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NEUROPROTECTIVE EVALUATION OF ETHANOLIC LEAF EXTRACT OF *DALBERGIA SISSOO* IN MONOSODIUM GLUTAMATE INDUCED NEUROTOXICITY IN RATS

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ABSTRACT: Neurodegenerative disorders are heterogeneous group of diseases of the nervous system, including the brain, spinal cord and peripheral nerves that have many different etiologies. Due to the prevalence, morbidity, and mortality of the neurodegenerative diseases, they represent significant medical, social, and financial burden on the society. *Dalbergia sissoo* (family: Fabaceae) is an Asian deciduous rosewood tree and state tree of Punjab called as Shisham used for aphrodisiac, expectorant, anthelmintic, antipyretic, emesis, ulcers, skin disease, memory enhancer etc. The leaf of extract of *D. sissoo* having more concentration of flavonoids and its neuroprotective effect in animals is unclear. So this study was to explore the neuroprotective activity of ethanolic leaf extract of *Dalbergia sissoo* (ELDS) against chemically induced neurotoxicity in rats. This study was designed with 30 albino wistar rats divides into 5 groups. They were treated for 7 days with glutamate salt followed by ELDS. During treatment physico-pharmacological parameters were recorded, 24hr after experiment i.e on 8th day antioxidant profile from brain isolate were estimated and histopathology of brain was performed. Monosodium glutamate (MSG) significantly altered animal behavior, oxidative defense (raised levels of LPO, depletion of antioxidant levels), mitochondrial enzymes activity and loss of hippocampus neurons in the brain. Treatment with ELDS significantly attenuated behavioral alterations, oxidative damage, and hippocampus damage in MSG treated rats. So it was suggested that ELDS showed protection against chemical (MSG) induced neurotoxicity in rats. The antioxidant, anti-inflammatory and estrogenic property of ELDS may be responsible for its neuroprotective action.

INTRODUCTION: Monosodium glutamate is the sodium salt of the amino acid glutamic acid. Glutamic acid or glutamate is one of the most common amino acids found in nature. It is the main component of many proteins and peptides, and is present in most tissues [1].

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Glutamate is the most abundant excitatory neurotransmitter in the brain, in addition to its vital role as a neurotransmitter excessive amounts of glutamate may be potent neurotoxin. Although glutamate induced cell death is associated with apoptotic and necrotic changes, the mechanism of cell death remains to be established. Two distinct pathways for glutamate induced cell death have been described the excitotoxic pathway involves the over activation of glutamate receptors that leads to both rapid and slow triggered cytotoxic events. The rapid effect involves the activation of the NMDA-R that lead to a large Ca^{++} influx that may

be detrimental to cell viability. The oxidative pathway involves breakdown of the glutamate cysteine antiporter and a drop in glutathione levels which allows for aberrant formation of neurotoxic reactive oxygen species [2]. It is reported that MSG could impair memory retention and induce damage in the hypothalamic neurons in mice. In addition, alteration in mitochondria lipid peroxidation and antioxidant status in different regions, namely the cerebral hemispheres, cerebellum, brain stem and diecephalon [3], hence the i.p. injections of MSG administration provide a successful model to screen the drugs on brain oxidative stress and excitotoxicity induced neuronal damage.

Pharmaceutical approach for novel prevention and treatment strategies of neurodegeneration involves the use of neuroprotective agents in order to delay or stop neuronal cell death or to strengthen cellular defense system. But effective therapies still remain elusive [4]. A number of natural compounds are being used as brain tonic to help restore debilitated conditions. Since plants produce significant amount of antioxidants, they represent a potential source of new compounds with antioxidant activity. In view of this, *Dalbergia sissoo* Roxb. has been selected based on its use in traditional systems of medicine for augmenting neurological health and was reported to be brain tonic [5], which appear to offer very promising outcomes for neuroprotection.

Dalbergia sissoo Roxb.(family-fabaceae) (DS) also called 'shisham' is used time immemorial for treatment of various ailments like burning sensations, dysentery, dyspepsia, leucoderma, and skin ailment, ant inflammatory, memory enhancer and leaves have significant levels of flavonoids which showed antioxidant activity twice of commonly used antioxidants like vitamin C and selenium [6]. Pharmacologically, it is understood that DS has combination of many phyto constituents that are beneficial in curing mental inefficiency, illness and useful in the management of various other disorders. DS possesses antioxidant and central nervous system property however the effectiveness of DS in controlling glutamate induced excitotoxicity is not carried out. The present study aims to evaluate the effect of DS on the neurodegeneration induced with monosodium glutamate (MSG) and on oxidative markers in MSG treated rats.

MATERIALS AND METHODS:

Drugs and chemicals: Gabapentin (Intas Pharmaceuticals, Ahmedabad), Trichloroacetic acid (TCA), 2 thiobarbituric acid (TBA), 5-5- dithiobis (2 nitrobenzoic acid) (DTNB), Phenazine metho-sulphate, Nicotinamide adenine dinucleotide (NADH), Nitro blue tetrazolium (NBT) were purchased from Sigma-Aldrich Co, Spruce Street, St. Louis, MO, USA. All other chemicals were of the highest purity commercially available.

Kits: Total protein (TRANASIA BIO-MEDICALS LTD. Malpur, Solan, (HP)).

Instruments: Soxhlet apparatus, Heating mantle (Shital scientific industries, Mumbai); Rotary flush evaporator, Tray drier, Teflon-glass homogenizer, Cooling centrifuge (REMI Industries, Mumbai); Desiccator, Incubator, Photo actometer (Inco, Ambala, India); Bio analyzer (Recording and medicare systems(P) Ltd. India); Micro pipette (Nichiryo, Japan), Microtips, Ependroff tubes (Tarsons products Pvt Ltd. Kolkata); UV spectrophotometer (UV 1800) (Shimadzu corporation, India); Light Microscope (LOBMED), Animal weighing balance (Docbel Industries, Bangalore); Elevated Plus maze.

Plant material and extraction: The fresh leaves of *Dalbergia sissoo Roxb*, were collected from GKVK Karnataka, India in the month of july 2012. The plant was identified and authenticated by Mr. KP Sreenath, taxonomist Department Botany, Bangalore University, Bangalore, India and voucher specimen of the plant was kept in the college herbarium.

The collected fresh leaves of around 1.5kg were shade dried or tray dried for 2weeks and then grinded to a fine powder. In the continuous hot extraction method, the plant leaves powder was extracted in ethanol for 3 regular days at temperature of 78-80°C.

The mixture was subsequently filtered and concentrated under reduced pressure at 40°C in rotary flush evaporator. The extract yield was 26%w/w [7]. The extract was stored in desiccator.

Preparation of *Dalbergia sissoo* leaf extracts suspension: Weighed quantity of ELDS was suspended in distilled water using 0.5%v/v Dimethyl sulphoxide and administered orally to rats. The suspension of extract was prepared freshly every day. The extract was administered at a constant volume of 1ml for each animal [8].

Preliminary phytochemical investigation: The extracts was used for preliminary phytochemical screening with a battery of chemical tests viz., Molisch's, Fehling's, Benedict's and Barfoed's test for carbohydrates; Biuret and Millon's tests for proteins; Ninhydrin's test for amino acids; Salkowski and Libermann-Burchard's reactions for steroids; Borntrager's test for anthraquinone glycosides; Foam test for saponin glycosides; Shinoda and alkaline tests for flavonoids glycosides; Dragendorff's, Mayer's, Hager's and Wagner's tests for alkaloids; and ferric chloride, lead acetate tests for tannins and phenols. The results showed presence of all chemical constituents except alkaloids [9].

Acute oral toxicity studies: Acute toxicity studies were carried out on mice accordingly, alcoholic extracts at dose of 50, 100, 300, 1000, and 3000mg/kg body weight were administered to separate groups of the mice (n=6) after overnight fasting. Subsequent to administration of ELDS, the mice observed closely for the first 3hours for toxic manifestations like increased motor activity, salvation, clonic convulsions, coma and death. The Observations were taken at regular intervals for 24hr. The animals were observed for 1 week. The study revealed that ELDS was not toxic up to 3000mg/kg body weight [10].

Animals: Adult female Wistar rats (180 – 220 g) bred in animal house of PES College of Pharmacy, Bangalore, were used. The animals were procured from Ragavendra enterprises, Bangalore maintained on a 12 h light: 12 h dark cycle and free excess of food and water. Animals were acclimatized to laboratory conditions before the test. The experimental protocols were approved by the Institutional Animal Ethics Committee (PESCP/IAEC/02/11, Date: 14/12/11) and conducted according to CPCSEA guidelines, Govt. of India.

Study Plan: MSG was freshly diluted with saline (adjust pH 7.4) and administered intra-peritoneal to rats for period of 7 days to induce the toxicity. Total 30 rats were randomly divided in to five groups of 6 rats each and treated for 7 days as follows;

Control group: Receives 0.5ml of normal saline (i.p) followed by 1ml of 0.5%v/v of DMSO (p.o) for 7days.

MSG inducing group: Receives 0.5ml of MSG 2g/kg (i.p) followed by 1ml of 0.5%v/v of DMSO (p.o) for 7days.

ELDS (300mg/kg) + MSG group: Receives 0.5ml of MSG 2g/kg (i.p) followed by 1ml of ELDS 300 mg/kg (p.o.) for 7days.

ELDS (600mg/kg) + MSG group: Receives 0.5ml of MSG 2g/kg (i.p) followed by 1ml of ELDS 600 mg/kg (p.o.) for 7days.

Gabapentin + MSG group: Receives 0.5ml of MSG 2g/kg (i.p) followed by 1ml of Gabapentin 20mg/kg (p.o.) for 7days.

The gap between ELDS and MSG is 1 h. The dose of MSG was selected based on previous literatures [2]. During the treatment rats were observed for the behavioral changes for 50 min daily. On 8th day the rats were evaluated for neurological scoring, ambulatory behavior, elevated plus maze test, rota rod performance and hanging wire test. On 9th day rats were sacrificed and brain was isolated for estimation of GSH, CAT, LPO, SOD, total protein and histopathological study.

Parameters monitored:

Measurement of body weight change: Animal body weight was noted on the first day and last day of the experimentation. Percentage change in body weight was calculated in comparison to the initial body weight on the first day of the experimentation [11].

Behavioral parameters: All the behavioral tests were conducted during the day time between 08:00 -12:00 a.m. to avoid circadian influences.

Movement analysis: Neurotoxins are associated with several motor disturbances which prevent normal ambulatory movement of the animal. Severity of the motor abnormalities in these groups was therefore evaluated using a quantitative neurological scale. A neurological score was determined for each animal on 14th day after 4 h of last dose in comparison to control animals. (Score = 0, normal behavior; score=1, general slowness of displacement resulting from mild hind limb impairment; score=2, in coordination and marked gait abnormalities; score=3, hind limb paralysis; score= 4, incapacity to move resulting from fore limb and hind limb impairment; score=5, recumbecy) [11, 12].

Loco motor activity: The spontaneous loco motor activity was monitored using photo actometer (INCO Pvt. Ltd., Ambala, India) equipped with infrared sensitive photocells, the apparatus was placed in darkened, light and sound attenuated and ventilated testing room. Before loco motor task, animals were placed individually in the activity meter for 2 min for habituation. Thereafter, loco motor activity was recorded for a period of 5 min. The loco motor activity was expressed in terms of total photo beam counts/ 5min [13].

Elevated plus maze test for spatial memory: Memory dysfunction is evaluated using elevated plus maze, which consists of two opposite open arms (50 cm × 10 cm), crossed with two closed arms of same dimensions with 40 cm high wall. The arms are connected with Central Square (10 cm × 10 cm). Acquisition of memory was assessed on day 7th after initiating 3-NP treatment. Rat was placed individually at one end of an open arm facing away from the central square. The time taken by animal to move from open arm and enter in to one of the closed arm was recorded as initial transfer latency (ITL). Rat was allowed to explore the maze for 30 s after recording ITL and returned to its home cage. Retention transfer latency (RTL) was noted again on 8th day of first 3-NP dose. The percent retention of memory was calculated by the formula. Similarly the activity was repeated in 14th and 15th day [14].

$$\% \text{ Memory retention} = (ITL - RTL)/RTL \times 100$$

Rota-rod performance assessment for motor coordination: The rota rod (rotating rod) test is widely used in rodents to assess their ‘‘minimal neurological deceit” such as impaired motor function (e.g., ataxia) and coordination. The Rota rod unit consists of a rotating rod, 75mm in diameter, which was divided into four parts by compartmentalization to permit the testing of four rats at a time. Briefly, in a training session, the rats were placed on the rod that was set to 25 rpm and the performance time that each rat was able to remain on the rota rod was recorded. The rats were subjected to three training trials at 3- to 4-h intervals on two separate days for acclimatization purposes. In the test session, the rats were placed on the rota rod and their performance times were recorded [15].

String test for grip strength: The rat was allowed to hold with the forepaws a steel wire (2 mm diameter and 80 cm in length), placed at a height of 50 cm over a cushion support. The length of time the rat was able to hold the wire was recorded. This latency to the grip loss is considered as an indirect measure of grip strength cut off time was taken as 90 s [16].

Animal autopsy and isolation of brains: After the treatment period, experimental and control mice were sacrificed by decapitation under mild anesthesia. Brains were immediately isolated, rinsed in ice cold saline to remove blood and stored at -20°C immediately until used in assays described below.

Estimation of antioxidant enzyme levels in brain tissue:

Preparation of tissue homogenate: The whole brain dissected out, blotted dry and immediately weighed. The brain regions cerebral cortex (Ct), cerebellum (Cb), hippocampus (Hc) and striatum (St) were subsequently dissected from the intact brain carefully on ice plate (4 ± 2 °C). A 10% brain homogenate was prepared with ice-cold phosphate buffered saline (0.1 M, pH 7.4) using Teflon-glass homogenizer. The homogenate was centrifuged at 10,000 rpm at - 4 C for 15 min and the pellet discarded. The supernatant obtained was used for the quantification of antioxidant levels like GSH, CAT, LPO, SOD, total protein levels [17].

Catalase (CAT): In brief, the incubation mixture contained in a final volume of 2.0ml, 0.1ml of

diluted homogenate, 1.0ml of phosphate buffer and 0.4ml of distilled water to which 0.5ml of H_2O_2 solution was added to initiate the reaction, while the H_2O_2 solution was left out in control tubes. After incubating for 1 min at 37°C the reaction was stopped by addition of 2 ml of potassium dichromate acetic acid reagent. The samples were kept in boiling water bath for 15 minutes, finally cooled and the absorbance measured at 570 nm against control. The catalase content was calculated by using molar extinction coefficient = 58.03 x 10^{-3} M^{-1} cm^{-1} and the values are expressed as nmoles/mg protein [18].

Lipid peroxidation (LPO): Briefly, the reaction mixture contained 0.1 ml of brain regions homogenate/ mitochondria (1mg protein), 1.5 ml of 20% acetic acid (pH 3.5), 1.5 ml of 0.8% thiobarbituric acid (0.8% w/v) and 0.2 ml SDS. Following these additions, tubes were mixed and heated at 95 °C for one hour on a water bath and cooled under tap water before mixing 1 ml of distilled water and 5ml mixture of n-butanol and pyridine (15:1). The mixture was centrifuged at 2200g for 10 min. The amount of MDA/TBARS formed was measured by the absorbance of upper organic layer at a wave length of 532 nm. The results are expressed as nmol MDA/mg protein. The absorbance of the clear pink color supernatant was measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using molar extinction coefficient 1.56 x 10^5 M^{-1} cm^{-1} and the results were expressed as nmoles MDA/g of protein [19].

Reduced Glutathione (GSH): The assay is based on the principle of Ellman's reaction. The sulfhydryl group of glutathione reacts with DTNB (5, 5'-dithiobis-2-nitrobenzoic acid) and produces a yellow colored 5-thio- 2-nitrobenzoic acid (TNB). Measurement of the absorbance of TNB at 412 nm provides an accurate estimation of glutathione in a sample. Briefly, 0.5 ml of homogenate is mixed with 0.1 ml of 25% TCA to precipitate proteins and centrifuged at 4000 rpm for 5 min. Then, 0.3 ml of the supernatant was mixed with 0.5 ml of 0.1M phosphate buffer (pH 7.4) and 0.2 ml of 10 mM DTNB. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The glutathione content was calculated by using extension coefficient 13.6 x 10^3 M^{-1} cm^{-1}. The values are expressed as nmoles/mg protein [20].

Super oxide dismutase (SOD): The assay mixture contained 0.1ml of sample, 1.2ml of sodium pyrophosphate buffer (pH 8.3, 0.052M), 0.1 ml of phenazine methosulphate (186µm), 0.3 ml of nitro blue tetrazolium (300µ m), 0.2 ml of NADH (750 µm). Reaction was started by addition of NADH.

After incubation at 300 C for 90 sec, the reaction was stopped by the addition of 1 ml of glacial acetic acid. The mixture was allowed to stand for 10 min. The color intensity of the chromogen was measured at 560 nm against blank and concentration of SOD was expressed as units/min/mg of protein [21, 22].

Total protein: The total protein of brain tissue was determined by biurett method in ERBA diagnostic kit [23,24].

Total protein (g/dl) = Absorbance of test/Absorbance of standard*concentration of standard (g/dl)

Histopathological study of rat brain: A section of the brain was fixed with 10% formalin and embedded in paraffin wax and cut into sections of 5 µm thickness. The sections were stained with haemotoxylin and eosin dye for histopathological observations. Depending on the model, either hippocampal or striatal neurons were observed for morphological changes [25].

Statistical evaluation: The data were expressed as Mean ± S.E.M. Statistical comparisons were performed by one way ANOVA followed by Dunnet's post-test using Graph Pad Prism version 5.0. *P<0.5, **P<0.01, ***P<0.001 will be considered as significant compared to toxic control.

RESULTS:

Body weight: Administration of MSG resulted in significant (P<0.01) decrease in body weight when compared to normal rats. Treatment with ELDS in MSG induced rats significantly (P<0.001 and P<0.05) prevented the MSG induced decrease in body weight, the effect of ELDS at 300 mg/kg was found to be much better than its higher dose when compared to MSG control group **(Table 1)**.

Behavioral parameters:

1. **Neurological scoring:** Intra-peritoneal administration of MSG resulted significant (P<0.001) motor abnormalities, out of six rats, four rats showed in-coordination and hind limb paralysis, two animal showed hind limb and forelimb paralysis and gained significantly high neurological score, when compared to normal control rats. Pretreatment with ELDS (300 and 600 mg/kg) in MSG induced rats showed significant (P<0.001 and P<0.01) decrease in behavioral changes when compared to MSG induced rats **(Table 1)**.

2. **Locomotor activity:** Administration of MSG for 7 days in normal rats resulted in significant (P<0.001) decrease in movement of animals compared to normal control animals. Pretreatment with ELDS (300 and 600 mg/kg) in MSG induced rats significantly (P<0.01 and P<0.001) increased locomotor counts **(Table 1)**.

3. **Hanging wire test:** Pretreatment with ELDS (300 and 600 mg/kg) in MSG induced rats, improved significantly (P<0.001 and P<0.01) grip strength (32.50±2.68 and 31.83±2.52 s) compared to MSG induced rats (**Table 1**).

4. **Elevated plus maze paradigm:** In this study, mean initial transfer latency (ITL) on day 7th was relatively stable in all the animals within the group. MSG alone administered rats for 7 days showed significant (P<0.01) increase in retention transfer latency (RTL) compared to normal control animals. In contrast, MSG treated rats performed poor memory retention. This indicates there is cognitive dysfunction in MSG treatment. Chronic pretreatment with ELDS (300 and 600 mg/kg p.o.) in MSG treated rats showed significant (P<0.01and P<0.001) improvement in memory performance when compared to 3-NP alone treated rats **(Table 1)**.

5. **Rotarod test:** Administration of MSG for 7 days for normal rats resulted significantly (P<0.001) decreased motor coordination and body balance compared to normal control rats. Pretreatment with ELDS (300 and 600 mg/kg) in MSG induced rats significantly (P<0.01 and P<0.001) increased motor coordination and body balance compared to MSG induced rats **(Table 1)**.

Effect of ELDS on levels of CAT, LPO, GSH, SOD and TP: Results clearly revealed increase in the levels of MDA and hydroperoxides in MSG induced toxic group compared to control group. Treatment with extracts significantly prevented this raise in levels.CAT, GSH, SOD and TP content have significantly increased in extract treated groups whereas toxic group has shown significant decrease in levels compare to control group. Ethanolic extract (600mg/kg) has shown maximum protection **(Table 2)**.

TABLE 1: EFFECT OF ELDS ON BODY WEIGHT CHANGE AND BEHAVIORAL CHARACTERS IN MSG TREATED RATS

Treatment	% Body weight change	Neuro-logical score	Locomotor Activity (counts/5min)	Grip strength test (s)	Plus maze (transfer latency time sec)	Plus maze (% memory retention)	Rota rod performance (s)
Normal control group	93.75 ± 2.82^{b}	0.0^{a}	326.7 ± 8.43^{a}	31.33 ± 2.94^{b}	24.33 ± 0.55^{b}	69.77	28.67 ± 1.83^{a}
Inducing group (MSG)	78.62±2.32	3.20±0.20	205.7±14.39	14.50±1.11	32.33±1.64	32.87	13±0.96
Standard group (Gabapentin)+MSG	102.5 ± 0.89^{a}	0.0^{a}	344.0 ± 9.42^{a}	33.50 ± 3.45^{a}	15.33 ± 1.78^{a}	64.28	27.50 ± 1.43^{a}
Low dose (D.S 300mg/kg)+MSG	98.05 ± 4.22^{a}	1.40 ± 0.24^{a}	285.8 ± 11.43^{b}	32.50 ± 2.68^{a}	18.33 ± 1.05^{b}	48.64	21.67 ± 0.55^{b}
High dose(D.S 600mg/kg)+MSG	91.41 ± 3.21^{c}	1.80 ± 0.37^{b}	325.0 ± 4.19^{a}	31.83 ± 2.52^{b}	14.17 ± 1.16^{a}	54.55	26.50 ± 1.45^{a}

Each value are expressed as mean ± SEM (n = 6), ^{a}P < 0.001, ^{b}P < 0.01, ^{c}P < 0.05 when compared to MSG alone treated rats. One-way ANOVA followed by Dunnet's post test

TABLE 2: EFFECT OF ELDS ON CAT, LPO, GSH, SOD, TP IN MSG INDUCED NEUROTOXICITY IN RATS

Treatment	Catalase (µm H_2O_2/min/mg of protein)	Lipid peroxidation (nmoles of MDA/g protein)	Reduced glutathione (nmoles/min/mg of protein)	Super oxide dismutase (units/min/mg of protein)	Protein estimation (g/dl of total protein)
Normal control group	62.58±4.54[b]	202.5±28.62[a]	1.57±0.08[b]	18.26±0.51[a]	6.59±0.43[a]
Inducing group (MSG)	33.50±3.86	682.1±39.93	0.85±0.18	11.85±0.19	3.87±0.38
Standard group (Gabapentin)+MSG	72.36±2.18[a]	205.3±30.62[a]	1.51±0.012[b]	19.60±0.29[a]	6.79±0.26[a]
Low dose (D.S 300mg/kg)+MSG	59.60±6.10[b]	313.5±26.55[b]	1.28±0.11	14.55±0.34[b]	5.27±0.40
High dose(D.S 600mg/kg)+MSG	62.05±3.78[b]	269.6±30.82[a]	1.402±0.09[c]	16.13±0.37[a]	6.91±0.26[a]

Each value are expressed as mean ± SEM (n = 6), [a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$ when compared to MSG alone treated rats. One-way ANOVA followed by Dunnet's post test

HISTOPATHOLOGY:

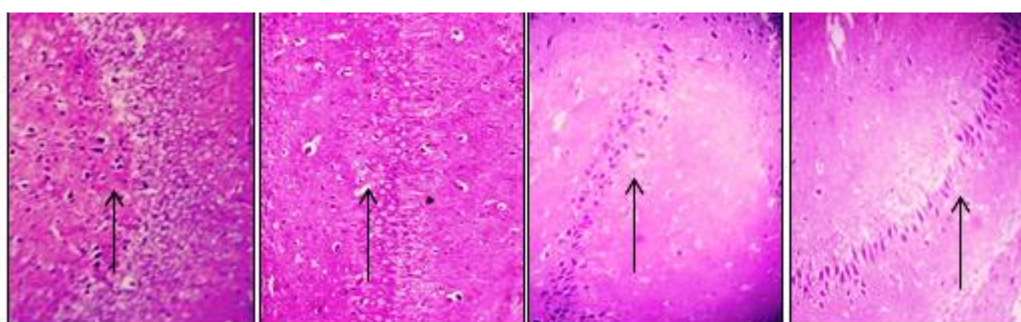

Fig: I Fig: II Fig: I Fig: II

A. Control group B. MSG Induced group

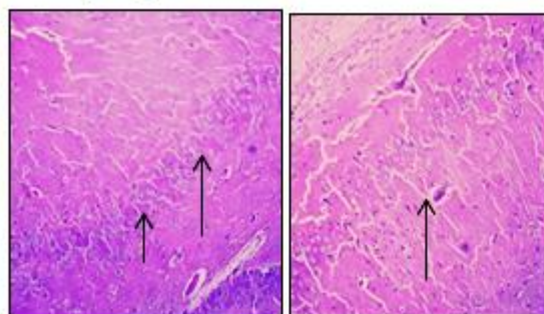

Fig: I Fig: II

C. Low dose ELDS (300mg/kg) + MSG

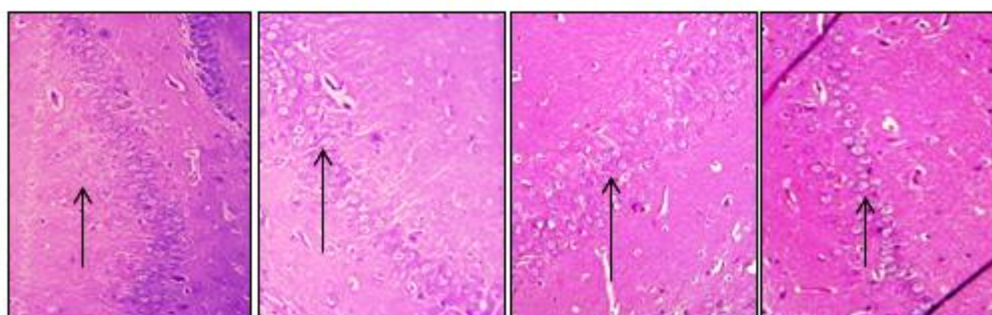

Fig: I Fig: II Fig: I Fig: II

D. High dose ELDS (600mg/kg) +MSG E. Gabapentin + MSG

Histopathological study:

A. Control group: The CA3 region shows intact pyramidal cells in tight clusters [**Fig. 1, Arrow**]. The interconnected neuropil fibers in CA3 region appear intact. The CA1 region shows intact pyramidal cells along with intact neuropil fibers [**Fig. 2, Arrow**].

B. MSG inducing group: The CA1region [Fig. 1, Arrow] shows degeneration of most of the pyramidal cells with intact neuropil fibers. The CA3region [Fig. 2, Arrow] shows degeneration of some of the pyramidal cells along with focal loss of neuropil fibers.

C. Low dose ELDS (300mg/kg) +MSG: The interconnected neuropil fibers in CA3 region show degeneration at focal areas [Fig: 1, Short-Arrow]. The CA1 region shows focal loosely packed pyramidal cells and intact neuropil fibers [Fig. 2, Arrow].

D. High dose ELDS (600mg/kg) +MSG: The CA3 region shows intact pyramidal cells [Fig: 1, Arrow]. The interconnected neuropil fibers in CA3 region appear intact. The CA1 region shows intact pyramidal cells along with intact neuropil fibres [Fig. 2, Arrow].

E. Gabapentin standard +MSG: The CA3 region shows intact pyramidal cells with interconnected neuropil fibers [Fig: 1, Arrow]. The CA1 region shows intact pyramidal cells along with intact neuropil fibres [Fig. 2, Arrow].

DISCUSSION: One week administration of MSG in rats exhibited aggressive behavior and in some animals fighting behavior was also observed seen from results of locomotor and rotarod performance. The animals showed depletion of glutathione, catalase and superoxide dismutase levels and increased the lipid peroxides in HP and ST regions.

The loss of hippocampal structure was noted in MSG treated rats as the result cognitive impairment occur and decrease in % memory retention in elevated plus maze test. These results clearly show that glutamate in rats can lead to excitation and oxidative stress resulting in neurodegeneration [26].

In glutamate toxicity the neuronal death is linked closely to glutamate-evoked excitotoxicity [27]. Glutamate plays a central role in neuro-degeneration, and increases extracellular glutamate concentrations from 30 to 200 µM in ischemic brain [28].

A characteristic response to glutamate challenge is the increase in the cytosolic Ca^{2+} level, which is due to either influx from the extracellular space or release from the intracellular stores. In this excitotoxic condition the survival of a cell depends largely on functioning of the mitochondria. The mitochondrial potential, the driving force necessary to satisfy the cellular energy demands, is also involved in the reactive oxygen species (ROS) generation, which in turn are suspected to cause cell death if they get out of control [29].

The ELDS could control the neurodegeneration and elevated the antioxidant enzymes stated that ELDS possess antioxidant properties in this model of excitatory response through controlling the glutamate induced radical generation. Further, the reversal of MSG induced behavioral alteration with ELDS may be attributed to its central antioxidant activity [30]. In addition to its effect on behavior, the active component of the ELDS showed neuroprotective property and inhibited free radical induced cell death hippocampal slices.

All these reports clearly show that ELDS can act as a neuroprotective agent and supports our present study. These effects of ELDS were comparable with gabapentin. Gabapentin increased the superoxide dismutase, catalase and decreased TBAR levels in hippocampus and striatal regions. This effect of gabapentin may be attributed to their antagonizing activity, leading to controlling the glutamate excitotoxicity and preventing the free radical generations resulting in the preservation of brain antioxidant enzymes.

Another interesting observation made in the present study was depletion of glutathione levels with glutamate treatment. It has been stated that synthesis of glutathione in astrocytes involves glutamate, cysteine and glycine. Excess of glutamate will inhibit the transport of cysteine there by blocking the glutathione formation [31].

Further it is also reported that the oxidative pathway involves the breakdown of the glutamate-cystine antiporter leading to decrease in glutathione levels that allows for aberrant formation of free radicals that are neurotoxic [32].

In the present work administration of MSG (2g/kg) would have blocked the synthesis of glutathione and resulted in the depletion of glutathione invariably in all the groups. On pretreatment with ELDS the glutathione levels were restored. In histopathological reports the neuronal damage caused by exicitotoxicity of MSG is attenuated by ELDS and gabapentin by preventing free radical generation.

CONCLUSION: Hence it can be concluded that ELDS protected MSG induced neurodegeneration attributed to its antioxidant and behavioral properties. The study also suggests that the protection of antioxidant enzymes activity along with the direct antagonism of glutamate receptor may be beneficial. This activity of *Dalbergia sissoo* can be further explored in stroke, epilepsy and other degenerative conditions in which the role of glutamate was known to play vital role in the pathogenesis.

REFERENCES:

1. Rosemary CP, Andrew JM, Clark US and Bennett GJ: A painful peripheral neuropathy in the rat produced by the chemotherapertic drug, paclitaxel. Pain 2001; 94:293-304.
2. Ramanathan M, Sivakumar S, Anandvijayakumar PR, Saravanababu C and Rathinavel PP: Neuroprotective evaluation of standardized extract of *Centella asiatica* in monosodium glutamate treated rats. Indian J Exp Biol 2007; 45:425-431.
3. Seiva FR, Chuffa GA, Camila PB and Amorim P: Quercetin ameliorates glucose and lipid metabolism antioxidant status in postnatally monosodium glutamate-induced metabolic alterations. Food Chem Toxicol 2012; 50:3556-3561.
4. Matthias L and Reichmann H: Clinical neuroprotection in Parkinson's disease — Still waiting for the breakthrough. J Neurol Sci 2010; 289:104–114.
5. Madhava chetty K, Sivaji K and Tulasi rao K: Flowering plants of chittoor district Andhra Pradesh, India, First Edition 2008.
6. Mukhtar HS, Irum M and Khan SN: Medicinal importance and association of pathological constraints with *Dalbergia sissoo*. Pak J Phytopathol 2010; 22:135-138.
7. Pankaj SN, Dharmendra S and Kiran P: Antidiabetic activity of ethanolic extract of *Dalbergia sissoo* L. Leaves in alloxan-induced diabetic rats. IJCPR 2010; 2:24-27.
8. Adedotun AA and Alexander BO: Laboratory assessment of molluscicidal activity of crude aqueous and ethanolic extracts of *Dalbergia sissoo* plant parts against Biomphalaria pfeifferi. TMAID 2008; 6:219–227.
9. Josephin NR, Venkatarathnakumar T, Gowra R and Raadhika K: Pharmacognostic and preliminary phytochemical evaluation of the leaves of *Dalbergia sissoo* Roxb. Asian J Pharm Clin Res 2012; 5:115-119.
10. Mohammad A and Arun K: Phytochemical investigation and evaluation of antinociceptive activity of ethanolic extract of *Dalbergia sissoo* (Roxb.) bark. J Nat Sc Biol Med 2011; 2:76-79.
11. Manuj A, Mahendra B and Chopra K: Protective effect of minocycline, a semi synthetic second-generation tetracycline against 3-nitropropionic acid(3-NP)-induced neurotoxicity. Toxicology 2008; 244:111-122.
12. Chao G, Li T, Miaomaio X, Haifan Y and Hailong D: Neuroprotective effect of calycosin on cerebral ischemia and reperfusion injury in rats. J Ethnopharmacol 2012; 144:768–774.
13. Shalavandl MH, Chandrasekhar VM, Avinash SP and Sowmya c: Neuroprotective activity of stereospermum suaveolens DC against 6-OHD induced Parkinson's model. Indian J Pharmacol 2012; 44:737-743.
14. Puneet K and Kumar A: Protective role of sertraline against 3-nitropropionic acid-induced cognitive dysfunction and redox ratio in striatum, cortex and hippocampus of rat brain. Indian J Exp Biol 2009; 47:715-722.
15. Moshahid KM, Ajmal A, Tauheed I, Badruzzaman K and Nasrul HM: Resveratrol attenuates 6-hydroxydopamine-induced oxidative damage and dopamine depletion in rat model of Parkinson's disease. Brain Res 2010; 1328:139–151.
16. Nicolas A, Gillet JP, Fialip J and Coudore F: An animal model of nociceptive peripheral neuropathy following repeated cisplatin injections. Expl Neurol 2003; 182:12-20.
17. Sandeep K, Usha KM, Jayantee K, Vinay KK and Yahiya KM: Imbalance in oxidant/antioxidant system in different brain regions of rat after the infection of Japanese encephalitis virus. Neurochem Int 2009; 55:648–654.
18. Anil kumar MD and Inamdar NM: Evaluation of beneficial effects of antioxidant properties of aqueous leaf extract of *andrographis paniculata* in STZ-induced diabetes. Pak J Pharm Sci 2009; 22:49-52.
19. Hiroshi O, Nobuko O and Yagi K: Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction. Anal Biochem 1979; 95:35l-358.
20. Ellman GL: Tissue Sulphydral Groups. Arch Biochem Biophys 1959; 82:70-77.
21. Deepak KD, Veerendra CY, Siva SN, Tirtha G and Rajalingam D: Evaluation of hepatoprotective and antioxidant activity of *Ichnocarpus frutescens* (Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. Trop J Pharm Res 2007; 6:755-765.
22. Anusha J and Flora SJ: Dose related effects of nicotine on oxidative injury in young, adult and old rats. J Environ Biol 2012; 33:233-238.
23. Young DS, Pestaner LC and Gibberman V: Effects of drugs on clinical laboratory tests. Clin. Chem 1975; 21:351-353.

24. Friedman RB. Anderson RE and Hirshberg SB: Effects of diseases in clinical laboratory tests. Clin.Chem 1980; 26:209-211.
25. Chandrashekhar VM, Ranipariya VL, Ganapaty S, Parashar and Muchandi AA: Neuroprotective activity of *Matricaria recutita* Linn against global model of ischemia in rats. J Ethanopharmacol 2010; 127:645-651.
26. Jose MM, Cristina PG, Ignacio N, Maite A and Javier M: Glutamine and its relationship with Intercellular redox status, oxidative stress and cell proliferation/death. Int. J Biochem & Cell Bio 2002; 34:439-458.
27. Gupta YK, Gupta M and Kohli K: Neuroprotective role of melatonin in oxidative stress vulnerable brain. Indian J Physiol Pharmacol 2003; 47:373-386.
28. Arundine M and Tymianski M: Molecular mechanisms of glutamate-dependent neurodegeneration in ischemia and traumatic brain injury. Cell Mol Life Sci 2004; 61:657-668.
29. Nicholls DG: Mitochondrial dysfunction and glutamate excitotoxicity studied in primary neuronal cultures. Curr Mol Med 2004; 4:149-177.

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