	Present
Quinone	Absent	Absent
Lignin	Absent	Absent
Saponin	Present	Present
Flavonoid	Present	Present
Terpenoid	Absent	Absent
Alkaloid	Present	Present
Tannin	Present	Present
Starch	Absent	Absent
Protein	Present	Present
Glycosides	Present	Present

Phytochemical Analysis of *Artemisia Nilagrica* Linn

Phytochemical analysis of the dry leaf powder and aqueous leaf extract of *Artemisia nilagirica* Linn showed the presence of alkaloids, flavonoid, saponin, steroid, terpenoid, tannin and glycoside. The saponin and flavonoid have been reported to be responsible for stimulation of spontaneous motor activity in mice. Thus, the reported pharmacological activities of *Artemisia nilagrica* Linn could be attributed to the presence of saponins and flavonoid.

Antiepileptic Activity

Table 3: Effect of aqueous leaf extract of *Artemisia nilagrica* Linn on immobility during forced swimming

Groups	I	II	III	IV	V	VI
FST (Sec)	31.6± 1.1401	91.4± 0.8944*	75.8± 0.8366	40± 0.7071**	34.8#± 0.4472	34.8**± 0.4472#

**p<0.05 statistically significant when compared with control group*

 # p<0.05 statistically significant when compared with disease control group

*** p<0.05 statistically significant when compared with disease control group*

Effect of *Artemisia Nilagrica* Linn on Immobility of Rats

The different dose (100, 200 mg/kg) of aqueous leaf extract of of *Artemisia nilagirica* Linn on ethanol induced rats showed different effects in immobility during FST (Table 3).The forced swimming test (FST) is the most widely used animal test procedure of antiepileptic treatment [11] The present investigation showed that ethanol exposure causes chemical stress on group II rats that caused depression like behavior in exposed animals. The true mechanisms of antiepileptic activity of *Artemisia nilagirica* Linn is unknown but behavioral parameters in forced swimming test (FST) confirmed potential antiepileptic activity as serotonergic agents. In this study, it was found that increase in the dose of extract decreased the immobility time and increased swimming time.

Table 4: Effect of *Artemisia nilagrica* Linn on motor activity of central nervous system

Groups	I	II	III	IV	V	VI
0 minutes	14.4 ± 0.54	13.4 ± 0.89	14.4 ± 0.54	13.6 ± 0.5477	14.6 ± 0.5477	14.6 ± 0.5477
10 minutes	13.6 ± 0.5477	12.2 ± 0.8366	13.2 ± 0.8366	13.2 ± 0.8366	13.2 ± 0.8366	11.2 ± 0.8366
20 minutes	12.4 ± 0.8944	10.2 ± 0.4472	12.2 ± 0.8366	11.8 ± 0.8366	12.4 ± 0.8944	12.4 ± 0.8944
30 minutes	11.6 ± 0.5477	7.2* ± 0.8366	11.8 ± 0.8366	11.8** ± 0.8366	12.6 # ± 0.5477	12.6** ± 0.5477
40 minutes	11.4 ± 0.5477	6.6* ± 0.5477	11.4 ± 0.8944	11.2** ± 0.8366	11.6 # ± 0.5477	11.6** ± 0.5477
50 minutes	9.4 ± 0.5477	4.4* ± 0.5477	8.6 ± 0.5477	8.6** ± 0.5477	10.6 # ± 0.5477	11.2** ± 0.836
60 minutes	8.6 ± 0.8944	2.6* ± 0.5477	6.6 ± 05477	8.4** ± o.5477	10.6 # ± 0.5477	10.6** ± 0.5477

**p<0.05 statistically significant when compared with control group*
***p<0.05 statistically significant when compared with disease control group*
#p<0.05 statistically not significant when compared with control group

Effect of *Artemisia Nilagrica* Linn on Hole Cross Test of Rats

The effect of aqueous leaf extract of *Artemisia nilagrica* Linn on Hole cross test in ethanol induced epileptic rats compared with normal, epileptic rats and reference drug is given in Table 4. Ethanol (3g/Kg bw) induction for 30 successive days to Group II rats decreased the nociceptive activity periods significantly ($p<0.05$) when compared to normal rats. Ethanol induction followed by treatment with aqueous leaf extract of *Artemisia nilagirica* Linn at doses of (100 and 200mg/kg bw) in group III and group IV rats did not show any alteration in the nociceptive function of central nervous system. Imipramine (15mg/1g bw) treated rats also showed no modulation in the nociceptive activity of central nervous system in group VI rats. Ethanol showed significant reduction in spontaneous motor activity. The activity is a measure of the level of excitability of the central nervous system. The decrease in activity may be closely related to sedation resulting from depression of the central nervous system. The reduction spontaneous motor activity could be due to inhibitory effects of the ethanol on the central nervous system. An immediate lifting of limb by rats which were treated with ethanol and AEAN in group III and IV could be due to normal motor activity of the central nervous system. In this study, it was also found that increasing the dose of extract decreased the immobility time. The same finding also has been shown in previous studies [12] Mechanism of this effect is unknown. However, it has been shown that *Artemisia nilagirica* Linn has CNS stimulant effect .

Table 5: Effect of *Artemisia nilagirica* Linn on Nociceptive function of central nervous system

Groups	I	II	III	IV	V	VI
NFT	7.2 ± 0.83	17.2 * ± 0.83	15.6 ± 0.54	9.6 ** ± 0.54	8.6 # ± 0.54	6.4 ** ± 0.54

**p<0.05 statistically significant when compared with control group*

***p<0.05 statistically significant when compared with disease control group*

#p<0.05 statistically not significant when compared with control group

Effect of *Artemisia Nilagrica*linn. on Nociceptive Activity of Rats

The effect of aqueous leaf extract of *Artemisia nilagirica* Linn on nociceptive function test in ethanol induced epileptic rats was compared with normal, epilepsy and reference drug is given in Table 5. Ethanol (3g/ Kg bw) induction for 30 successive days to Group II rats decreased the nociceptive activity periods significantly ($p<0.05$).

Ethanol induction followed by treatment with aqueous extract of *Artemisia nilagirica* Linn at doses of (100 and 200mg/kg bw) in group III and group IV rats did not show any alteration in the nociceptive function of central nervous system. Imipramine (100mg/1g bw) treated rats also showed no modulation in the nociceptive activity of central nervous system group Vs rats. Ethanol showed significant reduction in spontaneous motor activity. The activity is a measure of the level of excitability of the central nervous system. The decrease in activity may be closely related to sedation resulting from depression of the central nervous system. The reduction spontaneous motor activity could be due to inhibitory effects of the ethanol on the central nervous system. An immediate lifting of limb by rats which were treated with ethanol along group V could be due to normal motor activity of the central nervous system. In this study, we also found that increasing the dose of extract increased the immobility time. The same finding also has been shown in previous studies. [13]

Table 6: Effect of *Artemisia nilagrica* Linn on levels of Lipid per oxide and Antioxidants in ethanol induced epileptic rats

Groups	I	II	III	IV	V	VI
LPO U/mg	7.693 ± 0.15	70.66* ± 1.2	54.76 ± 0.33	15.35** ± 0.34	7.38 # ± 0.34	16.35** ± 0.18#
SOD U/mg	358 ±2.6457	95.66* ±1.5275	252.66 ±2.5166	329.66** ±2.0816	344.3 # ±0.5773	351** ±1.00
GSH U/mg	74.78 ±o.9986	55.01* ±0.7733	68.05 ±0.2511	72.804** ±0.5804	70.30 # ±0.6225	66.25** ±0.2462

**p<0.05 statistically significant when compared with control group*

***p<0.05 statistically significant when compared with disease control group*

#p<0.05 statistically not significant when compared with control group

Effect of *Artemisia Nilagrica* Linn on Lipid Peroxide and Antioxidants in Experimental Rats

The effect of aqueous leaf extract of *Artemisia nilagrica* Linn on lipid per oxide, antioxidants (enzymatic and non-enzymatic) levels in ethanol induced epileptic rats compared with normal, epileptic rats and reference drug is given in Table 6.

In the present study, the concentration of aqueous leaf extract of *Artemisia nilagirica* Linn as an indicator of lipid peroxidation. The results showed that chronic consumption of ethanol caused oxidation of cellular lipids in brain thus significantly elevated the level of lipid peroxide and decreased the levels of antioxidant such as superoxide dismutase (SOD) and reduced glutathione (GSH). The assay of superoxide dismutase (SOD) (enzymatic

antioxidant), and the level of reduced glutathione (GSH) (non-enzymatic antioxidant) were carried out and the results depicted that the elevated oxidative status in brain exposed to ethanol increased lipid peroxide level and decreased reduced glutathione level. On contrast, administration of aqueous extract of *Artemisia nilagirica* Linn in ethanol induced rats did not show any significant reduction in the lipid peroxidation and elevation in the levels of enzymatic and non-enzymatic antioxidant.

High concentrations of peroxidizable fatty acids present in brain are common cause for the elevation in the level of MDA due to ethanol administration. Ethanol induces lipid per oxidation through iron that is present in certain regions of brain which catalyses the generation of oxygen derived free radicals. [14] Hydroxy ethyl free radicals from ethanol having long half-life which damage the membrane that leads to an increase in lipid per oxidation. [15] MDA is the end product of lipid per oxidation whose level was elevated in the brain, kidney, liver and heart of the ethanol treated rats. [16] In the present study, a significant reduction in the levels of MDA was observed in the brain tissue of rats receiving ethanol and aqueous leaf extract of *Artemisia nilagirica* Linn thus showing the beneficial effect of the extract against ethanol-induced epilepsy. Increased lipid per oxidation causes a decrease in cellular defense system. The activity of enzymatic antioxidants has been decreased in ethanol induced rats. Superoxide anion radical produces damage to the membranes and biological structures that is protected by SOD. Inhibition of activity of SOD results in the generation of partially reduced oxygen species. When SOD activity is decreased, neurons are more vulnerable to oxy radical injury. [17]

Decreases in the levels of non-enzymatic antioxidants like GSH and in ethanol-exposed rats were due to an inhibition in their protective effect against lipid per oxidation. Oxidative stress occurs due to the accumulation of oxidized glutathione (GSSG), which is due to the inability of GSH to maintain the redox state. A decrease in the GSH level results from the binding of acetaldehyde to the cysteine residues in GSH. [18] Treatment of rats with aqueous extract of *Artemisia nilagrica* Linn increased the levels of non-enzymatic antioxidants.

Table 7: Effect of *Artemisia nilagrica* Linn on Acetyl Choline Esterase activity in ethanol induced epileptic rats

Groups	I	II	III	IV	V	VI
Acetyl choline esterase U/L	383.3 ± 0.57	147.6* ± 0.57	235.3 ± 0.57	372.3** ± 0.57	378.3 # ± 0.57	380.3** ± 0.57

**p<0.05 statistically significant when compared with control group*

***p<0.05 statistically significant when compared with disease control group*

#p<0.05 statistically not significant when compared with control group

Effect of *Artemisia Nilagrica* Linn on Activity of Acetylcholine Esterase in Epileptic Brain

The effect of aqueous leaf extract of *Artemisia nilagrica* Linn on Acetyl Choline Esterase activity in ethanol induced epileptic rats compared with normal, epilepsy and reference drug is given in Table 7.

The effect of chronic ethanol consumption and its withdrawal produced significant increase in serum Acetyl cholinesterase (AChE) activity in ethanol induced group II rats compared with normal rats ($p < 0.05$). Acetyl choline esterase degrades acetylcholine in effector organs or surrounding fluids and is of importance for cognitive functions and anesthetic medication in both human and experimental animals**.** Acetyl choline esterase activity in the brain is assumed to be a biochemical marker for clinical diagnosis and prognosis of several central and peripheral nervous system dysfunction such as Alzheimer's diseases, Parkinson's disease, dementia, Sctrizopheremia and Chronic alcoholism. This enzyme metabolize acetyl choline that may be important therapeutic target and measurement of acetyl cholinesterase activity in serum and brain may be important for estimating ethanol effects or ethanol related damage in both central and peripheral cholinergic systems. Hence in the present study, it was found that the activity of acetylcholine esterase was higher in serum and lower in brain of group II rats exposed to ethanol (3g/kg/bw for 30 days). On contrast, daily administration of plant extract along with ethanol showed no alteration in the activity of AChE in brain. The findings of earlier study reported that the effects of chronic ethanol ingestion (2g/kg,b.w/day for 6.5 weeks) decreased the activity of AChE in brain. [19] Hence, from the earlier work, it was confirmed that chronic ethanol exposure decreased the activity of AChE in brain.

Histopathological Studies

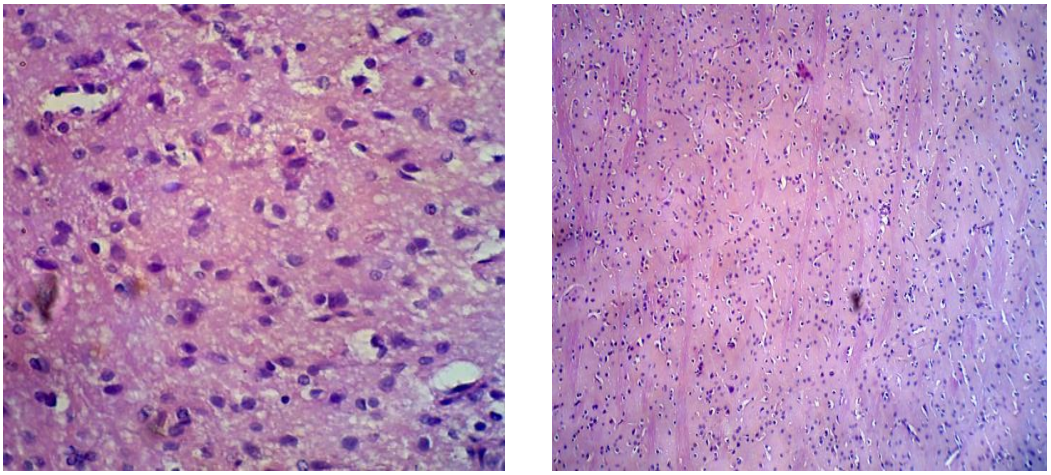

Fig. 1: Photomicrograph of Normal rat brain showing normal architecture of tissue

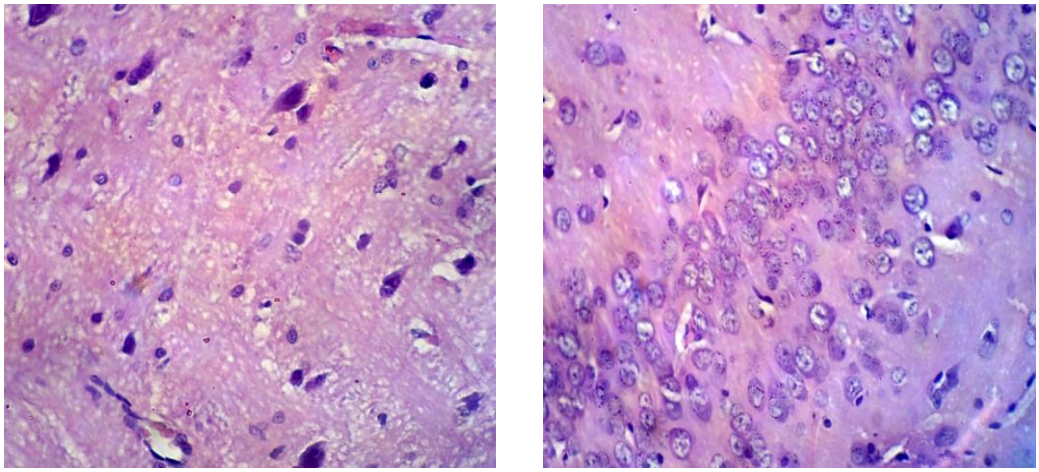

Fig. 2: Photomicrograph of Ethanol exposed epileptic rat brain showing gliosis

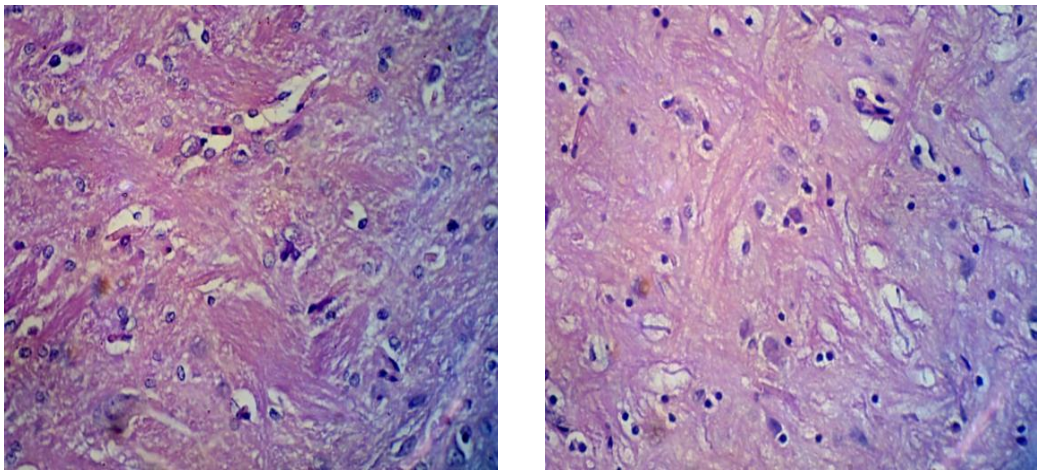

Fig. 3: Photomicrograph of Ethanol exposed epileptic rat brain showing gliosis

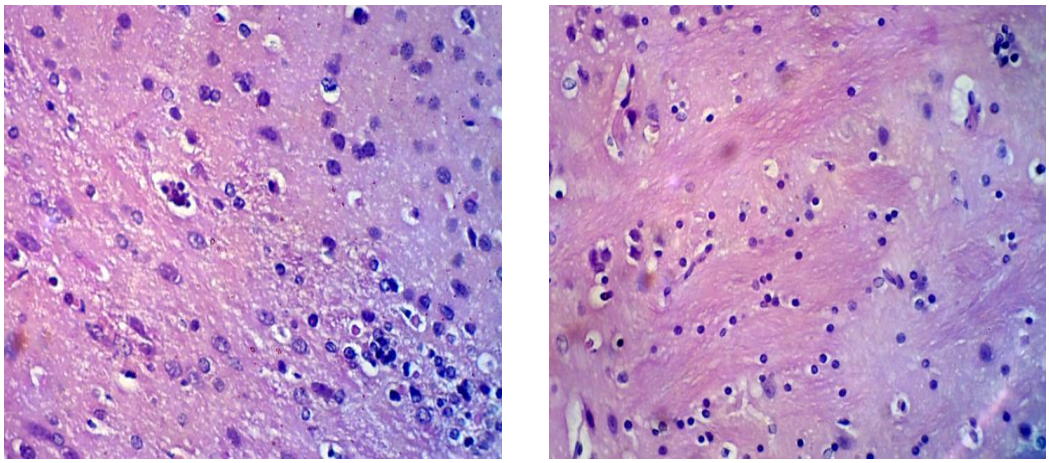

Fig. 4: Photomicrograph of *Artemisia nilagirica* Linn. treated epileptic rat brain tissue showing regeneration neuronal cells

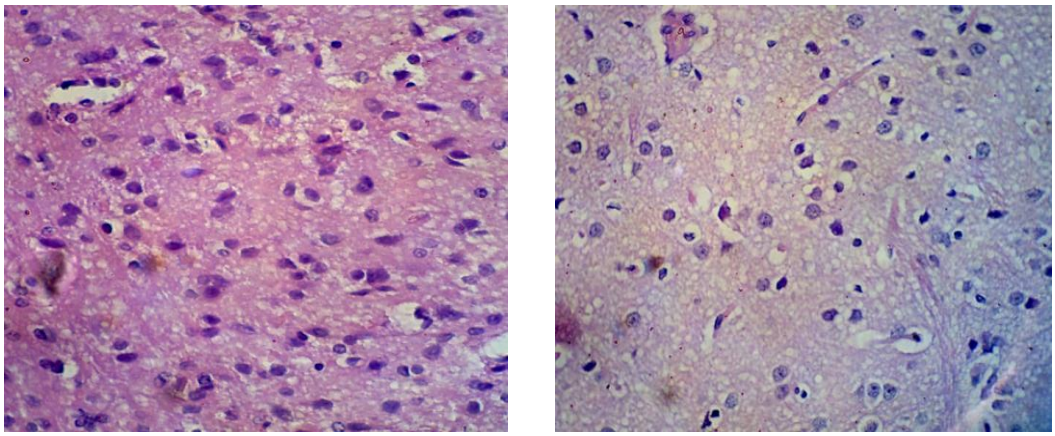

Fig. 5: Photomicrograph of Imipramine treated rat brain tissue showing regeneration of neuronal cells

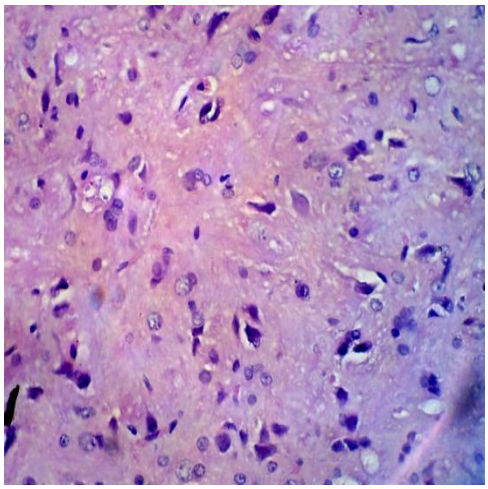

Fig. 6: Photomicrograph of *Artemisia nilagirica* Linn. treated Rat brain tissue showing normal histoarchitecture

The results of histopathological studies are presented in Fig. 1 - 6. Section of the brain tissue of normal rats showed normal architecture with glial tissue (Fig. 1). The brain section of alcohol exposed rats showed profound gliosis and degeneration of neural cells (Fig. 2 & 3). On the other hand, rats treated with the aqueous extract of *Artemisia nilagirica* Linn (200mg/kgbw) showed regeneration of neural cells with minimal gliosis and regeneration of glial cells (Fig. 4). Rats treated with *Artemisia nilagirica* Linn alone did not show any abnormalities in brain tissue (Fig. 5). Rats treated with standard drug showed minimal gliosis and regeneration of neural cells (Fig. 6). Normal brain functions require not only neurons, but also non neuronal cells called glia, that support growth and development of neurons. The radial glial cells normally change into another type of glial cell star shaped Astrocytes. [20] In the present study alcohol exposure in the brain of Group II rats converted radial glia into astrocytes which is supported by earlier studies. [21] Thus, the results of the present study reported that alcohol exposure alters several aspects of astrocyte structure and function. The alcohol exposure also reduced the overall number of astrocytes in the brain and reduced or delayed the production of proteins which interfere with the cells production of specific grow the factors. However, co-administration of aqueous extract of *Artemisia nilagirica* Linn and alcohol did not produce pathological observation in the brain of Group-III- IV rats.

CONCLUSION

Based on results of the present study, it can be concluded that the aqueous leaf extract of *Artemisia nilagirica* Linn protects and exhibits antioxidant activities against alcohol-induced oxidative stress in brain tissue. This effect may be attributed to the phytochemical constituents of *Artemisia nilagirica* Linn which might include alkaloids, flavonoids, glycosides and terpenoids. This approach might be useful in determining an acceptable treatment condition by plant extract in the management of alcohol induced convulsion.

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