

# The Protective Effect of Vitamin E against the Neurotoxic Effect of Aluminum Chlorid in Male Albino Rat.

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**Abstract:** Aluminum (Al) is an important element with known toxicity in the human body, mainly in the central nervous system. Vitamin E (Vit E) is known as antioxidant, free radical scavenger and metal chelator. This study was aimed to investigate the neurotoxic oxidative damage of aluminum chloride (AlCl<sub>3</sub>) and the protective effect of vit E on neurotoxic effect of AlCl<sub>3</sub> on brain of male albino rats exposed to the metal. Sixty male rats were divided into three treatment groups. The first group treated with AlCl<sub>3</sub> 1600 mg/L. In the second group, the rats were given AlCl<sub>3</sub> with vitamin E in a dose of 2.5 mg/kg BW orally. The third group was received no treatment and was kept as control group. Administrations of AlCl<sub>3</sub> with or without Vit E were carried out daily for one month. The present data revealed a significant increase in acetylcholinesterase (AChE) activity and malondialdehyde content (MDA) while the enzymatic antioxidant activities as glutathione-s-transferase (GST), glutathione peroxides (GPX) and glutathione reductase (GR) were significantly decreased in aluminum treated group. Also in the same group significant elevation in nitric oxide (NO) content and amino acid concentrations (glutamic acid, gamma amino butyric acid (GABA), tyrosine, methionine, aspartic acid, leucine, alanine, valine, phenylealanine and isoleucin were recorded, with marked histopathological changes as focal and diffuse gliosis with pericellular edema in cerebral cortex in addition to neurophagia and neuronal degeneration. Vit E supplementation resulted in a marked appreciable improvement in all previous abnormal alteration observed in AlCl<sub>3</sub> treated rats. Therefore, the study revealed that Vit E has a potential ability to exhibit neuroprotective role in conditions of Al-induced oxidative stress and neurotoxic effect in rat brain.

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**Keyword:** neurotoxicity, aluminum, vitamin E and rat

## 1. Introduction:

Nervous system is a vulnerable target for toxicants due to critical voltages which must be maintained in the cells and the all responses when voltages reach threshold levels. Aluminum (Al) has the potential to be neurotoxic in human and animals. It presents in many manufactured foods and medicines and is also added to drinking water for purification purposes (Newairy *et al.*, 2009). Al is widely used in antacid drugs, as well as in food additives and tooth paste (Abbasali *et al.*, 2005). Environmental pollution with different aluminium containing compounds, especially those in industrial waste expose people to higher than normal levels of Al (Kloppel *et al.*, 1997). Particulate matters distributed by cement – producing factories contain, high amount of Al, and animals and populations residing in the vicinity are exposed to the pollution (Shehla *et al.*, 2001). Although aluminum has been implicated in Alzheimer's disease, Parkinsonism, Dementia complex and causes extensive damage to

the nervous system, to date the mechanism of Al neurotoxicity has not been fully elucidated (Niu *et al.*, 2007). In recent researches, aluminum have been reported to accelerate oxidative damage to biomolecules like lipid, protein and nucleic acids (Jyoti *et al.*, 2007). Vit E ( $\alpha$ - Tocopherol and its derivatives) is a predominant chain breaking lipid - soluble antioxidant and is believed to be the primary free radical scavenger and prevent lipid peroxidation (Cerolini *et al.*, 2000).

Therefore, the present study was carried out to investigate the alteration in biochemical parameters including free radicals, enzymes activities, amino acids concentrations and histopathological alterations induced by AlCl<sub>3</sub> in brain tissue of male rats in addition to the role of vitamin E in altering the oxidative damage and neurotoxic effects of aluminum.

## 2. Material and methods:

Materials

#### I- Tested substances:-

- 1-  $\text{AlCl}_3$  is a white powder, was purchased from Aldrich chemical company (Milwaukee, USA).
- 2- Vitamin E: As E-viton capsules (Vit E tocopherol acetate) 100 mg obtained from Kahira Pharmaceutical and Chemicals Ind. Co.

#### II- Experimental animals:

Sixty apparently healthy adult male albino rats with initial weight of 90 – 100 g were supplied from Animal House of National Research Center. Animals were left for one week before the start of experiment for acclimatization. They were fed on balanced commercial rat diet with free access of food and water. Rats were randomized and divided into three groups, treated and control groups twenty rats each, the rat in the first group were given daily  $\text{AlCl}_3$  via their drinking water containing 1600 mg / L  $\text{AlCl}_3$  for one month (Nedzvetsky *et al.*, 2007). In the second group, rats were administered  $\text{AlCl}_3$  as in the first group in addition to vit E as E. Viton in a dose level of 2.5 ml / kg BW (Paget and Barnes, 1964) orally by using stomach tube. The animal in the third group served as control and received no treatment. Administrations of  $\text{AlCl}_3$  with or without Vit E were carried out daily for 30 successive days.

#### Sampling:

Five animals from each experimental group were sacrificed every week and their brains were taken in order to carry out biochemical and histological examination. For biochemical analysis, brain samples were stored at  $-20^\circ\text{C}$ , while another brain samples were preserved in 25% neutral formalin for histopathological examination.

#### 2- Methods:

##### 1- Preparation of brain samples:

a- preparation of brain samples for determination of some biochemical constituents: Brain samples were rapidly removed, rinsed from blood using distilled water, blotted between two damp filter papers, then weighted. The whole brain was placed in pre-chilled glass tube with calculated volume of cold saline and the tube surrounding by cooling mixture (ice + sodium chloride + acetone) then homogenized by homogenizer, the final homogenate was 10% weight / volume, this homogenate is centrifuged and the supernatants were taken for different biochemical studies.

b- Preparation of brain samples for estimation of GABA, glutamic acid and other amino acids concentrations: Brain samples were homogenized with 1:4 w/v 0.5% sodium dodecylsulphate (SDS) with homogenizer then incubate at room temperature for 15 min. then 4% sulpho salicylic acid solution

(1:6 V/V) was added and the samples were vortexed and centrifuged at 12000 rpm for 15 min. The supernatant was frozen at  $-20^\circ\text{C}$  until the analysis of GABA, glutamic acid and other amino acids concentrations.

2- Biochemical studies: Determination of the activities of acetylcholinesterase and lipid peroxidation (malondialdehyde concentration) were performed according to the method of Kendel and Bottger (1967) and Okhawa *et al.* (1979) respectively. Determination of glutathione-S-transferase, glutathione peroxidase and glutathione reductase activities were carried out using the methods described by Goldberg and Spooner, (1983) Pagila and Valentin, (1967) and Habig *et al.* (1974) respectively. Also Nitric oxide was determined after the method of Montogometry and Doymock (1961).

3- Concentration of GABA, glutamic acid and other amino acids: They were determined in brain samples on the basis of retention time and quantified using a Beckman 6300 automate amino acid analyzer according to the method of Beckman method for analysis of physiological fluids (Beckman system application notes October, 1985 cited by Romano *et al.*, (1990).

4- Histopathological examination: histopathological examination of brain samples were performed according to the method of Bancroft *et al.*, 1996

5- Statistical analysis: Data were compared across group using one way analysis of variance ANOVA followed by least significant difference (LSD) test at 5% and 1% within groups according to (Sendecor and Cochran, 1982).

### 3. Results

#### Effect on AchE activity:-

The present data revealed significant increase in the activity of AchE in brain homogenate of  $\text{AlCl}_3$  -treated rats all over the experimental period while in group of rats treated with  $\text{AlCl}_3$  and Vit. E, the present data showed significant decrease in the mean value of the elevated enzymatic activity of AchE in comparison with the control group (Table, 1).

#### Effect on brain lipid peroxidation:-

Results presented in table (1) indicated that in  $\text{AlCl}_3$ - treated rats the mean values of lipid peroxidation (malondialdehyde concentration) in brain tissue significantly increased from the 1<sup>st</sup> week of the experiment and lasted till the end while in the 2<sup>nd</sup> group that received Vit. E in association with  $\text{AlCl}_3$ , the mean values of lipid peroxidation showed no alteration in comparison with the control group.

Effect on antioxidant enzymes activities and nitric oxide content:-

Effect of  $AlCl_3$  on brain antioxidant enzyme activities of treated male rats was recorded in table (1). It was evident from this data that oral administration of  $AlCl_3$  significantly inhibited the activities of GST, GPX and GR with significant increase in NO content. Vit E in association with  $AlCl_3$  posses a protective effect against the effect of  $AlCl_3$  on the antioxidant enzymes status and nitric oxide content which represented by a significant improvement in the previous abnormal parameters.

Effect on amino acids concentrations in brain tissue:-

Results presented in table(2) showed that glutamic acid and aspartic acid concentration significantly increased from 1<sup>st</sup> week till the end of the experiment while the concentration of GABA, tyrosin and methionine significantly increased from 1<sup>st</sup> week till the end of the third week, while co-administration of  $AlCl_3$  and Vit E decreased the elevated amino acid concentration to levels that are nearly similar to control group (Table, 2).

Data in table (3 ) denoted that  $AlCl_3$  when administered to male rat in a dose level of 1600 mg/L for one month induced a significant increase in leucin concentration from the beginning till the end of the experiment while the increase in alanine concentration was observed from the beginning till the third period of the experiment. Effect of  $AlCl_3$  on valine, phenyl aniline and isoleucin concentrations in brain homogenates were evaluated in table 3. Concentrations of these amino acids significantly increased during 1<sup>st</sup> and 2<sup>nd</sup> week in Al-treated rats while cotreatment of Vit E with  $AlCl_3$  decreased the previous elevated concentrations.

Histopathological finding:-

Microscopical examination of brain in  $AlCl_3$  treated rats that administrated  $AlCl_3$  for 15 days revealed focal as well as diffuse gliosis in the cerebral cortex with sever dilatation and congestion in meningeal blood vessels (Fig. 1). The cerebral cortex of rat's brain of this group showed pericellular odema and congestion of cerebral blood vessels and capillaries ( Figs. 2,3). In group of rats administrated  $AlCl_3$  for one month, the histopathological examination showed sever and massive diffuse gliosis as well as sever pericellular odema in the cerebral tissue (Fig 4), encephalomyelacia with focal gliosis (Fig. 5) and neuronophagia with neuronal degeneration were detected in the brain matrix of rats in this group (Fig. 6).

Histopathological examination of the 2<sup>nd</sup> group that received Vit E in association with  $AlCl_3$  for 15 days showed mild diffuse gliosis in cerebral cortex (Fig. 7). Mild pericellular odema were noticed in cerebral tissue (Fig.8) in the brain of rat

administrated aluminum and protected by Vit E for one month.

#### 4. Discussion

The obtained data revealed that  $AlCl_3$  induced significant increase in AchE activity in brain tissue of treated male rates. This result was parallel to those recorded by (Bihagi *et al.*, 2009). They observed a significant increase in AchE activity in rat treated with  $AlCl_3$  (50 mg/kg) daily for three months. Also Kumar (1998) reported that AchE activity in different brain regions increased after 4 and 14 day from oral administration of  $AlCl_3$  at a dose of 320 mg/kg BW. This elevation in AchE activity in may be attributed to the direct neurotoxic effect of metal or perhaps a disarrangement of the cell membrane caused by increased lipid peroxidation as reported by Kaizer *et al.*( 2005). Lipid peroxidation is one of the main manifestations of oxidative damage and it has been found that LPO plays an important role in toxicity and carcinogenicity of many xenobiotics (Anane and creppy, 2001). Concerning the data obtained, our results revealed elevation of LPO in brain of Al-treated rats which were evidenced by the increased production of MDA . Similar result were mentioned by Nedzvetsky *et al.* (2007) who stated that Al induced significant increase in MDA concentration in hippocampus and frontal cortex of rats administered daily  $AlCl_3$  via drinking water for six weeks. Nearly similar finding to our data that was obtained by Jyoti *et al.* (2007) who demonstrated that administration of  $AlCl_3$  in dose level of ( 50 mg/kg/day) in drinking water for a month induced oxidative damage with accumulation of lipid damage peroxidation. Yang *et al.* (2006) reported that intraperitoneal injection of  $AlCl_3$  solution for 60 days at different dose can accelerate lipid peroxidation in rat's brain which may be one of the most important intoxication mechanisms. This elevation of MDA could be attributed to the ability of aluminum itself and its different salts to accelerate oxidative damage to biomolecules like lipids, protein and nucleic acids (Strong *et al.*, 1996). Also, Al is able to cross the blood brain barrier and is deposited in rat brain and increase LPO (Deloncle *et al.*, 1990). Therefore, the estimation of free radical generation and antioxidant defense has become an important aspect of investigation in mammals. The obtained data revealed significant inhibition in activities of GST, GPX and GR in brain tissue of Al- treated rats, these results were recorded in table1. Our findings were consistent with results of several investigators who revealed marked decrease in endogenous antioxidant after administration of different salts of Al (Katyal *et al.*, 1997 and Dua and Gill, 2001).

**Table (1): Acetyl choline esterase (AchE) activity malondialdehyde(MDA) concentration, enzyme activities of glutathione –s- transferase( GST) , glutathione peroxidase (GPX), and glutathione reductase (GR) and nitric oxide NO content in the brain of treated and control rat groups (mean ± SE).**

Time	Group	AchE (U/L)	MDA nmol / g)(	GST U / g)(	GPX (mU/ mL)	GR (U/ L)	NO ( $\mu$ mol / L)
1 <sup>st</sup> Week	AL	218.56 ± 2.37	47.64 ± 1.94	0.01175± 0.0007	0.2037 ± 0.0195	39.19 ± 0.31	22.81± 0.09
	AL+E	162.05 ± 0.3	23.46 ± 0.29	0.023± 0.0011	0.3132 ± 0.0027	55.62 ± 0.55	11.39± 0.19
	C	143.15 ± 0.77	24.67 ± 0.56	0.027± 0.0019	0.3265 ± 0.0027	86.29 ± 0.35	7.86 ± 0.42
2 <sup>nd</sup> Week	AL	239.85 ± 1.38	48.36 ± 0.11	0.0155± 0.0007	0.1622 ± 0.27	42.035 ± 0.42	30.45 ± 0.37
	AL+E	184.92 ± 0.71	31.34 ± 0.53	0.0295± 0.0015	0.332 ± 0.0032	61.99 ± 0.26	15.93 ± 0.50
	C	172.96 ± 0.67	35.94 ± 0.63	0.0305± 0.0019	0.3402 ± 0.0078	92.42 ± 0.74	8.93 ± 0.48
3 <sup>rd</sup> Week	AL	205.75 ± 1.45	44.68± 0.53	0.0645± 0.0242	0.1632 ± 0.0007	43.49 ± 0.97	23.27± 0.15
	AL+E	181.75 ± 0.34	32.41± 1.88	0.0787± 0.0213	0.3315 ± 0.0031	54.155 ± 0.45	12.35± 0.31
	C	160.72 ± 0.41	32.12± 0.47	0.0870 ± 0.0286	0.353± 0.0061	92.6 ± 0.46	9.06 ± 0.33
4 <sup>th</sup> Week	AL	232.8 ± 0.51	45.85± 1.01	0.0137± 0.0006	0.1622 ± 0.0003	44.61 ± 0.43	25.55± 0.19
	AL+E	176.11± 0.19	34.54± 0.54	0.0322± 0.002	0.3232 ± 0.0032	61.12 ± 0.25	14.05 ± 0.25
	C	164.83 ± 0.65	32.33 ± 0.40	0.033± 0.0009	0.347 ± 0.0089	90.72± 0.27	10.88 ± 0.18
LSD	LSD at 5%	20.46	6.43	0.007	0.0232	5.009	3.86
	LSD at 1%	29.40	9.25	0.009	0.0333	7.196	5.54

Data were expressed as mean and standard errors (means±SE)

AL: Aluminum

E: Vit E

C: control group

**Table (2): Mean values  $\pm$  SE of Glutamic acid, GABA, Tyrosine, Methionine and Aspartic acid amino acids concentrations in brain tissue of treated and control rat groups.(means  $\pm$ SE)**

Time	Groups	Glutamic acid	GABA	Tyrosine	Methionine	Aspartic acid
1 <sup>st</sup> week	AL	6.12 $\pm$ 0.72	5.57 $\pm$ 0.46	2.34 $\pm$ 0.21	1.90 $\pm$ 0.22	1.16 $\pm$ 0.06
	AL+E	1.57 $\pm$ 0.32	4.37 $\pm$ 0.42	0.96 $\pm$ 0.04	0.95 $\pm$ 0.06	0.57 $\pm$ 0.02
	C	0.62 $\pm$ 0.02	2.16 $\pm$ 0.22	0.119 $\pm$ 0.01	0.30 $\pm$ 0.02	0.15 $\pm$ 0.01
2 <sup>nd</sup> week	AL	2.66 $\pm$ 0.22	6.62 $\pm$ 0.69	3.55 $\pm$ 0.32	5.05 $\pm$ 0.23	1.33 $\pm$ 0.12
	AL+E	1.72 $\pm$ 0.34	4.89 $\pm$ 0.46	1.65 $\pm$ 0.113	3.03 $\pm$ 0.25	0.53 $\pm$ 0.02
	C	0.57 $\pm$ 0.02	1.68 $\pm$ 0.13	0.16 $\pm$ 0.01	0.28 $\pm$ 0.02	0.18 $\pm$ 0.04
3 <sup>rd</sup> Week	AL	2.34 $\pm$ 0.37	4.88 $\pm$ 0.37	1.15 $\pm$ 0.07	2.34 $\pm$ 0.25	0.79 $\pm$ 0.03
	AL+E	1.30 $\pm$ 0.13	2.92 $\pm$ 0.28	0.77 $\pm$ 0.03	1.60 $\pm$ 0.08	0.51 $\pm$ 0.02
	C	0.55 $\pm$ 0.03	1.56 $\pm$ 0.09	0.115 $\pm$ 0.01	0.34 $\pm$ 0.02	0.19 $\pm$ 0.02
4 <sup>th</sup> Week	AL	1.98 $\pm$ 0.08	2.20 $\pm$ 0.08	1.35 $\pm$ 0.08	1.25 $\pm$ 0.05	0.62 $\pm$ 0.02
	AL+E	0.97 $\pm$ 0.07	1.25 $\pm$ 0.07	0.72 $\pm$ 0.04	0.37 $\pm$ 0.03	0.42 $\pm$ 0.01
	C	0.52 $\pm$ 0.03	1.21 $\pm$ 0.08	0.134 $\pm$ 0.01	0.22 $\pm$ 0.01	0.16 $\pm$ 0.01
LSD	<b>LSD at 5%</b>	1.115	3.270	1.569	1.553	0.364
	LSD at 1%	2.712	4.609	2.555	1.944	0.534

Data were expressed as mean and standard errors (means $\pm$ SE)

AL: Aluminum

E: Vit E

C: control group

**Table (3): Mean values  $\pm$  SE of Leucine, Alanine, Valine, Phenyle alanine and Iso-leucine amino acids concentrations in brain tissue of treated and control rat groups.**

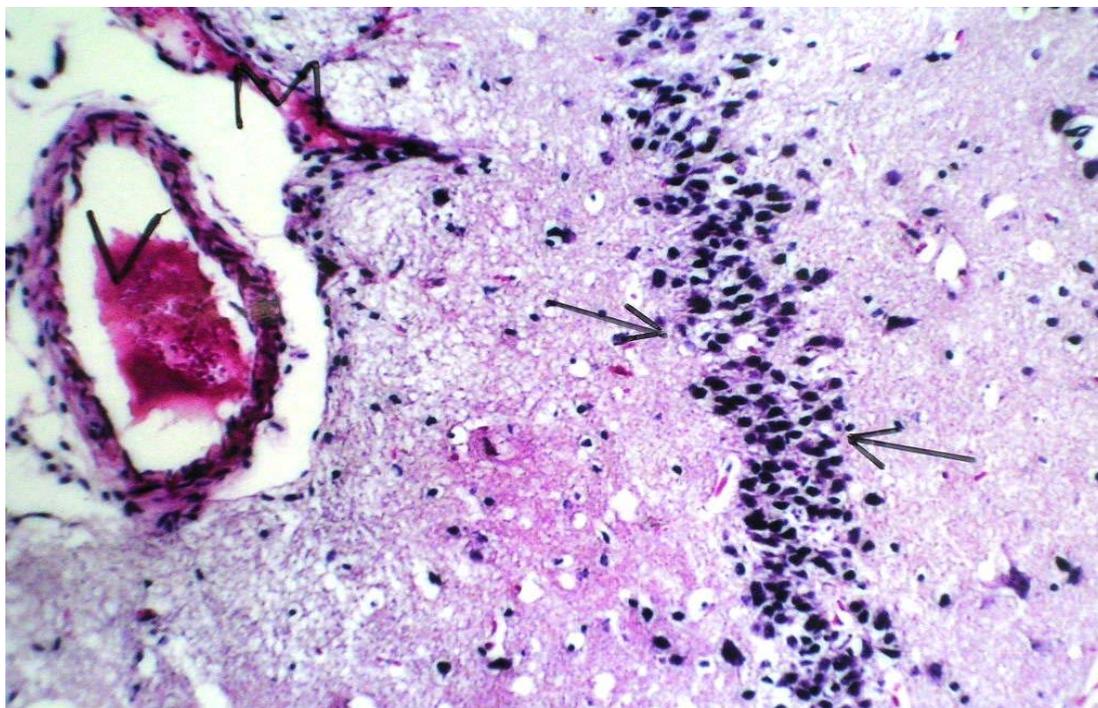
Time	groups	Leucine	Alanine	Valine	Phenyle alanine	Iso-leucine
1 <sup>st</sup> week	AL	9.63 $\pm$ 0.58	9.82 $\pm$ 1.28	6.12 $\pm$ 0.55	2.98 $\pm$ 0.26	4.34 $\pm$ 0.33
	AL+E	5.42 $\pm$ 0.38	4.70 $\pm$ 0.17	4.87 $\pm$ 0.26	1.18 $\pm$ 0.07	1.63 $\pm$ 0.06
	C	0.62 $\pm$ 0.02	1.25 $\pm$ 0.06	0.72 $\pm$ 0.05	0.118 $\pm$ 0.01	0.119 $\pm$ 0.01
2 <sup>nd</sup> Week	AL	11.25 $\pm$ 1.12	8.59 $\pm$ 0.56	3.52 $\pm$ 0.41	3.50 $\pm$ 0.37	5.56 $\pm$ 0.42
	AL+E	7.45 $\pm$ 0.62	5.98 $\pm$ 0.18	1.38 $\pm$ 0.07	1.87 $\pm$ 0.11	2.75 $\pm$ 0.18
	C	0.52 $\pm$ 0.02	1.21 $\pm$ 0.08	0.636 $\pm$ 0.02	0.117 $\pm$ 0.01	0.137 $\pm$ 0.02
3 <sup>rd</sup> Week	AL	8.16 $\pm$ 0.53	6.52 $\pm$ 0.71	1.86 $\pm$ 0.05	1.30 $\pm$ 0.05	2.27 $\pm$ 0.14
	AL+E	4.56 $\pm$ 0.42	3.45 $\pm$ 0.35	0.97 $\pm$ 0.03	0.74 $\pm$ 0.04	1.98 $\pm$ 0.07
	C	0.48 $\pm$ 0.03	1.23 $\pm$ 0.09	0.512 $\pm$ 0.02	0.16 $\pm$ 0.01	0.116 $\pm$ 0.01
4 <sup>th</sup> week	AL	4.52 $\pm$ 0.36	4.53 $\pm$ 0.13	0.93 $\pm$ 0.03	0.94 $\pm$ 0.04	2.22 $\pm$ 0.21
	AL+E	2.60 $\pm$ 0.14	1.97 $\pm$ 0.07	0.45 $\pm$ 0.02	0.24 $\pm$ 0.01	1.25 $\pm$ 0.09
	C	0.51 $\pm$ 0.03	0.98 $\pm$ 0.05	0.36 $\pm$ 0.01	0.15 $\pm$ 0.01	0.115 $\pm$ 0.01
LSD	LSD at 5%	2.758	4.125	2.347	1.374	2.941
	LSD at 1%	6.005	6.523	5.372	2.549	4.226

Data were expressed as mean and standard errors (means $\pm$ SE)

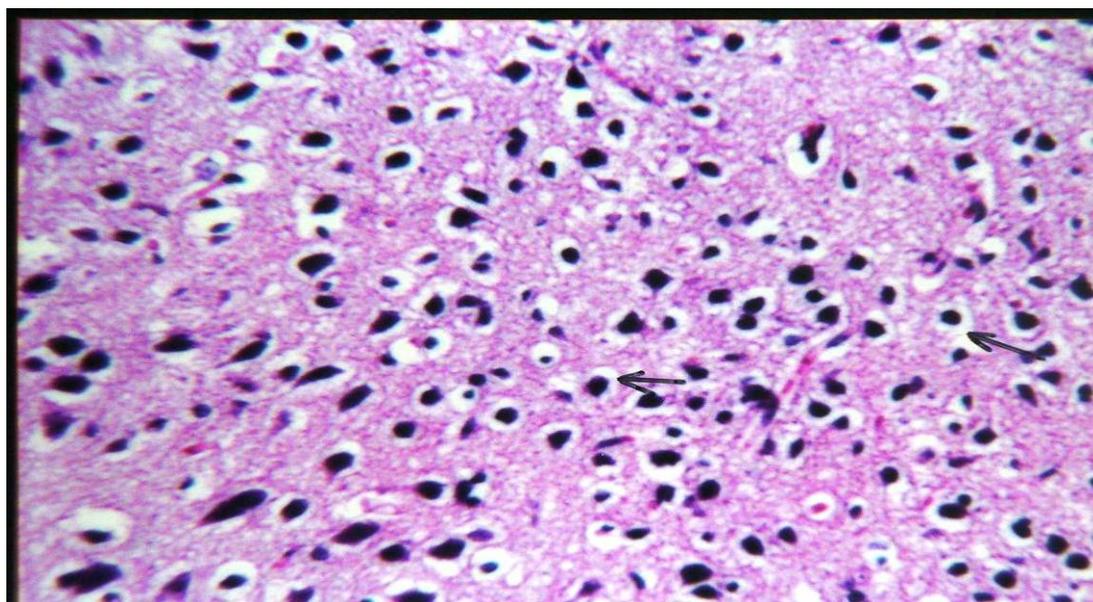
AL: Aluminum

E: Vit E

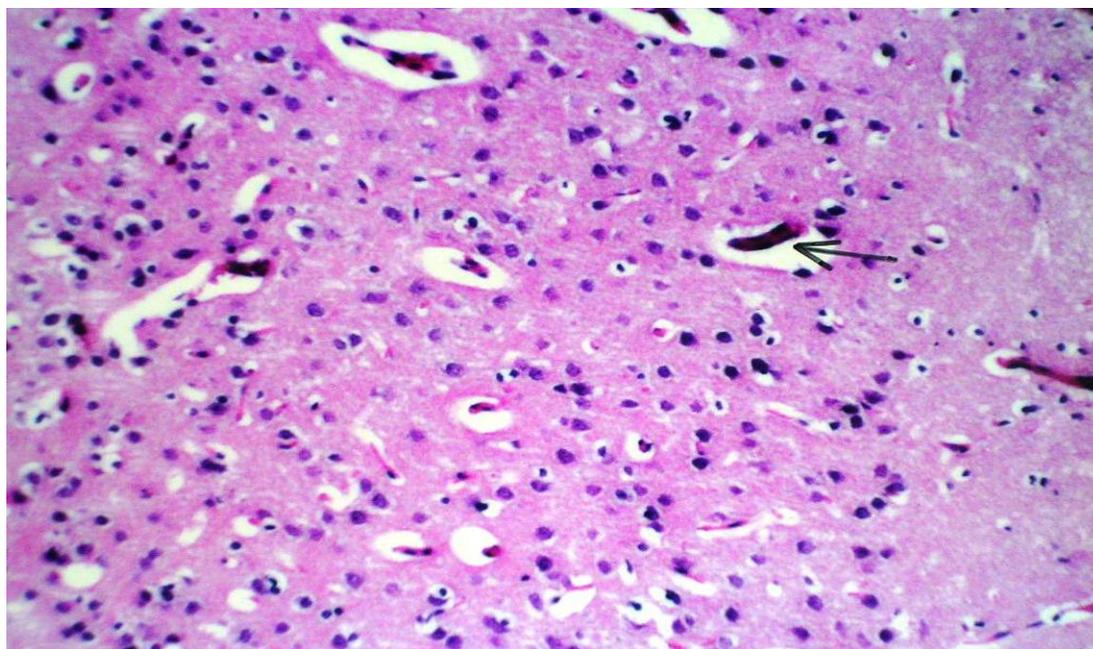
C: control group



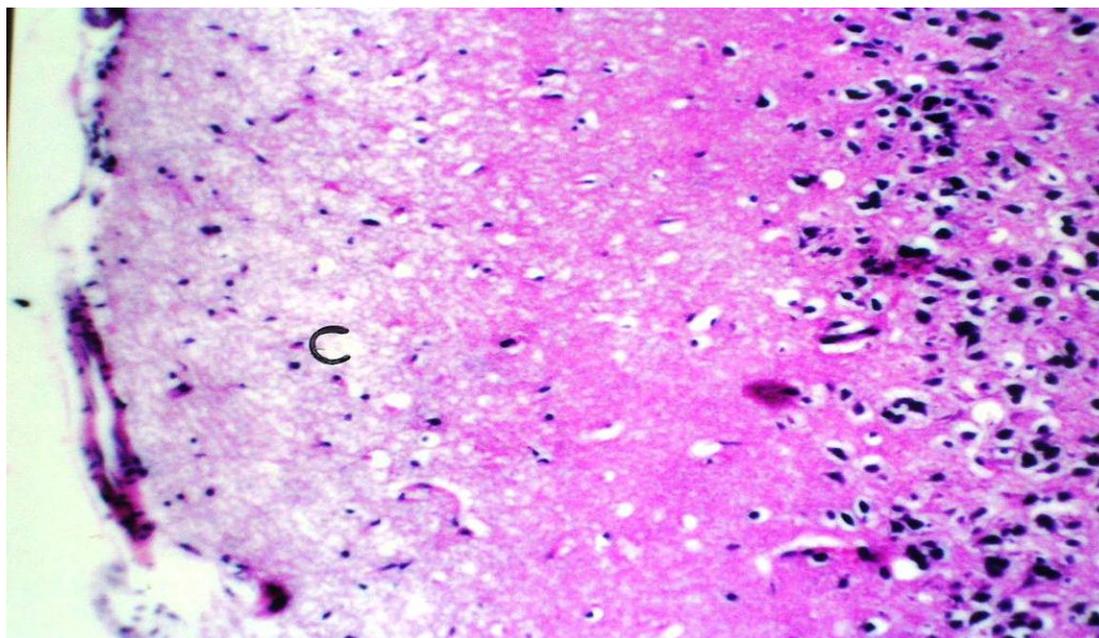
**Fig (1):- Brain of rat administrated aluminum for 15 days showing focal as well as diffuse gliosis (arrow)in cerebral cortex with sever dilatation and congestion in meningeal blood (v)H&E X 40**



**Fig. (2):- Brain of rat administrated aluminum for 15 days showing Pericellular oedema in cerebral cortex (arrow) H&E X 64.**



**Fig. (3):- Brain of rat administrated aluminum for 15 days showing sever congestion in the blood vessels and capillaries of cerebral cortex. H&E X40**



**Fig. (4):- Brain of rat administrated aluminum for one month showing sever and massive diffuse gliosis as well as sever pericellular oedema in the cerebral tissue H&E X 4**

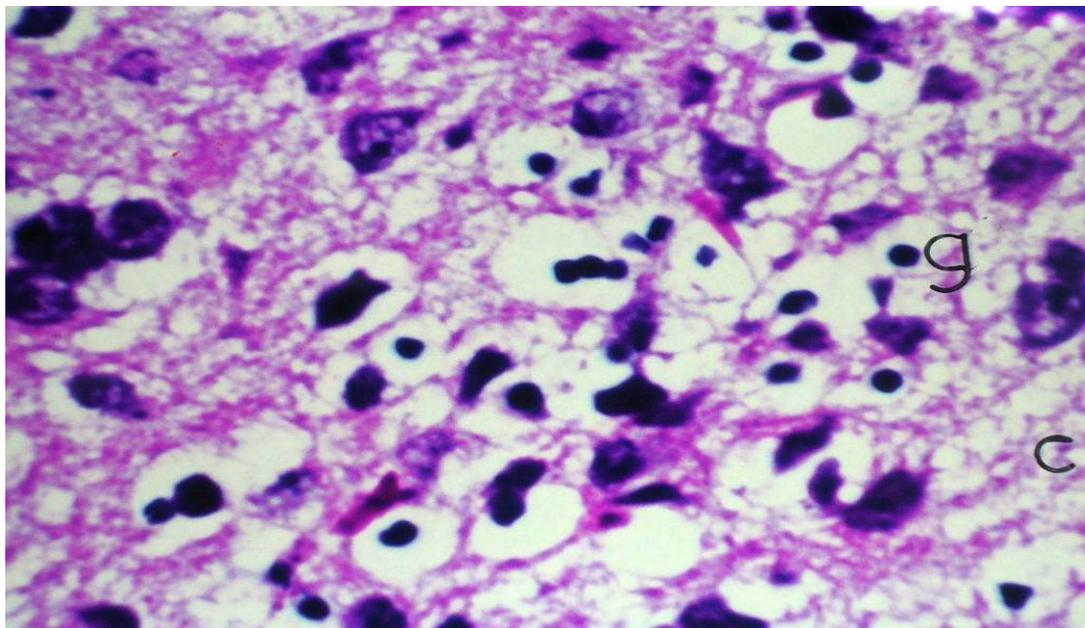


Fig. (5):- Brain of rat administrated aluminum for one month showing encephalomyelacia o brain matrix ( C ) with gliosis (g) H&E X 160.

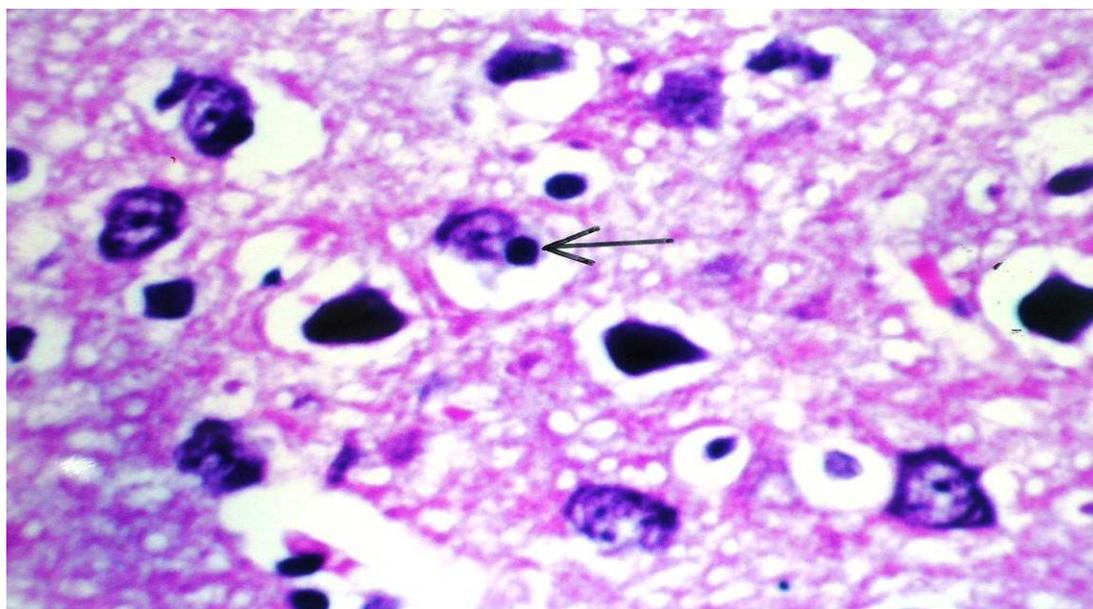


Fig. (6):- Brain of rat administrated aluminum for one month showing neurophagia (arrow) with degeneration of neuronal cells. H&E X 160.

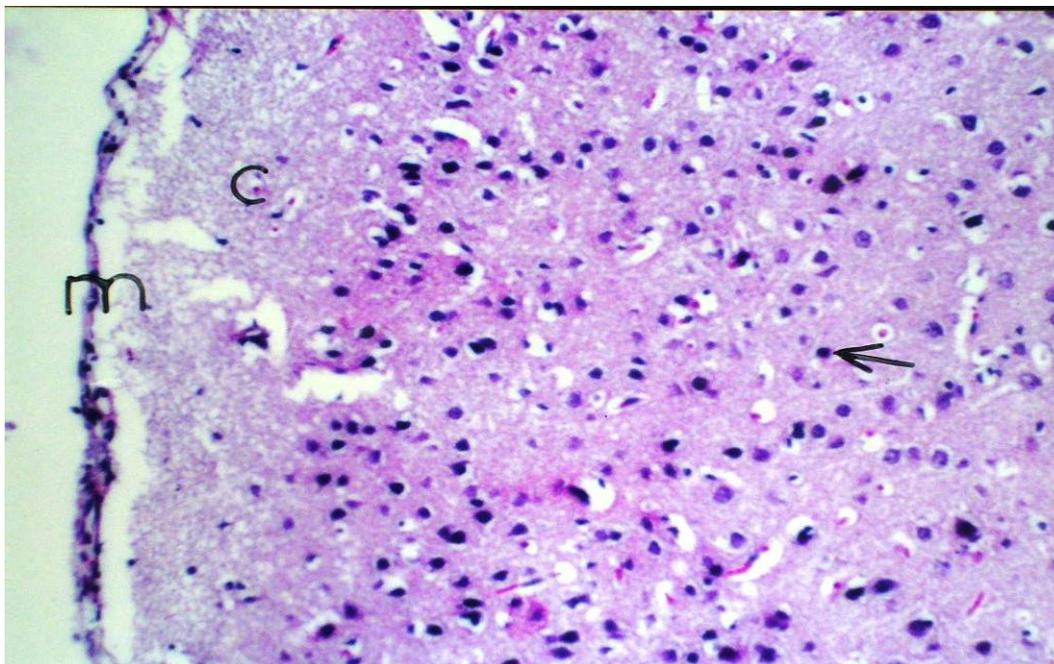


Fig. (7):- Brain of rat administrated aluminum and vitamin E for 15 days showing mild diffuse gliosis in cerebral cortex (C). H&E X 40.

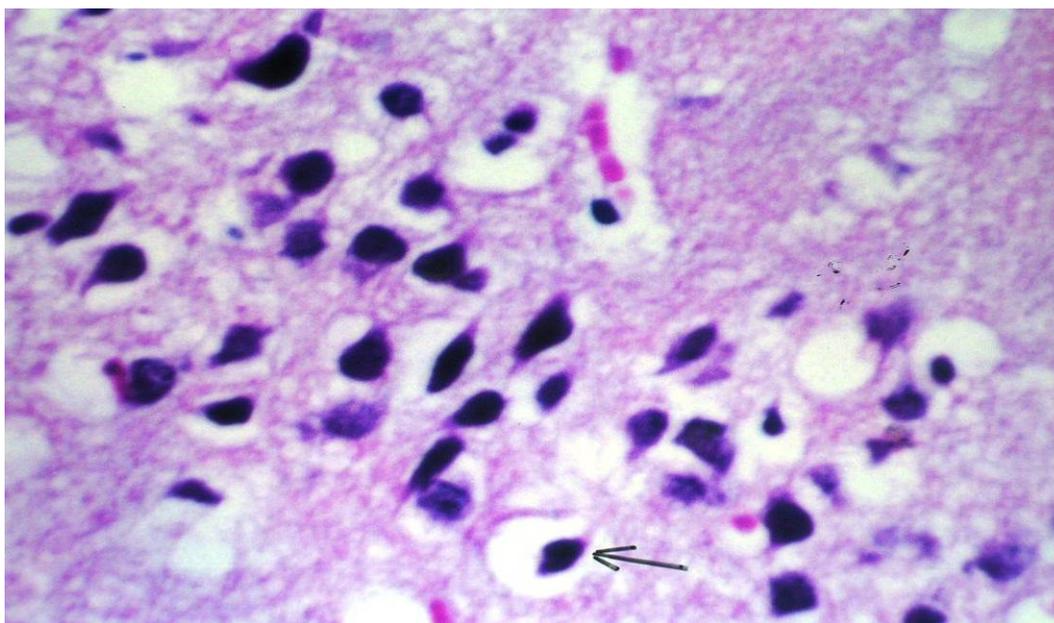


Fig. (8):- Brain of the rat administrated aluminum and vitamin E for one month showing mild precellular oedema (arrow). H&E X 64.

El-Demerdash (2004) reported that administration of 34 mg  $\text{AlCl}_3$  /kg Bw significantly ( $p < 0.05$ ) induced free radicals (TBARS) and decreased the activity of glutamate-S-transferase. Also Mokhtar (2004) mentioned that administration of  $\text{AlCl}_3$  34mg/kg Bw (1/25 LD 50) to male rabbit every other day for 16 weeks induced decline in the activity of GST and level of sulfhydryl group (SH) in brain, liver, plasma, kidney and testes. Bihagi *et al.* (2009) observed a significant decrease in the enzymatic activities of antioxidant enzymes like glutathione peroxidase and glutathione S transferase during treatment with 50 mg/kg BW  $\text{AlCl}_3$  daily for 3 months. Newairy *et al.* (2009) revealed that the level of TBARS was increased and the activities of GST, super oxide dismutase (SOD), catalase and GPX were decreased in liver, kidney and brain of rat treated with 34 mg/kg BW  $\text{AlCl}_3$  daily for 70 days. Furthermore, Dua and Gill (2001) reported that the exposure to Al phosphide resulted in significant increase in neuronal lipoperoxidation damage with concomitant alteration in the antioxidant defence status thus having serious bearing of the functional and structural status of CNS. The reduction in activities of GST, GPX and GR may be attributed to the elevated level of protein and lipid oxidative products due to oxidative damage effect of Al on biomolecules. This inhibition of these enzymes activities also may be referred to the effect of Al in declining the expression of mRNA of endogenous antioxidants (Gonzalez *et al.*, 2007).

The obtained data revealed a significant increase in nitric oxide content in  $\text{AlCl}_3$ - treated group parallel to our results those recorded by Stevanovic *et al.* (2009) who reported that intrahippocampal injections of  $\text{AlCl}_3$  in wistar rat induced significant increase in nitric oxide and malondialdehyde concentrations and reduced the glutathione contents at 3hrs. and 30 days after treatment. Also Satoh *et al.* (2007) recorded that treatment of PC12 cells with 150 microM aluminum-maltol complex for 48 hrs induced significant increase in nitric oxide generation which was involved in the onset mechanism of aluminum-maltol induced cell death. This elevation in nitric oxide could be attributed to the ability of aluminum to generate reactive oxygen species and free radicals (Parakash and Rao, 1996). One of the multiple pathways to increase free radicals mediated neurotoxicity is the formation of peroxynitrite by reaction of nitric oxide and superoxide radical (Yao *et al.*, 2004).

The statistical analysis of the present results revealed a significant increase in concentration of glutamic acid, GABA, tyrosin, methionine and aspartic acid. Also, a significant increase of leucine,

alanine, valine, phenyl alanine and isoleucine which were recorded in table (3). In this aspect, our results were correlated with those mentioned by Nayak and Chatterjee (2001) who mentioned that intraperitoneal injection of  $\text{AlCl}_3$  and hexahydrates by for 4 week induced significant increase in brain glutamic acid and GABA with other alteration in enzymes of glutamate-GABA system. One of the contributory factors for this increase in glutamic acid level may be the Al- induced inhibition of glutamate transport by synaptic vesicles (Wong *et al.*, 1981) or inhibition of glutamate release by aluminum (Provan and Yokel, 1992). The elevation of GABA concentration may be attributed to the increased level of glutamate which undergo decarboxylation by action of glutamic acid decarboxylase enzyme (GAD) to produce GABA, where GAD is the sole enzyme responsible for decarboxylation of glutamate. Also, Al induced modification of the enzyme activities may be either due to the direct impact of aluminum or due to aluminum-induced changes in the cellular environment and the differential regional accumulation of glutamate or other alteration in enzymes of the glutamate-GABA system which were induced by aluminum and may be considered as one of the causes of aluminum induced neurotoxicity (Nayak and chatterjee, 2001).

Jabaudon *et al.* (2000) revealed that when extracellular glutamate concentration increase, it become excitotoxic and reaching levels that activate N-methyl-D-aspartate type of glutamate receptor (NMDAR) thereby causing neuronal death. From the present study, it is clear that glutamate itself and its metabolizing enzymes as well as its metabolites are prone to Al-induced alteration. The role of glutamate in Al- induced neuronal degeneration may be multifaceted. Elevated glutamate can directly produce excitotoxicity in neuronal cell or affect cells through its metabolites. The present data indicated a significant increase in aspartic acid in  $\text{AlCl}_3$ -treated group. This result is in the harmony with those of Jia *et al.* (2001) who studied the effect of long term exposure to aluminum on amino acid transmitters in hippocampus of rats, and he found that the contents of aspartate and glutamine were significantly increased at the dose of 111.9 mg  $\text{AlCl}_3$ / kg BW.

The obtained data revealed significant increase in tyrosine, methionine, leucine, alanine, valine, phenylealanine and isoleucine. Our results are in agreement with high cumulative concentrations of aluminum absorbed over a lifetime result in increased amounts of proline, lysine, arginine and other amino acids in brain cells, leading to an alteration in neuronal excitability, resulting in the erratic and convulsive effects seen in aluminum neurotoxicity (Wenk and Stemmer, 1981,1982). The altered content

of amino acid neurotransmitters might be one of the important mechanisms of aluminum neurotoxicity.

Exposure to Al may cause marked histopathological alterations in the brain tissue which were represented by focal as well as diffuse gliosis in cerebral cortex, edema and inflammatory cell infiltration and pericellular edema in cerebral cortex, encephalomyeliasis with neuronal degeneration. Parallel to our findings, those recorded by Bihagi *et al.* (2009) who found that AlCl<sub>3</sub> causes histopathological lesions in cerebral cortex including neuronal degeneration as cytoplasmic vacuolization hemorrhage, ghost cell and gliosis. Our histopathological findings are correlated to those of Matyja (2000) who noticed that exposure to aluminum causes marked histopathological alteration in the cerebral cortex including neuronal degeneration, pericellular edema and gliosis.

Antioxidants are known to reduce oxidative radical induced reaction.  $\alpha$  tocopherol is an important antioxidant in biological systems. It inhibits peroxidation of membrane lipid by scavenging lipid peroxyl radical with formation of tocopheroxyl radical as a consequence (Arita *et al.*, 1998).

The present data showed that the presence of vit E with Al alleviated its harmful effect on all above measured parameters and their levels become near to the normal values of control. These results are in good accordance with those obtained by Ithayarasi and Devi (1997) and El-Demerdash (2001) who found that  $\alpha$ -tocopherol maintained the levels of antioxidant membrane-bound enzyme and the activities of antioxidant enzymes near the normal value thus emphasizing their effect as antioxidant. Chinoy and Memon (2001) demonstrated that Vit E has a very significant bearing on the amelioration of Al toxicity in human. Parallel to our result, those mentioned by El-Demerdash (2004) who stated that Vit E or selenium in combination with Al significantly decreased level of free radicals and increased the activity of GST and SH. The protective effect of Vit E against the neurotoxic effect of AlCl<sub>3</sub> may be attributed to the ability of Vit E to appear as the first line of defense against peroxidation of polyunsaturated fatty acid contained in cellular and subcellular membrane phospholipids. Tocopherol act as antioxidant breaking free radical chain reaction as a result of their ability to transfer a phenolic hydrogen to peroxyl free radical of peroxidized polyunsaturated fatty acid (Murry *et al.*, 2000). Also Vit E ameliorates glial activation and reduces the release of proinflammatory cytokines induced by aluminum (Nedzvetsky *et al.*, 2007). Vit E counteracts aluminum harmful effects not only by preventing free radical formation but also by favoring Al disposal (Gonzalez *et al.*, 2007). Also,

histopathological examination of rat's brain in AlCl<sub>3</sub> and Vit E -treated group showed only mild gliosis and mild pericellular edema in cerebral cortex that confirm the marked protective effect of Vit E against the toxic effect of AL

In conclusion the present study demonstrated that Vit E in combination with aluminum minimized its harmful effect. Vit E proved to be effective in decreasing level of free radicals and increasing the activity of antioxidant enzymes with improvement of brain amino acids concentration to be close to normal values. Consequently, efforts must be done to reduce the exposure to aluminum as well as using diet rich in Vit E may be beneficial in alleviating aluminum toxicity.

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