

**Research Article****Assessment of cerebroprotective activity of *Sonchus asper* (L) Hill on induced cerebrototoxicity in experimental rats****Rajashekar S Chavan<sup>1</sup>, Vinuth Chikkamath<sup>2</sup>, Shanmukha I<sup>2\*</sup>**<sup>1</sup>PG Dept. of Pharmacology, KLE's College of Pharmacy, Belgaum, Karnataka, India.<sup>2</sup>PG Dept. of Pharmacology, S.C.S College of Pharmacy, Harapanahalli, Davanagere 583131 Karnataka, India.

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**Abstract**

**Objective:** This study was aimed to determine the quantity of total poly phenol, flavonoids, and tannic acid and, to assess the cerebroprotective potential of *Sonchus asper* (L) hill against monosodium glutamate (MSG) and aluminium fluoride (AlF<sub>3</sub>) induced cerebrototoxicity in wistar albino rats. **Materials and methods:** Quantitative determinations of phytochemicals of 95% ethanolic extract by estimation of total phenolic content and tannins. The albino rats received 95% ethanolic extract at a dose of 250 and 500 mg/kg and ascorbic acid 500 mg/kg (p.o) daily for 7 days. On all the 7 days, MSG (2g/kg, i.p.) was administered one hour before drug treatment and aluminium fluoride (AlF<sub>3</sub>) at 600 ppm for 7 days in drinking water. During the treatment the rats were observed for neurobehavioral performance for 50minutes daily. Oxidative damage and histopathological analysis of tissue were also assessed. **Results:** By preliminary phytochemical screening found that 95% alcoholic extract contains glycoside, flavonoid, tannin, alkaloid, saponins etc. It was qualitatively observed the extract contains higher concentration of polyphenol, flavonoid, and tannin components. Extract and ascorbic acid administration significantly improved body weight and attenuated locomotors activity, rota rod and balance beam test performance compared with diseased groups and reversed the enzyme levels to the normal. **Conclusion:** It was concluded that ethanolic extract of *Sonchus asper* (L) Hill (EESA) contain significant amount of phenols and tannins and these constituents play an essential role as an antioxidant and cerebroprotective effects in both diseased groups.

**Keywords:** *Sonchus asper* (L) Hill, cerebroprotective activity, monosodium glutamate, aluminium fluoride

**Introduction**

The brain is a highly sensitive organ, requiring a continuous supply of oxygen, glucose and other nutrients to maintain its function and viability. Several neurons die because of energy depletion, and increased oxidative stress (Moro, 2004). Many natural or manmade toxic substances also alter the normal activity of the nervous system leading to neurotoxicity, which is observed as various physiological and psychological disorders (Massaro, 2002).

Neurodegenerative diseases defined as progressive loss of the functional and structural integrity of central nervous system.

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The oxidative stress is a primary factor to the development of neurodegenerative diseases like Parkinson's, Alzheimer's diseases, neurological condition like seizures, epilepsy, stroke, CNS trauma and amyotrophic lateral sclerosis etc. Oxidative damage usually occurs in organs like brain, heart, skeletal muscles, since the organ are collected primarily post mitosis cell. All the cells and tissues of our body are also equipped with anti-oxidative enzymes like super oxide dismutase, Catalase, Lipid peroxidase and substances like reduced glutathione. Normally, the balance is maintained between the oxidative attack of the free radicals and, the antioxidative defence system in the cells and tissues of living body. They dispose the free radicals and when they are generated thereby protecting the cells and tissues from the oxidative attack (Mikhail and Richard, 1994).

Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Reactive oxygen

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species are also said to be responsible for the human aging. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenol and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Herbal plants are considered as good antioxidant since ancient times (Mahdi et al., 2012).

Glutamate is an excitatory neurotransmitter in the brain. It interacts with two main sub types of membrane receptors like Ionotropic, is coupled with a cation gated channel and metabotropic, is coupled with G-protein receptor, are further sub divided based on selective agonists into N-methyl-D-aspartate (NMDA), kainite and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). Interact of glutamate with its inotropic mainly NMDA receptor can lead to neurotoxic changes in some experimental situation by allowing excessive amount of calcium to enter the neuron.

*Sonchus asper* (L.) Hill is an herbaceous annual plant it grow up to 2 meters in height, the family of Asteraceae (Compositae). The entire plant contains sticky, white latex. There were reported that chemical constituents such as polyphenolic compounds and sesquiterpene, lactones glycosides such as sonchusides E, F, G, H and I, and three ion one's of sonchuniosides A, B and C. It also contains  $\alpha$ -amyrin,  $\beta$ -amyrin, germanicol, taraxasterol, lupeol, epifriedelinol acetate, and stigmasterol while in sesquiterpene lactones glycosides have flavonoids, tannin, saponins, terpenoids, ascorbic acid and carotenoids possess antioxidant, anticancer, anti-inflammatory property. In Pakistan, it is used as traditional (folk) medicine for hormonal disorder and oxidative stress. In Spain, it is commonly used as emollient and it is also used in various disorders like wounds and burns, gastrointestinal infection, kidney and liver disorders like jaundice, cancer etc. Hence, this plant was selected for study of Cerebroprotective activity (Narasimharao et al., 2011).

## Materials and methods

### Preparation of plant extract and phytochemical screening

The whole plant of *Sonchus asper* (L.) Hill was collected surrounding Harapanahalli, Karnataka, India in the month of June-July 2013. The plant materials were identified and authenticated by Prof. K Prabhu, Department of Pharmacognosy, SCS College of Pharmacy, Harapanahalli, India. A herbarium specimen No. SCSCOP.Ph.Col Herb.No.004/2013-2014. The dried plant were extracted successively with petroleum ether, chloroform, 95% ethanol, and distilled water at room temperature. The extract was

concentrated under reduced pressure (bath temperature 50C) and stored in airtight container in refrigerator below 10C. The preliminary phytochemical screening was carried out on different extracts, to identify the presence of qualitative identification type of phytoconstituents (Kokate, 1999; Khandelwal, 2000).

### Estimation of total phenolic content (TPC) by Folin-Ciocalteu method

The total phenolic content of 95% extract of *Sonchus asper* (L.) Hill was determined. Aliquot of the extract was taken in a 10 ml glass tube, and the volume was made upto 3 ml with distilled water. Then 0.5 ml Folin-ciocalteu reagent (1:1 with water) and 2 ml  $\text{Na}_2\text{CO}_3$  (20%) were added sequentially in each tube. A blue colour was developed in each tube and the intensity of the colour was directly proportional to the phenolic content. The blue coloration is obtained due to the formation of molybdenum blue as a result of complex redox reaction between phenols and Phosphomolybdic acid Folin-ciocalteu reagent in alkaline medium. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm. The calibration curve was prepared using catechol as standard. The phenolic content of the plant was expressed as a milligram equivalent of phenol per gram of extract. (Singh, 1980; Heljima et al., 2007)

### Estimation of tannins by Prussian blue method

The quantitative estimation of the tannin content in the samples has done prior to the presence of tannins was identified by using the  $\text{FeCl}_3$  and Gelatin tests (Urve et al., 2010). The quantitative estimation of tannin content in samples was calculated by the method of Price and Butler with some modifications. In short, of a dry plant sample was transferred to 100 ml flask; 50 ml water was added and boiled for 30 min. After filtration with cotton filter, the solution was further transferred to a 500 ml flask and water was added up to 500 ml mark. 0.5 ml aliquots were finally transferred to vials, 1 ml  $\text{K}_3\text{Fe}(\text{CN})_6$  and 1 ml  $\text{FeCl}_3$  were added and water was added up to 10 ml volume. After five min time period, the solutions were measured spectrophotometrically at 720 nm. The actual tannin concentrations were calculated on the basis of the optical absorbance values obtained for the standard solutions in range 5-25  $\mu\text{g}/10\text{ ml}$ .

### Animals

Wistar albino rats (weighing 150-250g) of either sex were procured from Sri Venkateshwara Enterprises, Bangalore. They were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at  $27^\circ\text{C} \pm 2^\circ\text{C}$  under 12 hrs dark / light cycle and

fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water *ad libitum* was provided. Ethical clearance for handling the animals was obtained from the Institutional animal ethical committee prior to the beginning of the project work, and the registration no. is SCSCP/626/6/2012-13 CPCSEA and date is 09.01.2013.

### Experimental design

#### Monosodium glutamate- induced cerebrototoxicity

Overnight fasted rats were randomly divided into five groups of six rats (n=6) each as treated for 7 days 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.) +EESA250mg/kg (p.o.)

**Group 4:** Received MSG 2 g/kg (i.p.) + EESA 500mg/kg (p.o.)

**Group 5:** Received MSG 2 g/kg (i.p.) + ascorbic acid 500 mg/kg, (p.o) (Reference standard)

The gap between 95% alcoholic extract and MSG is 1 hour. The dose of MSG was selected based on previous literature. During the drug treatment, rats were observed for the behavioural changes for 50 minutes daily. On 8<sup>th</sup> day the rats were evaluated for Balance beam test, digital rota rod test and locomotors. On 9<sup>th</sup> day rats were sacrificed and brains were isolated for estimation of glutathione, SOD, CAT, lipidperoxidation and protein (Ramanathan et al., 2007)

#### Aluminium fluoride-induced Cerebrototoxicity

The animals were divided into five groups of six rats each as follows;

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

**Group 2:** Received (AlF<sub>3</sub>) 600ppm + normal saline (p.o.).

**Group 3:** Received (AlF<sub>3</sub>) 600 ppm +95%EESA250mg/kg (p.o.)

**Group 4:** Received (AlF<sub>3</sub>) 600 ppm +95%EESA 500mg/kg (p.o.)

**Group 5:** Received (AlF<sub>3</sub>) 600ppm + ascorbic acid 500 mg/kg, (i.p.) (reference standard)

Animals from each group were fed with drug or vehicle for 10 days prior to aluminium fluoride treatment through their drinking water (7 days at a dose of 600 ppm). During the drug treatment, rats were observed for the behavioural changes for 50 minutes daily (Chandrashekhar, 2010).

#### Statistical analysis

To check the significance of data, following statistical test were performed: ANOVA: to see the variability within all the groups.

#### Results

It is observed from the preliminary phytochemical screening that glycoside, flavonoid, tannin, alkaloid, saponins, protein and

carbohydrate were found in 95% alcoholic extract. It was qualitatively observed that 95% ethanolic extract contain higher concentration of polyphenol, flavonoid, and tannin components and hence, selected this extract for further study.

#### Spectroscopy determination of total phenol and total tannin

The total phenolic content of 95% ethanolic extract was 18.18 mg/g expressed as equivalent to catechol and the tannin content was found to be 27.82mg/g, as shown in figure 1 & 2.

#### Effect of 95%EESA on Behavioural characters in MSG treated rats

Motor coordination, Body balance and Locomotor activity were significantly (P<0.001) affected in MSG administered rats, when compared to normal control rats (Table 1).

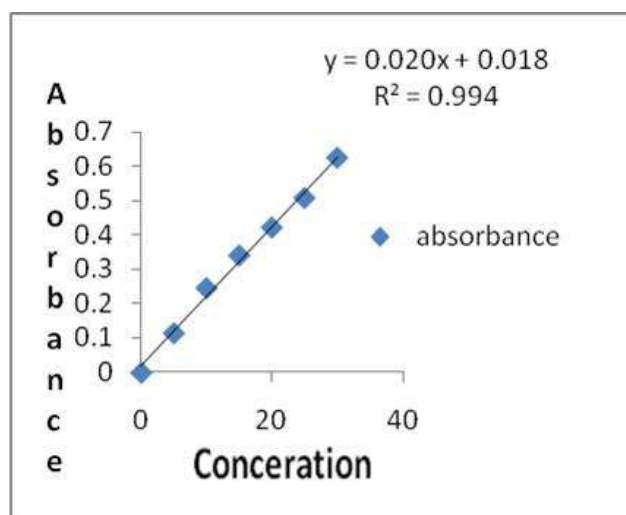


Figure 1. Standard graph of catechol

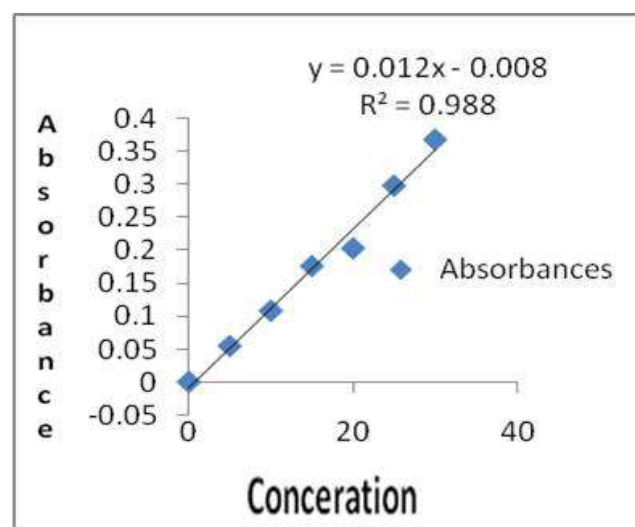


Figure 2. Standard graph of Tannin

**Table 1.** shows the effect of 95% EESA change on Behavioural characters and biochemical parameters in MSG treated rats

Treatment	Rota Rod	Balance Beam	Locomotor Activity	GSH		LPO		SOD		CAT		TP	
	Test (sec)	Test (sec)	(Count/5 min)	Mean±	%	Mean±	%	Mean±	%	Mean±	%	Mean±	%
	SEM	SEM	SEM	SEM	increase	SEM	Inhibition	SEM	increase	SEM	increase	SEM	increase
Negative control	199± 9.59	76.33± 6.30	98.33 ± 2.30	0.289± 0.020	.....	0.308±0.018	.....	0.139± 0.007	....	0.455± 0.016	.....	8.112± 0.255	.....
Positive control	117± 4.90	24.67 ± 1.74	50.67± 2.39	0.160± 0.003	.....	0.595± 0.023	.....	0.083± 0.003	.....	0.268± 0.010	.....	4.155± 0.348	.....
Standard	168± 1.72	53.17± 1.37***	78.83± 3.00***	0.249± 0.070***	55.03	0.411± 0.011	30.86	0.134± 0.003	83.96	0.448± 0.017	67.09	7.560± 0.370***	81.94
250mg/kg	141± 2.27	43.33 ± 2.09**	64.00± 4.71*	0.219± 0.016*	19.65	0.504± 0.013**	15.20	0.112± 0.004**	86.51	0.344± 0.008**	28.21	5.362± 0.223*	26.23
95%EESA	147± 2.03	44.67 ± 2.52***	66.00 ± 2.01**	0.215± 0.015*	33.89	0.479± 0.012***	19.52	0.109± 0.005**	86.87	0.360± 0.022**	34.25	5.245± 0.231*	29.67

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 ALF<sub>3</sub> alone treated rats. One-way ANOVA followed by Dunnett's comparison test.

**Table 2.** shows the effect of 95% EESA change on Behavioural characters and biochemical parameters in ALF<sub>3</sub> treated Rats.

Treatment	Rota Rod	BalanceBeam	Locomotor Activity	GSH		LPO		SOD		CAT		TP	
	Test (sec)	Test (sec)	(Count/5 min)	Mean±	%	Mean±	%	Mean±	%	Mean±	%	Mean±	%
	SEM	SEM	SEM	SEM	increase	SEM	Inhibition	SEM	increase	SEM	increase	SEM	increase
Negative control	177.0± 3.48	111.0± 6.66	74.17± 2.05	0.199± 0.006	.....	0.307± 0.032	.....	0.067± 0.001	.....	0.217± 0.003	.....	7.24±0.14	.....
Positive control	64.67± 4.12	32.33± 2.99	30.17± 2.92	0.076± 0.013	.....	0.430± 0.010	.....	0.028± 0.001	.....	0.130± 0.007	.....	3.91± 0.18	.....
Standard	115.3± 4.74***	62.33± 2.60***	50.67± 2.33***	0.177± 0.007***	133.00	0.252± 0.010***	41.39	0.059± 0.0009***	106.38	0.204± 0.003***	57.21	5.64± 0.20***	43.30
250mg/kg	83.67± 3.48**	51.33± 1.99**	45.50± 2.43**	0.113± 0.003**	49.25	0.149± 0.009***	25.90	0.041± 0.003**	45.34	0.150± 0.002*	15.36	4.84± 0.06**	23.70
95%EESA	89.17± 3.68***	54.17± 1.74**	52.83± 3.04***	0.146± 0.004***	96.91	0.319± 0.009***	25.96	0.053± 0.002***	87.75	0.167± 0.002**	28.49	5.01± 0.20***	27.9

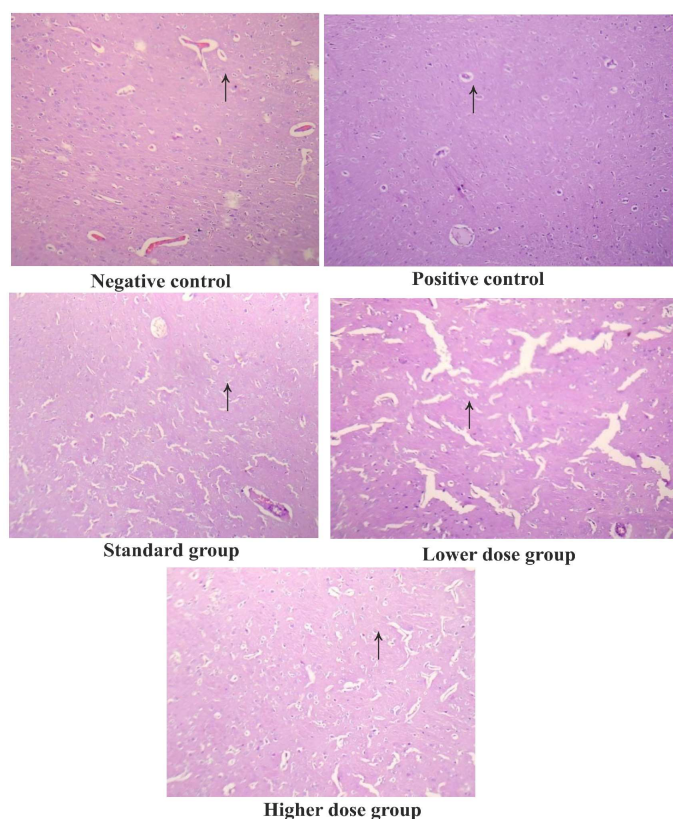
Each value is expressed as mean ± SEM (n = 6), where, NS represents non significant; \*\*\*P<0.001 – highly significant; \*\*P<0.01- very significant; \*P<0.05-significant, when compared to ALF<sub>3</sub> alone treated rats. One-way ANOVA followed by Dunnett's comparison test. \*GSH- Glutathione, LPO- Lipid peroxides, SOD- Superoxide dismutase, CAT- Catalase, TP- Total Protein.

Normal control animal showed the fall of time in 199.5±9.59s, 76.33 ± 6.30s & 98.33 ± 2.3 respectively, whereas MSG alone treated rats showed the fall of time in 117.8±4.9, 24.67 ± 1.7 & 50.67± 2.3s. Pre-treatment with 95%EESA and Ascorbic acid at 500 mg/kg significantly improved the motor coordination and body balance in MSG treated rats and traversed the beam in 168.3±1.72, 53.17± 1.37, 78.83 ± 3.005 and 147.0±2.037, 44.67± 2.525, 66.00 ± 2.017 respectively. 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. Histopathological observations of different groups in MSG are shown in figure 3.

#### Effect of 95% EESA on behavioural characters in AIF<sub>3</sub> treated rats

Motor coordination, Body balance and Locomotor activity were

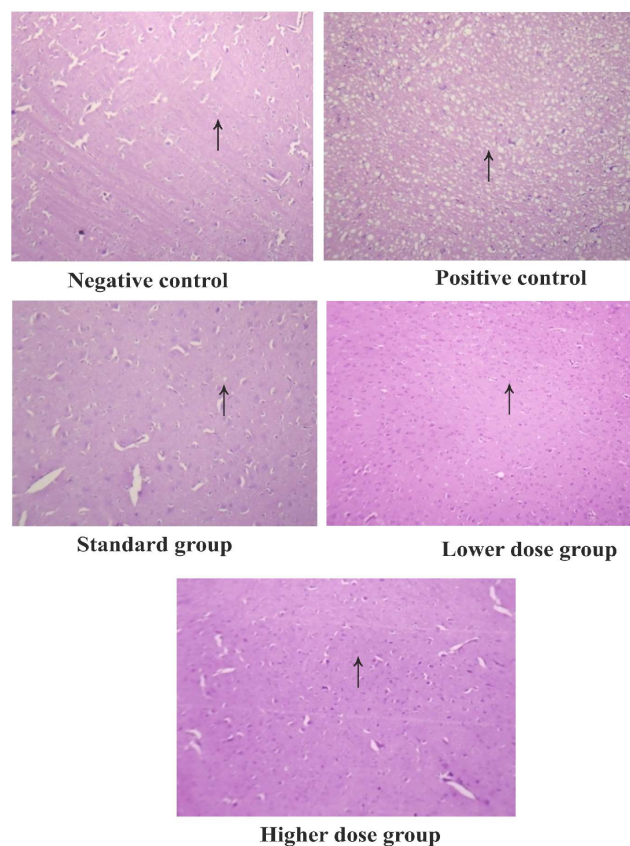
significantly (P<0.001) affected in AIF<sub>3</sub> administered rat, when compared to normal control rats (Table 2). Normal control animals showed the fall of time in 177.0±3.48; 111.0±6.66, 74.±2.05 respectively. Whereas, AIF<sub>3</sub> alone treated rats showed the fall of time in 64.67±4.12, 32.33±2.99 & 30.17± 2.92s respectively. Pre-treatment with Ascorbic acid and 95%EESA (250, and 500 mg/kg) significantly (P<0.001, P<0.01 and P<0.05) improved the motor coordination and body balance in AIF<sub>3</sub> treated rats 115.3±4.745, 83.67±3.480, 89.17±3.683 respectively. 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. Histopathological observations of different groups in AIF<sub>3</sub> are shown in figure 4.



**Figure 3.** Histopathological observations of different groups in ALF<sub>3</sub>

### Discussion

The human body cells are involved in the metabolic process to generation of energy with release of free radicals scavengers. These free radicals are useful for many oxidative processes of the cells. Reactive oxygen species (ROS) includes free radicals, as well as other oxygen related reactive compounds. The most important ROS like superoxide anion (O<sub>2</sub><sup>-</sup>), Hydrogen peroxide radical (H<sub>2</sub>O<sub>2</sub>), Nitric oxide (NO), Hydroxyl radical (OH) are closely involved in various Human diseases such as Alzheimer's disease, Aging, Cancer, Inflammation, Rheumatoid arthritis and Atherosclerosis<sup>125</sup>. Plants are the source presence of poly phenols, flavonoids and tannins which possess wide range of biological activities. Hence in this study, the plant *Sonchus asper* (L) hill reported to possess polyphenolic compounds and tannins, which are reported to possess antioxidant property. These antioxidant property of may be assessed by several screening for assessing the Cerebroprotective activity of MSG and ALF<sub>3</sub> induced cerebrotoxicity models were selected. The extracts were prepared separately. Thus prepared extracts were subjected to preliminary phytochemical studies, the results indicated that plant possess poly phenols, tannins, flavonoids, proteins, cardiac glycosides and saponins in 95% ethanolic extract of plant. The spectroscopic determination was observed that, total content of poly phenol and tannins contents in the plant, it was found the 95% EESA possess 18.18mg/gm and



**Figure 4.** Histopathological observations of different groups in MSG

27.82mg/gm expressed to catechol and tannic acid respectively.

The amount of total phenolic and tannin content was found higher amount in 95% ethanolic extract as compare to that of aqueous extract, and also we found that the amount of tannin was higher in 95% of ethanolic extract as compare to that of polyphenolic. In the present study the treatment with glutamate as monosodium glutamate (MSG) induced severe neurochemical damage and neurotoxic effect on some brain region. Various investigators have previously demonstrated some of the neurotoxicological signs induced by monosodium glutamate intake (2mg/kg i.p) in rat. Excessive accumulating of glutamate in the synaptic cleft has been associated with excitotoxicity is implicated in number of neurological disorder.

However there is accumulating evidence suggesting that glutamate-induced toxicity can be mediated through necrosis and apoptosis. The principle of modulating toxic effect of MSG by interfering with neurotransmitter role may have significant impact on understanding neurodegenerative disorder. Aluminium has the potential to be Neuro toxic in human and animals, and is present in many manufactured foods and medicines and also added to drinking water for purification purposes. The present study it is clear that the chronic administration of monosodium glutamate and its

subsequent withdrawal caused a significant decrease in monoamine content in most of the tested brain areas at different time intervals. In AIF<sub>3</sub> (600ppm p.o for 7 days) in drinking water revealed altered behavioural changes and causes oxidative damage to the brain was estimated by measuring GSH, LPO, SOD, CAT and total protein levels. The histopathological studies were performed to striatal region of the brain. A significant decline in the enzymatic activity of antioxidant enzymes like GSH, SOD, CAT and TP levels monosodium glutamate and aluminium treatment where as LPO levels showed a significant increase as compared to normal control. Synchronous administration of EESA during monosodium glutamate and aluminium treatment reversed the enzyme levels to the normal.

### Conclusion

The present Study was carried out to evaluate Cerebroprotective effects of 95% ethanolic extract *Sonchus asper* (L) hill contain significant amount of phenols and tannins. These constituents may play an essential role as antioxidant and organ protective. Thus our studies support the folklore use of the title plant in cerebroprotective disorders.

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### Conflict of interest

The author declares there is no conflict of interest.

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