Research Article

Preliminary neuroprotective activity of aerial parts of *Enicostemma littorale* Blume extract in albino rats

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Received: 16 September 2017 Revised: 9 October 2017 Accepted: 17 October 2017

Abstract

Objective: The main aim of this research paper is to evaluate the neuroprotective activity of *Enicostemma littorale* Blume of 70% ethanolic extract against Monosodium Glutamate (MSG) and Aluminium fluoride (AlF_3) treated albino rats. **Materials and methods:** The *Enicostemma Littorale,* extracted from Soxhlet apparatus unit, by using 70% ethanol and evaluated, in the course of free radical generation and excitotoxicity in Monosodium Glutamate (MSG) and Aluminium fluoride (AlF_3) treated albino rats. **Results and conclusion:** The extract showed significant improvement in body weight, total protein, Catalase, super oxide dismutase, and lipid peroxide levels. The general behavior, locomotor activity, and region of the hippocampus were significantly protected by organ protective properties of ethanolic extract of *Enicostemma Littorale* Blume attribute to its antioxidant and behavioral properties.

Keywords: Neuroprotective, *Enicostemma littorale* Blume, Monosodium glutamate, Aluminium fluoride.

Introduction

Neurodegenerative disorders are a heterogeneous group of diseases of the nervous system, including the brain, spinal cord, and peripheral nerves that have many different etiologies (Ravindra et al., 2004). Acute and chronic neurodegenerative diseases are illnesses, associated with high morbidity and mortality and few or no effective options are available for their treatment. A characteristic of many neurodegenerative diseases which include stroke, brain trauma, spinal cord injury, amyotrophic lateral sclerosis, Huntington's disease, Alzheimer's disease, and Parkinson's disease is neuronal-cell death (Robert, 2003). Neuropathlogically, these are characterized by abnormalities of relatively specific regions of the brain and specific populations of neurons. *Enicostemma littorale* Blume a glorious perennial herb, belonging to the family Gentianceae. Upon literature survey, it was found that the hot extract it is being used for the treatment of diabetes, fever, stomach ache, dyspepsia, and malaria. It is also reported to possess antitumor (Dash, 2000), antiarthritic, hypoglycemic, and antimalarial activities (Katewa and Arora, 2001). There are reports that the plant possesses flavonoids, xanthenes, and so on in the aerial parts of this plant (Ghosal and Jaiswal, 1980). The flavonoids are known to have antioxidant, antiulcer, and anti-inflammatory properties. However, there are no reports on the Neuroprotective activity of the herb. As this herb is reported to contain alkaloids, catechins, saponins, sterols, triterpenoids, phenolic acids, flavonoids, and xanthones other related compounds. The hydro alcoholic extract of this herb was investigated for Neuroprotective activity by using various experimental models in albino rats

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Materials and methods

Collection of plant Material and Preparation of extract

The herb of *Enicostemma littorale* Blume was collected from the Kanavihalli, near to Harapanahalli, Karnataka, India from June to August 2012, at the end of the flowering season. The herb was identified and authenticated by Prof .K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. A herbarium specimen No. SCSCOP.Ph.Col Herb.No.005/2011-2012. The dried powder of aerial parts were defatted with Petroleum Ether,

Chloroform, and then extracted with 70% ethanol using Soxhlet apparatus. The extracts were concentrated under reduced pressure, using rota flash evaporator and stored in airtight container in a refrigerator below at 10ºC.

Experimental animals and housing conditions and ethical approval

Albino Wistar rats weighing 150-250g were procured from Sri Venkateshwara Enterprises, Bengaluru. The animals were kept in quarantine section till monitoring of health status and subsequently transferred to the housing area. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages with dust free rice husk as a bedding material and standard laboratory conditions of temperature (23 ± 2°C), humidity (40 ± 10%) and 12:12 h dark/light cycles. They were fed with standard rodent feed, (Gold Mohur Lipton India Ltd.) and water *ad labium* was provided. The husk in the cages was renewed thrice a week, to ensure hygienity and maximum comfort for animals. Ethical clearances for handling the animals was obtained from the Institutional animal ethics committee (IAEC), prior to the beginning of the project work and the registration no. SCSCP/583/5/2011-12 and dated was 26.11.2011 and care of laboratory animals was done as per CPCSEA guidelines, Ministry of Forests & Environment and Government of India.

Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out on Petroleum ether, Chloroform, 70% Ethanolic, and Aqueous extract for qualitative identification of type of phytoconstituents present (Kokate, 1999; Khandelwal, 2000).

Induction of Neurotoxicity

Neurotoxicity was induced in wistar rats by intraperitoneal injection of freshly prepared Monosodium glutamate (MSG) 2 g/kg and Aluminium fluoride (AlF_3) 600 ppm in disease control, treatment and standard(Ascorbic acid) group animals as per experimental design.

Experimental design

Monosodium glutamate - induced Neurotoxicity
(Ramanathan et al., 2007)

Overnight fasted rats were randomly divided into five groups of six rats (n=6) each as follows and treated for 7 days as follows;

Group 1: Received normal saline (i.p.) + vehicle (p.o.).

Group 2: Received MSG 2 g/kg (i.p.) + normal saline (p.o.).

Group 3: Received MSG 2 g/kg (i.p.) + 70% EEEL 250mg/kg (p.o)

Group 4: Received MSG 2 g/kg (i.p.) + 70% EEEL 500mg/kg (p.o)

Group 5: Received MSG 2g/kg (i.p.) + ascorbic acid 100 mg/kg, (i.p.) (reference Std.)

Aluminum fluoride - induced Neurotoxicity
(Chandrashekhar et al., 2010)

The animals were divided into five groups of six rats (n=6) each as follows;

Group 1: Received normal saline (i.p.) + vehicle (p.o.).

Group 2: Received (AlF_3) 600ppm + normal saline (p.o.).

Group 3: Received (AlF_3) 600 ppm + 70% EEEL 250mg/kg (p.o).

Group 4: Received (AlF_3) 600 ppm + 70% EEEL 500mg/kg (p.o).

Group 5: Received (AlF_3) 600ppm + ascorbic acid 100 mg/kg, (i.p.) (reference Std.).

Results

Effect of 70% ethanolic extract on MSG-induced neurotoxicity

There was a marked change in the body weight in MSG-treated groups. Ethanolic extract showed a dose-dependent increase in the body weight of rats. Ethanolic extract at 500mg/kg dose increased the body weight by 66.11%. Motor coordination and body balance were significantly affected in MSG administered rats when compared to normal control rats. Normal control rats showed the fall of time in 60.89 ±2.376s; whereas MSG alone treated rats showed the fall of time in 22.46 ± 1.771s. Pretreatment with 70% Ethanolic extract (250 and 500 mg/kg), significantly improved the motor coordination and body balance in MSG-treated rats (29.52±1.966 and 40.68 ± 2.446) respectively. It was further confirmed by histopathological observations.

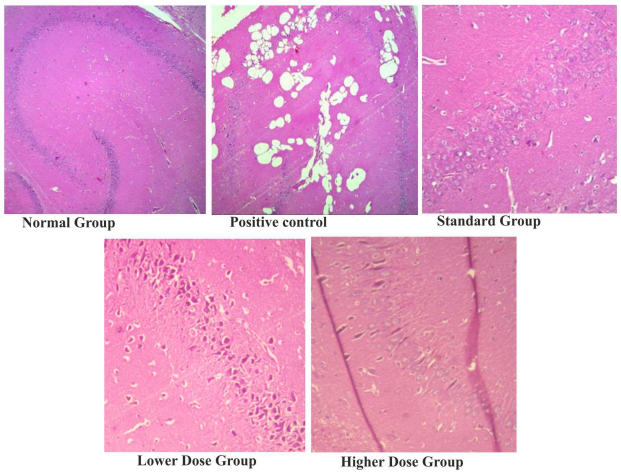

Figure 1A. Shows the Histopathological Changes of different groups in MSG-induced rats.

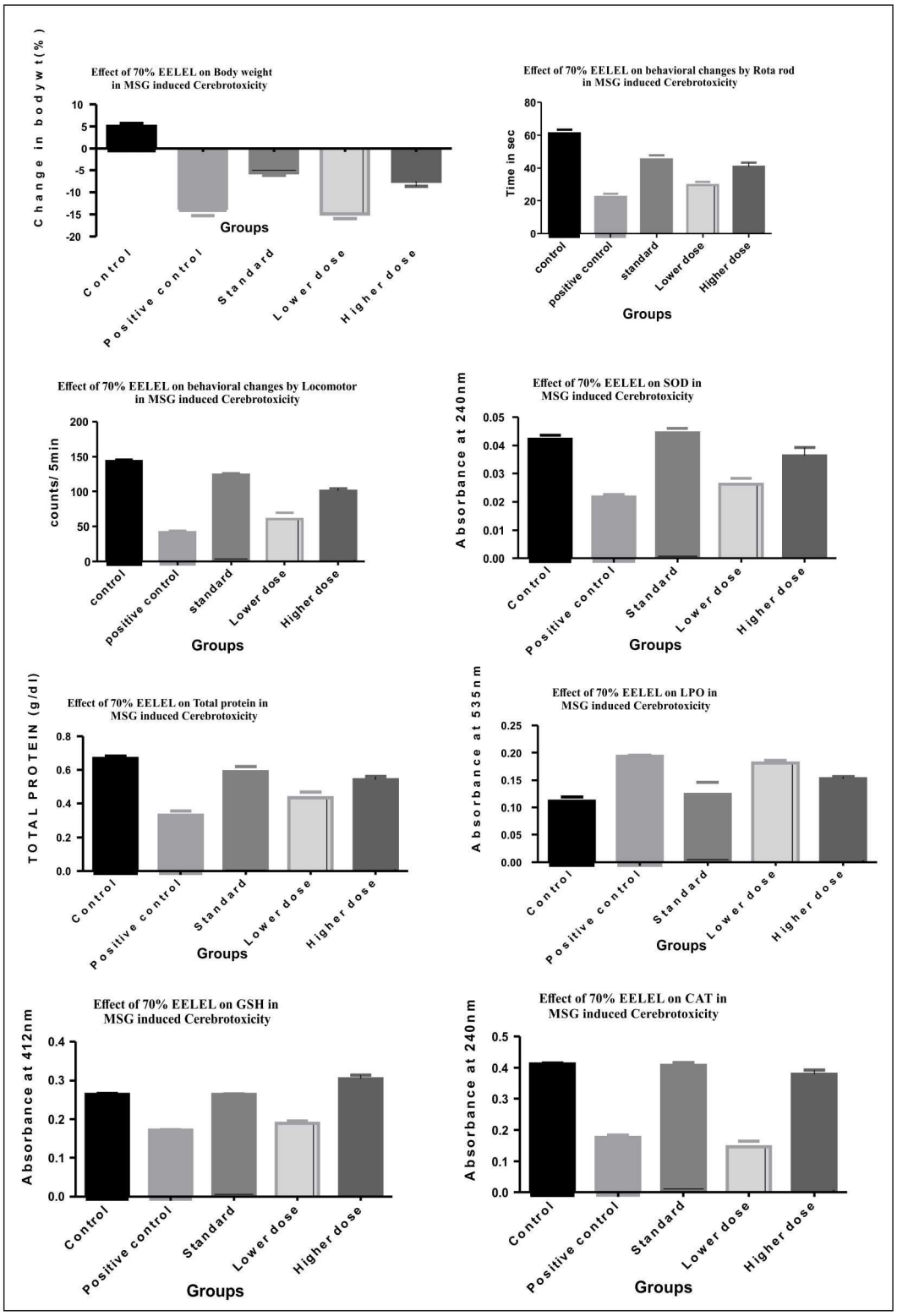

Figure 1B. Shows the Bar graphs of different groups in MSG-induced rats

Effect of 70% EEEL on Aluminium Fluoride (AlF_3) – induced Neurotoxicity:

There was also a marked change in body weight in AlF_3 treated groups. Ethanolic extract showed a dose-dependent increase in the body weight of animals. Ethanolic extract at 500 mg/kg dose, increased the body weight by 56.01%. Motor coordination and body balance were significantly affected in AlF_3 administered rats when compared to normal control rats. Normal control animals showed the fall of time in 58.58±6.628s; whereas AlF_3 alone treated rats showed the fall of time in 21.43±2.452s. Pretreatment with Vitamin-C and 70% (250 and 500 mg/kg) significantly improved the motor coordination and body balance in AlF_3 treated rat

Table 1. Effect of 70% Ethanolic Extract on Biochemical parameters, Body weight and Behavioral characters in MSG-treated rats

Treatment	GSH		LPO		SOD		CAT		TP		% Body weight change	Rota Rod Test (s)	Locomotor Activity (Count/5 min)
	Mean ± SEM	% increase	Mean ± SEM	% inhibition	Mean ± SEM	% increase	Mean ± SEM	% increase	Mean ± SEM	% increase			
Negative control	0.263± 0.002	----	0.111± 0.007	-----	0.042± 0.001	-----	0.411± 0.003	-----	0.666± 0.016	-----	------	60.89± 2.376	143.9 ± 2.300
Positive control	0.171± 0.001	-----	0.194± 0.001	-----	0.021± 0.008	-----	0.175± 0.007	-----	0.332± 0.023	-----	13.04	22.46 ± 1.771	41.37 ± 2.384
Standard (Ascorbic acid)	0.263± 0.001***	53.80	0.124± 0.001***	36.08	0.040± 0.001***	90.47	0.400± 0.009***	91.50	0.603± 0.031***	81.62	04.80	45.11± 2.683***	124.5 ± 1.743***
70% EEEL (250mg/kg)	0.220± 0.007*	31.81	0.145± 0.004*	21.94	0.030± 0.002*	58.80	0.295± 0.019*	42.42	0.435± 0.033*	31.02	12.18	29.52± 1.966*	60.71± 8.880*
70% EEEL (500mg/kg)	0.250± 0.010**	46.19	0.132± 0.003**	31.95	0.036± 0.003***	71.42	0.381± 0.013***	87.45	0.540± 0.021**	62.65	08.24	40.68 ± 2.446***	100.9 ± 3.653***

Each value is expressed as mean ± SEM (n = 6), where, NS represents non significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when compared to MSG alone treated rats. One-way ANOVA followed by Dunnett's comparison test. *GSH- Glutathione, LPO- Lipid peroxides, SOD- Superoxide dismutase, CAT- Catalase, TP- Total Protein.

Table2: Effect of 70% Ethanolic Extract on Biochemical parameters, Body weight change and Behavioral characters in AlF_3 treated

Treatment	GSH		LPO		SOD		CAT		TP		% Body weight change	Rota Rod Test (s)	Locomotor Activity (Count/5 min)
	Mean ± SEM	% increase	Mean ± SEM	% inhibition	Mean ± SEM	% increase	Mean ± SEM	% increase	Mean ± SEM	% increase			
Negative control	0.263± 0.002	----	0.111± 0.007	-----	0.042± 0.001	-----	0.411± 0.003***	-----	0.666± 0.016	-----	------	60.89± 2.376	143.9± 2.300
Positive control	0.171± 0.001	-----	0.194± 0.001	-----	0.021± 0.008	-----	0.175± 0.007	-----	0.332± 0.023	-----	13.04	22.46 ± 1.771	41.37 ± 2.384
Standard (Ascorbic acid)	0.263± 0.001***	53.80	0.124± 0.001***	36.08	0.040± 0.001***	90.47	0.400± 0.009***	91.50	0.603± 0.031***	81.62	04.80	45.11± 2.683***	124.5± 1.743***
70% EEEL (250mg/kg)	0.222± 0.005*	29.81	0.153± 0.004*	22.94	0.029± 0.002	27.80	0.279± 0.019 *	36.42	0.435± 0.033*	31.02	12.18	29.52± 1.966*	60.71± 8.880*
70% EEEL (500mg/kg)	0.250± 0.010**	46.19	0.132± 0.003**	31.95	0.036± 0.003***	71.42	0.381± 0.013***	87.45	0.540± 0.021**	62.65	08.24	40.68 ± 2.446***	100.9 ± 3.653***

Each value is expressed as mean ± SEM (n = 6), where, NS represents non significant; ***P<0.001 – highly significant; **P<0.01- very significant; *P<0.05- significant, when compared to MSG alone treated rats. One-way ANOVA followed by Dunnett's comparison test. *GSH- Glutathione, LPO- Lipid peroxides, SOD- Superoxide dismutase, CAT- Catalase, TP- Total Protein.

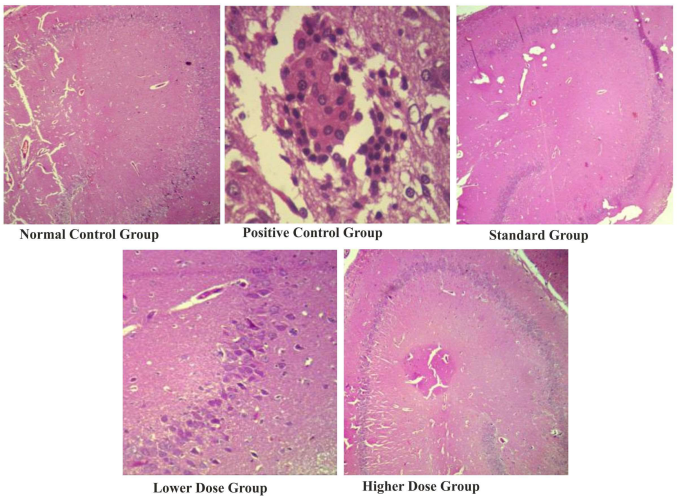

Figure 2A: Shows the Histopathological Changes of different groups in AlF_3-induced rats.

48.50±2.022, 29.34±2.239 and 42.78±1.997s) respectively. It was further confirmed by histopathological observations. There was a dose dependent inhibition of *in-vivo* LPO and increase in GSH, CAT and SOD in both the models. This was statically significant when compared to the positive control group.

Discussion

Treatment with glutamate, as monosodium glutamate (MSG) - induced severe neurochemical damage and neurotoxic effects on any brain region (Johnston et al., 1998; Ortwo et al., 1997, Orti et al., 2006). Various investigators have previously demonstrated, some of the neurotoxicological signs induced by monosodium glutamate intake. These neurotoxicological signs are

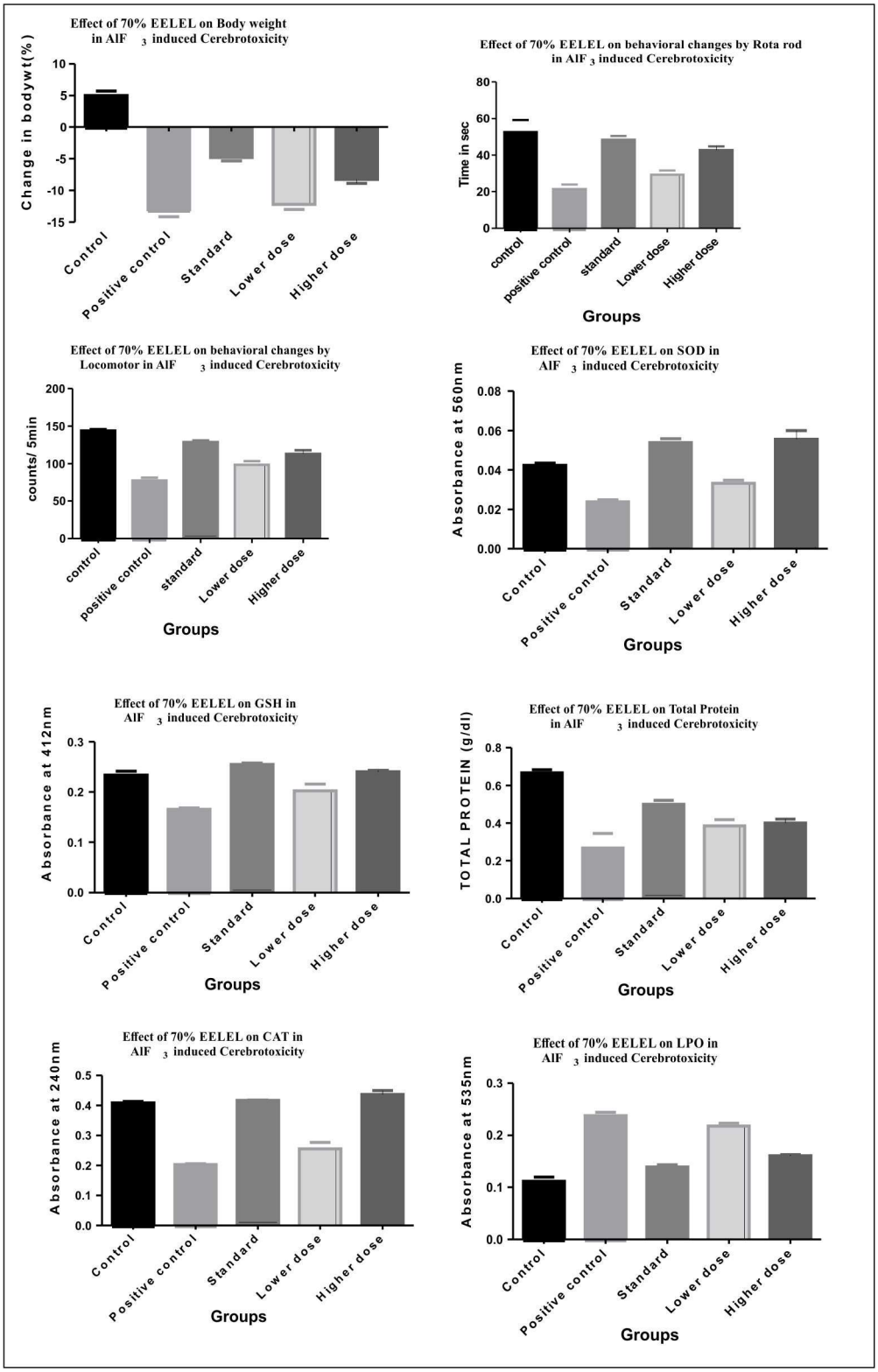

Figure 2B: Show the Bar graphs of different groups in AlF_3-induced rats.

observed, after the administration of monosodium glutamate (2g/kg, i.p. for 7 days) in mouse and rats, including hypoxia-ischemia, hepatotoxicity, musculoskeletal pain and metabolic failures (Pavlovic et al., 2007). Excessive accumulating of glutamate in the synaptic cleft has been associated with excitotoxicity and glutamate is implicated in a number of neurological disorders (Mallick, 2007). However, there is accumulating evidence suggesting that glutamate-induced toxicity can be mediated through necrosis and apoptosis (Ankarcrona et al., 1995; Martin et al., 2012). One week administration of MSG in rats depleted the GSH, CAT and SOD levels and increased the LPO. The animals also

exhibited aggressive behavior and in some animals fighting behavior was also observed.

The animals treated with AlF_3(600ppm p.o for 7days) in drinking water, revealed altered behavioral changes and causes oxidative damage to the brain was estimated by measuring LPO, SOD, CAT, GSH and total protein levels. The histopathological studies were performed in the striatal region of the brain (Thirunavukkarasu et al., 2012). A significant decline in the enzymatic activity like GSH, SOD, CAT and TP levels during monosodium glutamate and aluminum treatment, whereas LPO levels showed a significant increase, as compared to normal control. Synchronous administration of during monosodium glutamate and aluminum treatment reversed the enzyme levels to the normal. In the present study, it was observed that the plant possesses alkaloids, flavonoids and tannin and these constituents are reported to have organ protective properties. Hence, the organ protective properties may be attributed to the alkaloids, flavonoids and tannins constituents that are present in the test extract.

Conclusion

The 70% ethanolic extract of *Enicostemma littorale* Blume has a cerebroprotective activity against monosodium glutamate and aluminium fluoride induced cerebrotoxicity in Albino rats.

Acknowledgment

The authors would like to thank the Management, Principal, SCS College of Pharmacy, Harapanahalli for making available the infrastructure required for the study.

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