Available online at www.abhipublications.org
Research Article
International Journal of Pharmacy and Engineering (IJPE)
ISSN 2320-849X

Impact of methanol extract of *Andrographis paniculata* against ethanol induced toxicity in brain of mice

Tarasankar Maiti[1], Arunima Datta[1] and Somenath Roy*

[1]*Department of Physiology, Bhairab Ganguly College, Kolkata-700056, West Bengal, India,*

Abstract:

Chronic ethanol intake can cause cerebral atrophy with accompanying mental impairment. Reactive oxygen species (ROS), other radicals are generated during the metabolism of ethanol and can alter the redox balance in the body, causing toxicity in brain. *Andrographis paniculata*, (Ap) a medicinal plant has been used for alleviation many diseases. The aim of the present study was to evaluate the effect of a methanol extract of *Andrographis paniculata* (ME-Ap) and vitamin E (Vit. E) against ethanol-induced oxidative stress in cerebrum and cerebellum of mice. The significant elevation of lipid peroxidation and the decreased antioxidant enzyme activities (SOD, CAT, GPx and GR) were observed in ethanol induced toxicity as compared to vehicle treated group. Pretreatment of ME-Ap (50,100,150 mg/kg b. wt./day) and Vit E (25 mg/kg b. wt./day) significantly reduced the lipid peroxidation and increased the activities of antioxidant enzymes and glutathione (GSH) in comparison to the ethanol treatment. This indicates that methanol extract of *Andrographis paniculata* may have some important components, having the antioxidant property to diminish ethanol induced oxidative stress in cerebrum and cerebellum of mice.

Keywords: Ethanol, *Andrographis paniculata*, oxidative stress, ROS, Cerebellum, Cerebrum

Received: June20, 2014, Revised: June 25, 2014, Accepted: June 30, 2014.

Corresponding Author: *Dr. Somenath Roy, Professor, Immunology and Microbiology Laboratory, Department of Human Physiology with Community Health, Vidyasagar University, Midnapore-721 102, West Bengal, India,
Email: sroy.vu@mail.vidyasagar.ac.in

1. INTRODUCTION

Alcohol addiction is associated with physiological, mental and social problems. During the last century, alcohol abuse has emerged as a major problem with health implications. Central nervous system is more markedly affected by alcohol than other system of the body. It exerts depressive action on reticular system and certain cortical sites[1]. Cerebral white matter volume is reduced in chronic alcoholics [2]. In addition to cerebral white matter shrinkage, the volume of the cerebral gray matter appears to be slightly reduced in alcoholics [3]. About 42% of alcoholics show atrophic lesions of the midline cerebellar structures, the lesion being most marked in the anterior, superior part of the vermis [4,5]. Even though alcohol-induced damage has been observed throughout the entire brain, the frontal cortex appears to be particularly vulnerable [6]. Other affected brain regions include the hypothalamus, medial temporal lobe, and cerebellum and hippocampal area [7].

The brain is vulnerable to free radical damage because of high rate of oxygen utilization, a high content of transition metals (iron and copper), its abundant lipid content, and the relative paucity of antioxidant enzymes as compared with other tissues [8]. The production of free radicals is likely favoured by the increase in low-molecular weight chelatable (LMWC) iron derivatives that was observed in the rat cerebellum following acute ethanol administration [9].Super oxide anion was identified by electron spin resonance (ESR) spectroscopy in brain microsome incubated in presence of ethanol [10].Warner and Gustafsson demonstrated the presence of cytochrome P450 in rat brain and its induction by ethanol[11]. Cytochrome P450 2E1, a variant of cytochrome P450 (i.e., isozyme) that is capable of oxidizing ethanol efficiently in other tissues, also was found in the white matter of brain of animals chronically exposed to alcohol [12].It was suggested that ethanol induce cytochrome P450-2E1 (CYP2E1) enhances reactive oxygen species (ROS) generation in the brain [13].It has been proposed that NADPH oxidase (NOX) plays in role in alcohol-induced neurodegeneration and neurotoxicity. Activation of NOX in microglia and astrocytes results in the formation of reactive oxygen species (ROS) in the brain ultimately leading to neuroinflammation and neurodegeneration. [14].These multiple lines of evidence suggest that chronic and excessive ethanol consumption enhances generation of the free radicals in brain. Disturbance in the antioxidant system that could contribute to ethanol induced oxidative stress was reported by Uysal M.et al.,1989[15], Montoliu C et at., 1994[16], Reddy SK, 1999[17].Since the cellular redox status is closely associated with cell death, an attractive hypothesis is that alterations in this balance play a key role in ethanol-mediated neurotoxicity. A number of studies have demonstrated that co-administration of antioxidants with ethanol provide significant protection against deleterious ethanol effects lending support to this hypothesis [18-19]. So, there is interest in using dietary supplements containing antioxidants to protect the components of the human body from oxidative damage.

Andrographis paniculata also known commonly as "King of Bitters," is a member of the plant family *Acanthaceae.* It is a medicinal plant which has been used through the centuries against different diseases especially as hepatoprotective agent. *Andrographis paniculata* has demonstrated a number of different pharmacological actions in in-vitro and/or animal studies. Anticancer[20], immunomodulatory[21]., anti-inflammatory[22],

antipyretic[23], hepatoprotective[24-26], hypotensive[27], hypoglycemic[28], antiplatelet [29-31],antimicrobial[32] activity have all been reported. Accordingly, the aim of the present study was to evaluate if methanol extract of this plant and vitamin E treatments have a protective effect in ethanol induced oxidative stress in cerebrum and cerebellum of mice.

2. MATERIALS AND METHODS

2.1 **Animals:**

Male Swiss mice aged 7-12 weeks were used in present study and divided in different groups, housed in polypropylene cage. The animals were fed Bengal gram, homemade bread, carrot and water *ad libitum*. The animals were maintained under standard conditions of temperature (25 ± 2 °C) and humidity (60 ± 5%) with an alternating 12 h light/12 h dark cycle. All experiments performed were in compliance with the guidelines of the National Institute of Nutrition, Hyderabad, India and approved by animal ethics committee of Vidyasagar University that follow Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.2 Plant materials:

Whole plants (*Andrographis paniculata*) were collected from the campus of Indian Institute of Technology (IIT), Kharagpur, India in the month of August and air dried. A voucher specimen (IIT- VU/ AP-1) was deposited in the herbarium of Botanical Survey of India, Sibhpur, Howrah, west Bengal, India.

2.3 Chemicals and Reagents:

Ethanol was purchased from Merck chemical company (KgaA, 64271, Darmstadt, Germany). Bovine serum albumine (BSA) was purchased from Sigma chemical co. (St. Louis, MO 63178 USA). 2-Vinylpyridine was purchased from Lancaster synthesis (Eastgate, White lund, Morecambe, England). Reduced glutathione (GSH), HCl, NaH_2PO_4, Na_2HPO_4, TCA, EDTA, H_2O_2, NaCl, cyclohexane, 5,5-dithiobis 2-nitrobenzoic acid (DTNB), pyrogallol, Tris, diethylene triamine penta acetic acid (DTPA), thiobarbituric acid (TBA), sulfosalicylic acid, chloroform, methanol, NaN_3, NaOH, $CuSO_4$, Na_2CO_3, Na-K-tartrate, Folin-ciacalteu reagent were purchased from E. Merck (India) Limited (Worli, Mumbai 400 018). NADPH, GSSG were purchased from Sisco research laboratories PVT,Ltd. (Mumbai 400 099, India).

2.4 Preparation of Methanol Extract:

The fresh aerial parts of *Adrographis paniculate* was blended and extracted with methanol (10:1) for 48 hours. The mixture was filtered with Whatman filter paper (No.1) and concentrated at 40 °C by a rotary evaporator (HITACHI, Japan) and then in water bath until the paste was form. The yield of the extract was 11.8 %. The extract was stored in an amber glass bottle and refrigerated at -20 °C and used as when required.[33]

2.5 Experimental Design:

The animals were randomized to experimental and control groups and divided into 6 groups of 6 animals each. Ethanol (4gm/ kg b. wt.) was administered by intraperitoneal (i.p.) injection. Methanol extract of plant (ME-Ap) was suspended in 5 % dimethyl sulfoxide (DMSO) in physiological saline at the dose of 50,100,150 mg/kg body weight per day. The plant extract, Vitamin E were administered by oral gavage 2 hours before ethanol treatment for a period of 28 days. The dose volume was 0.2ml. Olive oil, as a vehicle for vitamin E, was administered by oral gavage to group I, III, IV, V.

Group – I (Vehicle treated)

These animals served as control and received 0.9% NaCl solution (i.p.) for 28 days.

Group-II

These animals received ethanol at the dose of 4gm/ kg body weight, i.p. and also maintained standard diet for 28 days**.**

Group-III

These animals were treated with ethanol as in group II as well as 50mg of ME-Ap/kg body weight (i.p.) in 5 % dimethyl sulfoxide (DMSO) in physiological saline for 28 days.

Group-IV

These animals were treated with ethanol as in group II as well as 100mg of ME-Ap/kg body weight in 5 % dimethyl sulfoxide (DMSO) in physiological saline for 28 days.

Group V

These animals were treated with ethanol as in group II as well as 150mg of ME-Ap/kg body weight in 5 % dimethyl sulfoxide (DMSO) in physiological saline for 28 days.

Group VI

These animals were treated with ethanol as in group II as well as 25mg of Vit. E/kg body weight for 28 days.

2.6 Tissue collection and sample preparation:

After stipulated period of treatment, the animals were kept fasting for over night to adjust their functional activities at basal level. The mice were sacrificed, the cerebrum and cerebellum were immediately dissected out and washed with cold saline and immediately immerses in liquid nitrogen and stored at -80 °C until use. For sample preparation, tissues were sliced and homogenized in ice cold 50mM ice cold sodium phosphate buffer (pH 7.4) using glass homogenizer to yield 10 % homogenate.

2.7 *Estimation of oxidative stress markers*

2.7.1 Malondialdehyde *(*MDA):

Lipid peroxidation was measured in terms of MDA according to the method of Ohkawa et al.(1979). [34] One hundred microliters of tissue homogenate was added to 100 µl of double distilled water and 50 µl of 8.1 % sodium dodecyl sulphate (SDS) and incubated at room temperature for 10 min. Three hundred seventy five micriliters of 20% acetic acid (pH 3.5), along with 375 µl of thiobarbituric acid (0.6%), was added to the tissue solution and placed in a boiling water bath for 60 min. After incubation, 250 µl of double distilled water and 1.25 ml of 15:1 butanol-pyrimidine solution were added to the mixture and centrifuged for 5 min at 2000×g. Resulting supernatant was removed and measured at 532 nm with the use of the Hitachi U-2000 spectrophotometer. The MDA in the sample was calculated by using the extinction co-efficient 1.56 x 10^5 mol^{-1} cm^{-1} and expressed in the unit of nmol/mg of tissue.

2.7.2 Conjugated Dienes*:*

Conjugated dienes was measured according to the method of Slater, 1984. [35] Lipids were extracted with chloroform methanol and the lipid residue was dissolved in cyclohexane and absorbance of the formed hydroperoxide is noted at 233 nm in spectrophotometer. The amount of conjugated dienes were calculated using the extinction co-efficient of 2.80 x 10^4 mol^{-4} cm^{-4}. Unit of conjugated dienes of experimental tissue was expressed in the from of nmol/mg of tissue.

2.7.3 Oxidized glutathione (GSSG):

GSSG was measured by the method of Griffith (1980). [36] The required amount of tissue homogenate was mixed with 12% sulfosalicylic acid and centriguged at 2000×g for 15 min to settle the precipited proteins. 0.1 ml of protein free supernatant incubated in room temperature with 0.005 ml of 2M 2- venyl pyridine for 1 h. Following incubation, 0.4 ml of 0.5 mM NADPH, 0.1 ml DTNB and 0.48 unit of glutathione reductase were added and measured at 412 nm. For calibration a standard curve of oxidized glutathione was prepared treating varied concentration of GSSG with DTNB. GSSG content of each sample was calculated from corresponding value of absorbance by referring to the standard curve. Concertration of GSSG is expressed as mM/g tissue.

2.8 Estimation of antioxidant systems

2.8.1 Activity of superoxide dismutase (SOD): SOD activity was estimated by measuring the percentage inhibition of the pyrogallol auto- oxidation by SOD according to the method Marklund & Marklund, (1974). [37] In blank cuvette 2.04 ml of 50 mM TRIS was taken. In a test cuvette 2.02 ml, TRIS & 20µl pyrogallol was taken. Then auto-oxidation was recorded at 420 nm for 3min. Thus, a standard auto -oxidation curve was plotted. Next, for measuring the inhibition of auto-oxidation by SOD of different samples in the test cuvette 2ml of 50 mM TRIS, 20µl pyrogallol and 20 µl, of each sample were added and the inhibition of auto-oxidation was noted for 3min and O.D. were taken. 1 unit of SOD was defined as the enzyme activity that inhibits the auto -oxidation of pyrogallol by 50%. Specific activity of SOD is expressed in terms of unit/mg protein.

2.8.2 Catalase activity: Catalase activity was measured by estimating the decomposed H_2O_2 from the molar extinction coefficient of 46.6 M^{-1} cm^{-1} at 240 nm in spectrophotometer (Beers and Sizer 1952). [38] Specific activity of catalase is defined in terms of mMol of H_2O_2 consumption / mg of tissue / minute.

2.8.3 Reduced Glutathione (GSH):

GSH was measured according to the modified method of Griffith, 1980. [36] The required amount of tissue homogenate was mixed with 12% sulfosalicylic acid and centriguged at 2000×g for 15 min to settle the precipited proteins. 0.1 ml of protein free supernatant, 0.7 ml of 0.3mM NADPH, 0.1 ml of 6 mM DTNB and 0.48 units of glutathione reductase were combined and the absorbance of 5-thio-2-nitrobenzoic acid (TNB) was read at 412 nm. The glutathione content of aliquot assayed was determined by comparison of the rate observed to a standard curved generated with known amounts of glutathione. The level of GSH was expressed micromoles/min/gm of tissue.

2.8.4 Glutathione peroxidase activity (GPx): ***GPx activity was measured according to method of Pagila and Valentine (1967). [39] The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 1mM EDTA, 1 mM sodium azide, 0.2 mM NADPH, 1 U glutathione reductase and 1 mM reduced glutathione. The sample, after its addition, was allowed to equilibrate for 5 min at 25ºC. The reaction was initiated by adding 0.1 ml of 2.5 mM H_2O_2. Absorbance at 340nm was recorded for 5 min. Values were expressed as nM NADPH oxidized to NADP by using the extinction coefficient of 6.22 x $10^{-3}M^{-1}$ cm^{-1}. The activity of GPx was expressed in terms of nanomole NADPH consumed per min per mg of protein.***

2.8.5 Glutathione reductase activity (GR): ***The activity of GR was measured by the method of Miwa, (1972). [40] The tubes for enzyme assay were incubated at 37ºC and contained 2 ml of 9 mM GSSG, 0.02 ml of 12 mM NADPH Na_4, 2.68 ml of 1/15 M phosphate buffer (pH 6.6) and 0.1 ml of tissue homogenate. The activity of this enzyme was determined by monitoring the decrease in absorbance at 340nm. Enzyme activity was expressed as substrate (nmol NADPH) transformed min^{-1}mg $protein^{-1}$.***

2.9 Protein Estimation:

The total protein was measured according to the method of Lowry *et al.*, (1951)[41] using bovine serum as standard. 10µl of tissue supernatant was diluted in 490 µl 0.9% NaCl and 5 ml of freshly prepared alkaline reagent was added. An incubation time of 10 minutes were allowed at room temperature. To all the tubes 0.5 ml of Folin-Ciacalteu was added and mixed thoroughly. After 30 minutes, absorbances of mixtures were taken against a reagent blank (0.5 ml 0.9% NaCl instead of supernatant) at 660 nm in a spectrophotometer.

2.10 Statistical analysis: The data were expressed as mean ± standard error. Comparisons of the means of control treated and treated groups were made by model I ANOVA with multiple comparison t test (Das and Das, 1998).[42] Probability value equal or below 0.05 ($p < 0.05$) was considered as significant.

3. RESULTS

3.1 Effect of ethanol and plant extract on body weight and organ weight:

The body weights of mice were noted before ethanol treatment. Then the body weights of the mice of all six groups were taken every seven days from the day of treatment to the day of sacrifice. Changes in body weight during the period of treatment were depicted in Table 1. It was observed that the body weights of the ethanol treated mice were not significantly altered when compared with that of the control groups. It was further observed that the body weights of ethanol-treated mice were remained not significantly unaltered even when they were supplemented with ME-Ap and Vit E (Table 1).

There was no significant decreased in the weight of cerebrum and cerebellum after ethanol administration with respect to vehicle controls. In contrast, supplementation with ME-Ap and Vit E exhibited no significant recovery towards the control level in cerebrum and cerebellum weight (Table 1).

Table 1: Effect of Methanol Extract of *Andrographis paniculata* on body weight and tissue weight in response to ethanol.

Groups	Body weight (gm)		Tissue weight (mg)	
	Initial	Final	Cerebrum	Cerebellum
CONTROL	20.6±0.42	25.8±0.51	212.3±12.69	90.1±4.40
ETHANOL (4 mg/kg b. wt.)	21.3±0.80	22.6±0.66	208.5±14.02	78.6±4.51
ETHANOL + 50 mg/kg ME-Ap	22.3±0.58	26.3±0.84	214.8±12.67	87.6±4.04
ETHANOL + 100 mg/kg ME-Ap	21.6±0.31	25.5±0.50	215.5±11.24	86.8±3.87
ETHANOL+ 150 mg/kg ME-Ap	22.2±0.31	26.6±0.76	213.3±13.54	85±6.85

ETHANOL + 25 mg/kg Vit. E	21.6±0.61	26.5±0.34	210.5±19.02	86.1±5.66

The results are expressed as means ± SEM of six mice.

[a] Indicates significant difference between two groups when compared with control (P<0.05)
[b] Indicates significant difference between two groups when compared with treated (P<0.05)

3.2 Content of Malondialdehyde (MDA) and Conjugated Dines (CD):

These are the important marker of lipid peroxidation by radicals. MDA and CD levels were significantly (P<0.05) increased in cerebrum and cerebellum of ethanol treated mice compared to the control group (Fig. 1-4). In cerebrum, supplementation with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) showed significant (P<0.05) diminution of MDA content by 27%, 40%, 84% and 111% respectively, as compared to ethanol treated group (Fig.1). Similarly, in cerebellum supplementation with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) significantly (P<0.05) decreased the MDA level by 35%, 43%, 72% and 97% respectively, as compared to ethanol treated group (Fig.2).

In cerebrum, supplementation with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) showed a significant (P<0.05) diminution of CD level by 56%, 68%, 85%, 88% respectively, as compared to ethanol treated group (Fig.3). In cerebellum supplementation with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) significantly (P<0.05) decreased the CD level by 52%, 64%, 72% and 80% respectively, as compared to ethanol treated group (Fig. 4).

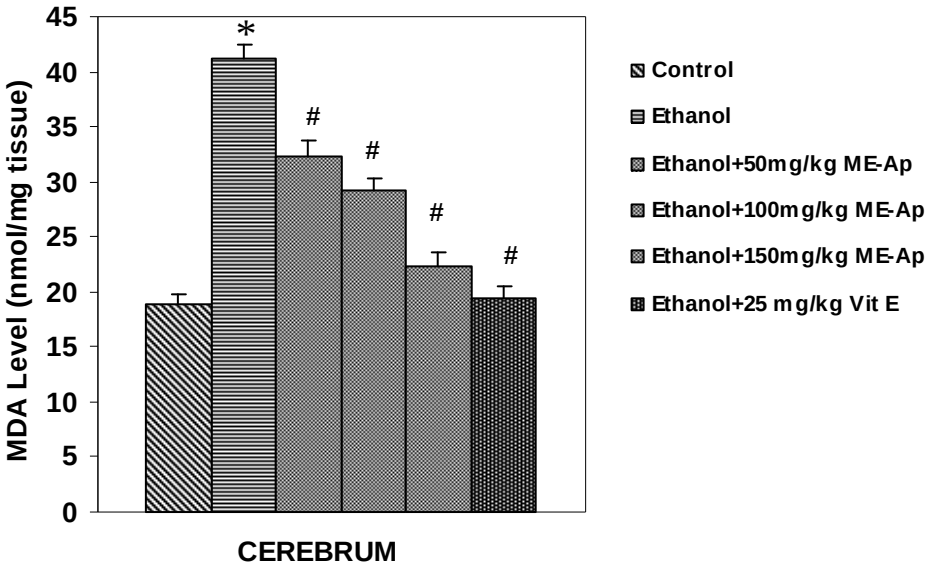

Fig. - 1 : Effect of Methanol extract of Andrographis paniculata on MDA level in Cerebrum of Mice Treated with Chronic dose of Ethanol.
*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

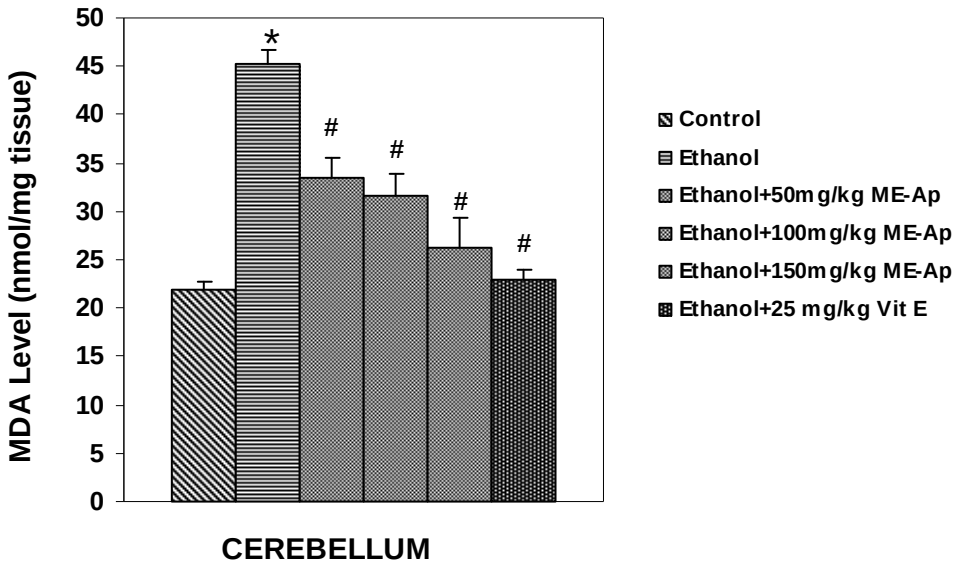

Fig. - 2 : Effect of Methanol extract of Andrographis paniculata on MDA level in Cerebellum of Mice Treated with Chronic dose of Ethanol.

*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

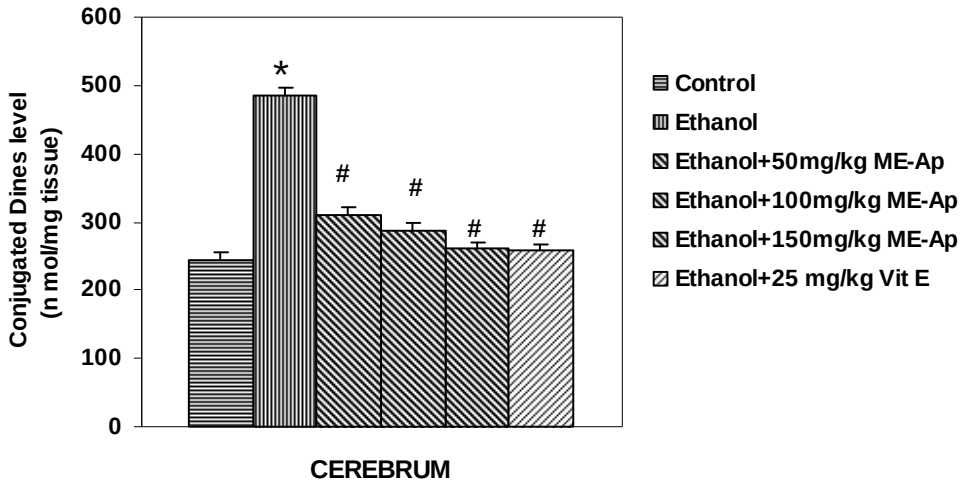

Fig. - 3 : Effect of Methanol extract of Andrographis paniculata on CD level in Cerebrum of Mice Treated with Chronic dose of Ethanol.

*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

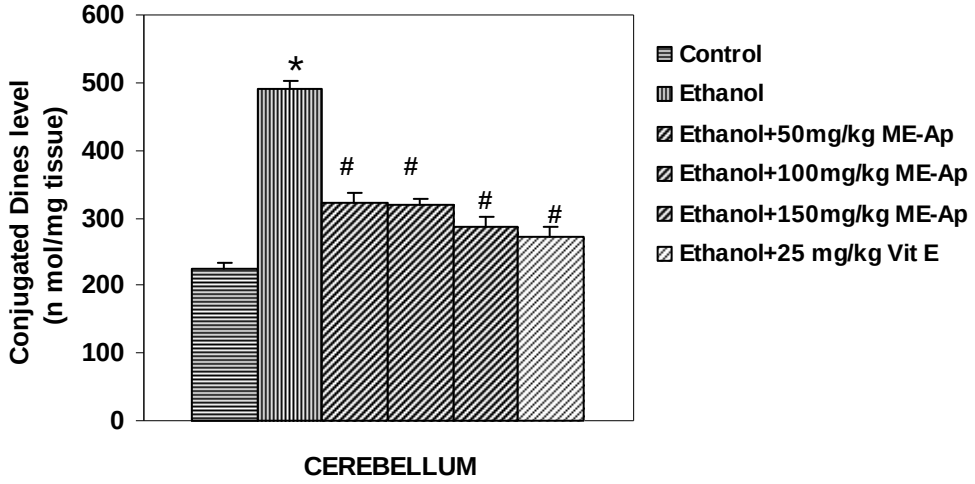

Fig. - 4: Effect of Methanol extract of Andrographis paniculata on CD level in Cerebellum of Mice Treated with Chronic dose of Ethanol.

*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

3.3 Content of GSH and GSSG:

In cerebrum and cerebellum, the GSH content decreased significantly (P<0.05) due to ethanol treatment when compared to vehicle treated control (Fig. 5-6). In contrast, pre-exposure of ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) followed by ethanol treatment results significant(P<0.05) elevation of GSH level by 78%, 102%, 157% and 158% respectively, in cerebrum as compared to ethanol treated group (Fig 5). In cerebellum, supplementation with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) showed significant (P<0.05) elevation of GSH content by 80%, 110%, 129% and 137% respectively, as compared to ethanol treated group (Fig.5).

The GSSG levels in cerebrum and cerebellum were significantly (P<0.05) increased due to ethanol treatment (Fig.7-8). Supplementation with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) showed significant (P<0.05) diminution of GSSG level by 46%, 56%, 63%, 84% respectively, in cerebrum as compared to ethanol treated group (Figure 7). In cerebellum, supplementation with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) showed significant (P<0.05) diminution of GSSG level by 35%, 40%, 60%, 64% respectively, as compared to ethanol treated group (Figure 8).

The GSH/GSSG ratio, an index of tissue oxidative stress, decreased significantly (P<0.05) in experimental tissues due to ethanol treatment when compared to control group (Table2,3). The supplemented groups showed the significant alteration (P<0.05) of this redox ratio in a dose dependent manner in experimental tissue in comparison to ethanol treated group (Table2,3).

Table 2. Concentration of Reduced Glutathione, Oxidized Glutathione and the GSH/GSSG Ratio of Cerebrum from mice treated with Ethanol

Groups	CEREBRUM		
	GSH (µmol/g tissue)	GSSG (µmol/g tissue)	GSH/GSSG Ratio
CONTROL	11.13±0.81	0.42±0.01	26.50
ETHANOL (4 mg/kg b. wt.)	4.08±0.17 [a]	0.72±0.02 [a]	5.66 [a]
ETHANOL + 50 mg/kg ME-Ap	7.27±0.37 [b]	0.49±0.015 [b]	14.83 [b]
ETHANOL + 100 mg/kg ME-Ap	8.28±1.09 [b]	0.46±0.012 [b]	18.0 [b]
ETHANOL+ 150 mg/kg ME-Ap	10.49±0.58 [b]	0.44±0.02 [b]	23.84 [b]
ETHANOL + 25 mg/kg Vit. E	10.56±0.91 [b]	0.39±0.01 [b]	27.07 [b]

The results are expressed as means ± SEM of six mice.

[a] Indicates significant difference between two groups when compared with control (P<0.05)
[b] Indicates significant difference between two groups when compared with treated (P<0.05)

Table 3. Concentration of Reduced Glutathione, Oxidized Glutathione and the GSH/GSSG Ratio of Cerebellum from mice treated with Ethanol

Groups	CEREBELLUM		
	GSH (µmol/g tissue)	GSSG (µmol/g tissue)	GSH/GSSG Ratio
CONTROL	10.81±0.15	0.39±0.02	27.71
ETHANOL (4 mg/kg b. wt.)	4.34±0.26 [a]	0.69±0.01 [a]	6.28 [a]
ETHANOL + 50 mg/kg ME-Ap	7.84±0.36 [b]	0.51±0.02 [b]	15.37 [b]
ETHANOL + 100 mg/kg ME-Ap	9.15±0.79 [b]	0.49±0.02 [b]	18.67 [b]
ETHANOL+ 150 mg/kg ME-Ap	9.95±0.13 [b]	0.43±0.01 [b]	23.13 [b]
ETHANOL + 25 mg/kg Vit. E	10.32±0.33 [b]	0.42±0.02 [b]	24.57

The results are expressed as means ± SEM of six mice.

[a] Indicates significant difference between two groups when compared with control (P<0.05)
[b] Indicates significant difference between two groups when compared with treated (P<0.05)

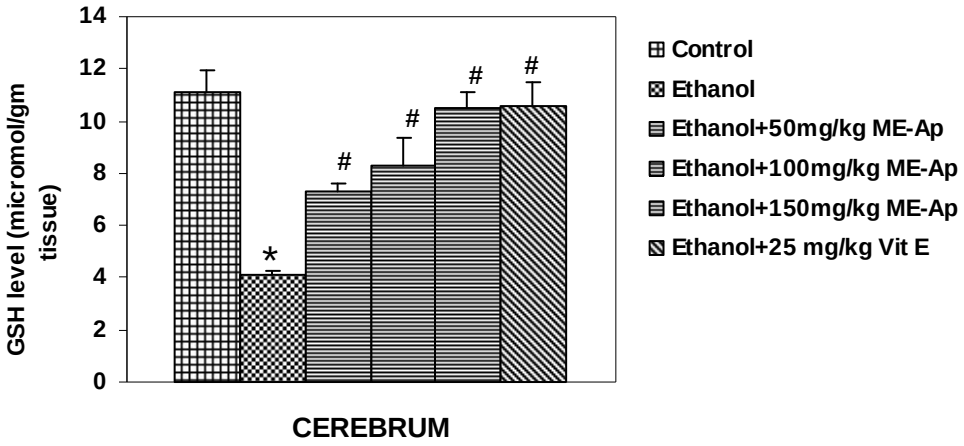

Fig. - 5 : Effect of Methanol extract of Andrographis paniculata on GSH level in Cerebrum of Mice Treated with Chronic dose of Ethanol.
*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

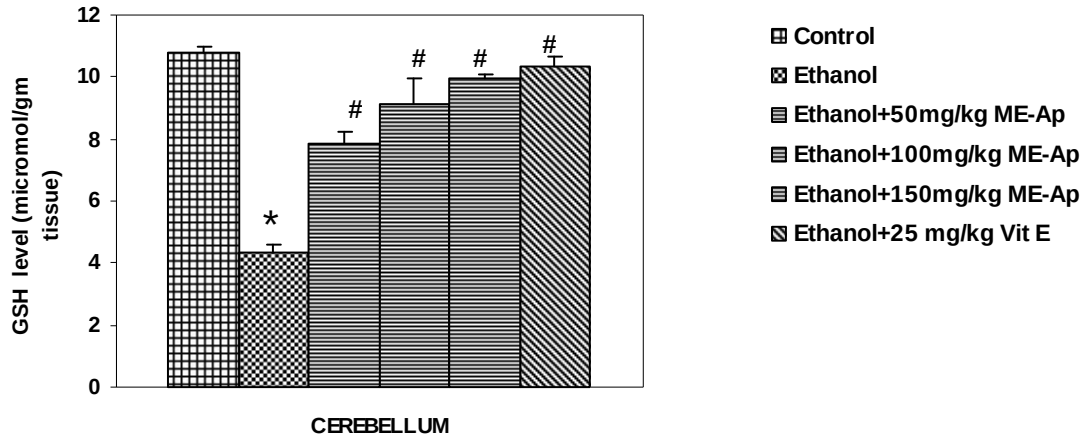

Fig. - 6 : Effect of Methanol extract of Andrographis paniculata on GSH level in Cerebellum of Mice Treated with Chronic dose of Ethanol.
*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

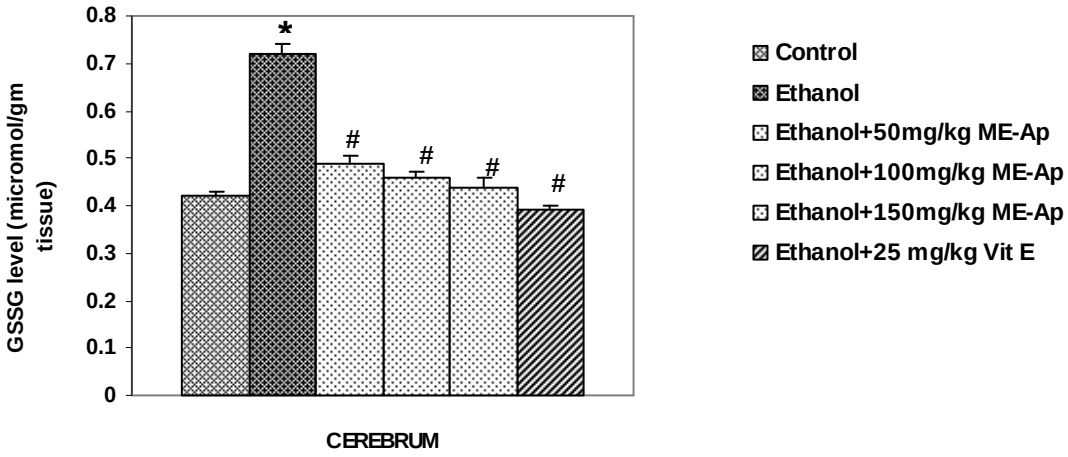

Fig. - 7 : Effect of Methanol extract of Andrographis paniculata on GSSG level in Cerebrum of Mice Treated with Chronic dose of Ethanol. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

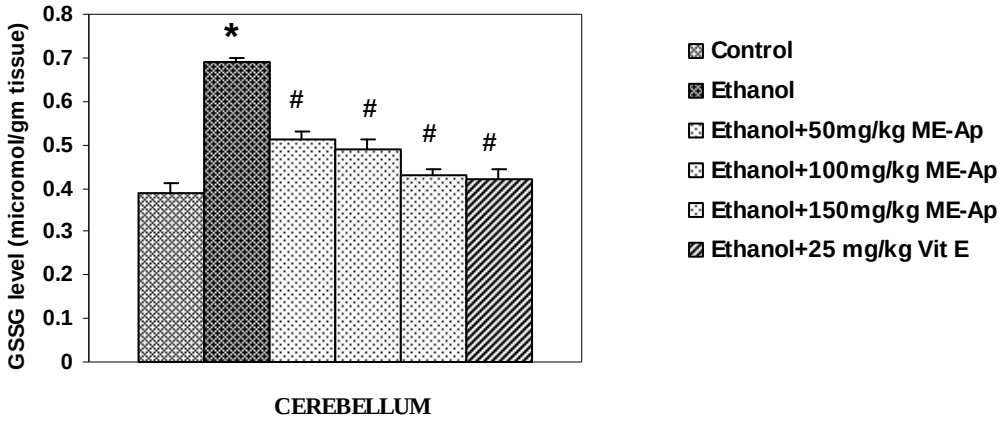

Fig. - 8 : Effect of Methanol extract of Andrographis paniculata on GSSG level in Cerebellum of Mice Treated with Chronic dose of Ethanol. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

3.4 Antioxidant enzyme activities

The superoxide dismutase (SOD) activity of cerebrum was significantly (P<0.05) reduced by 46.87% due to ethanol treatment in comparison to control. The pre-treatment of mice with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) restored significantly (P<0.05) the SOD activity by 72%, 83%, 109% and 113% respectively, as compared to ethanol treated group (Fig 9).

Similarly, in cerebellum the SOD activity was significantly (P<0.05) reduced by 48.05% due to ethanol treatment in comparison to control. Supplementation of mice with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) restored significantly (P<0.05) the SOD activity by 56%, 63%, 104% and 103% respectively, as compared to ethanol treated group (Fig 10).

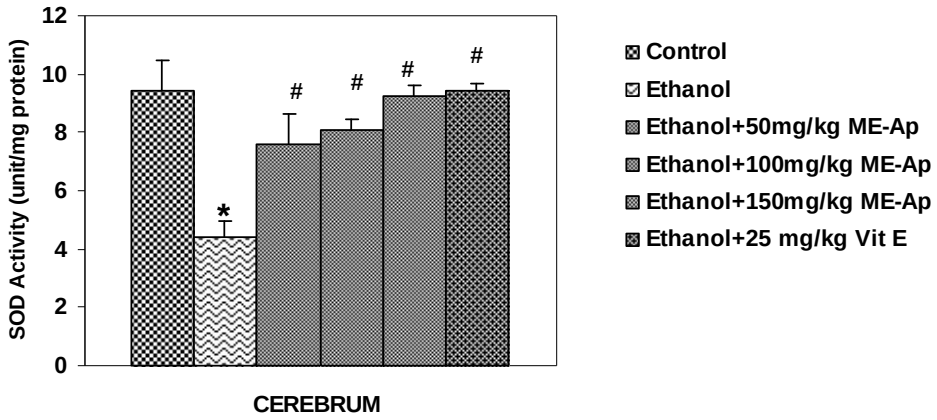

Fig. - 9 : Effect of Methanol extract of Andrographis paniculata on SOD activity in Cerebrum of Mice Treated with Chronic dose of Ethanol. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

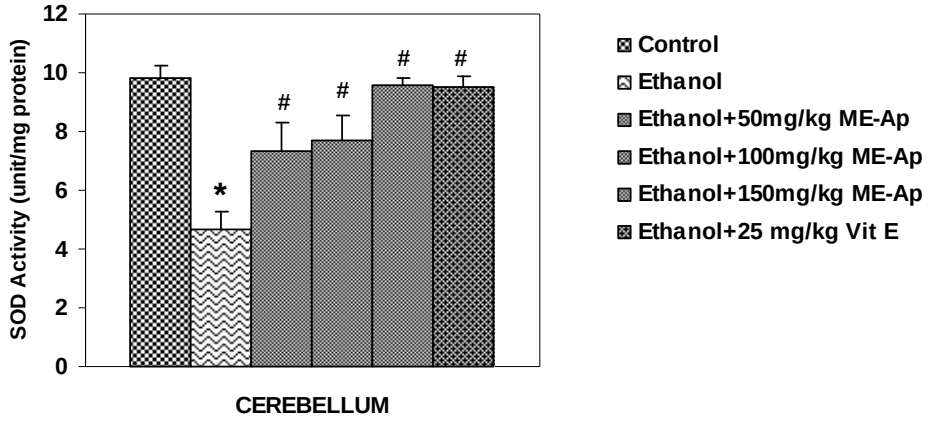

Fig. - 10 : Effect of Methanol extract of Andrographis paniculata on SOD activity in Cerebellum of Mice Treated with Chronic dose of Ethanol. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

In cerebrum, the catalase (CAT) activity was decreased by 51.13% due to ethanol administration. A significant rise (P<0.05) in CAT activity was noted on supplementation of mice with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) by 63.9%,68.59%,79.98% and 83.62% respectively, as compared to ethanol treated group (Fig 11).

There was significantly (P<0.05) reduced CAT activity by 46% in cerebellum due to ethanol administration. ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) supplementation to ethanol treated groups showed a significant rise (P<0.05) in CAT activity by 82.8%, 93.52%, 99.84% and 114.71% respectively (Fig. 12).

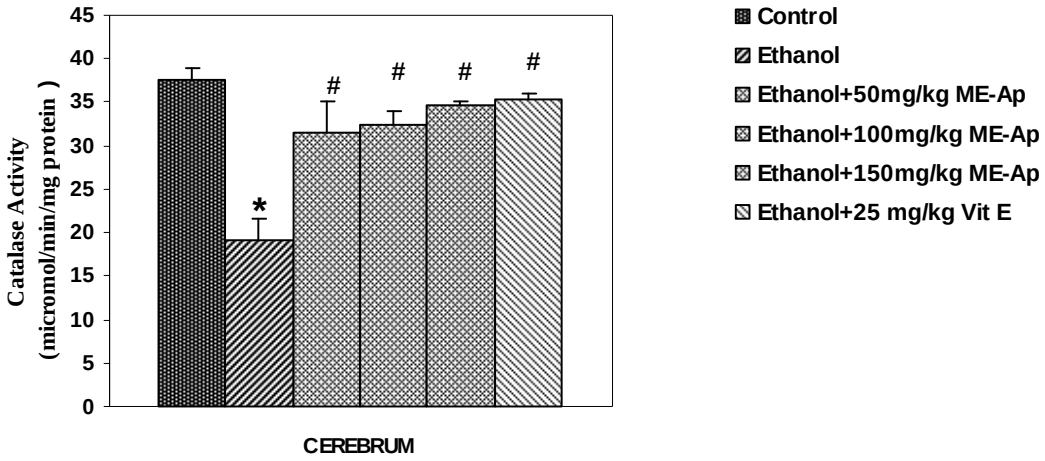

Fig. - 11 : Effect of Methanol extract of Andrographis paniculata on Catalase activity in Cerebrum of Mice Treated with Chronic dose of Ethanol.
*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

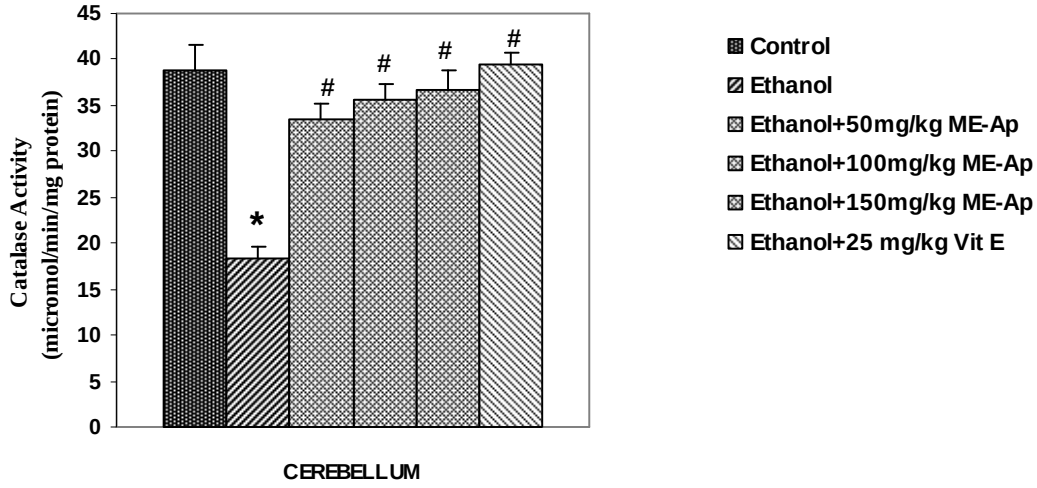

Fig. - 12: Effect of Methanol extract of Andrographis paniculata on Catalase activity in Cerebellumof Mice Treated with Chronic dose of Ethanol.
*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

Due to ethanol intoxication, the glutathione peroxidase (GPx) activity of cerebrum was significantly (P<0.05) decreased by 48.13% in comparison to control. The pre-treatment of mice with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) restored significantly (P<0.05) the GPx activity by 60%, 79.96%, 95.44% and 101.26% respectively, as compared to ethanol treated group (Fig 13).

In cerebellum the GPx activity was significantly (P<0.05) reduced by 53.37% due to ethanol treatment in comparison to control. Supplementation with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) restored significantly (P<0.05) the GPx activity by 65.33%, 79.13%, 83.54% and 90.99% respectively, as compared to ethanol treated group (Fig 14).

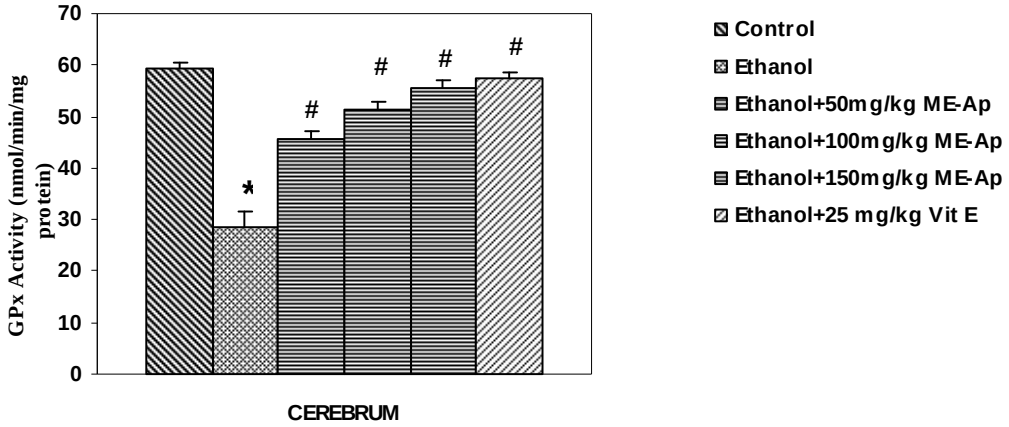

Fig. - 13 : Effect of Methanol extract of Andrographis paniculata on Glutathione peroxidase activity in Cerebrum of Mice Treated with Chronic dose of Ethanol.
*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

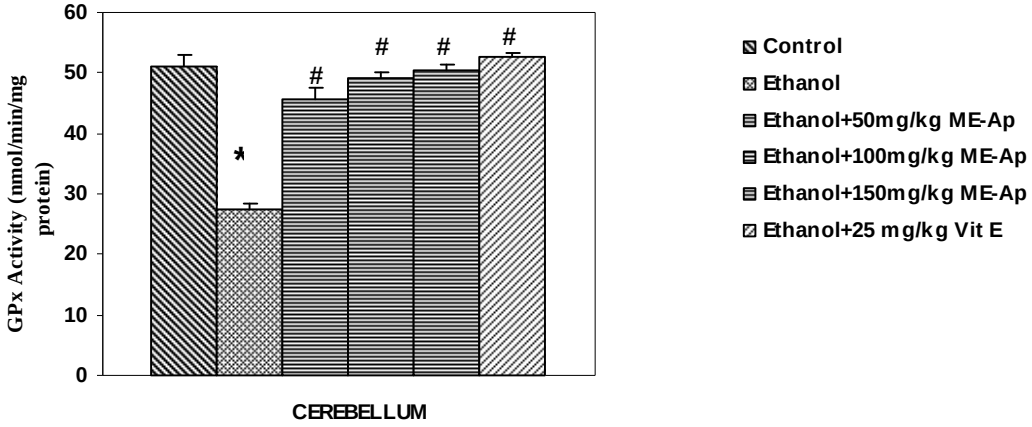

Fig. - 14 : Effect of Methanol extract of Andrographis paniculata on Glutathione peroxidase activity in Cerebellum of Mice Treated with Chronic dose of Ethanol.
*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

In cerebrum, the glutathione reductase (GR) activity was decreased by 32.26% due to ethanol administration. A significant rise (P<0.05) in GR activity was noted on supplementation of mice with ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) by 81.48%,118.96%,159.15% and 188.62% respectively, as compared to ethanol treated group (Fig 15).

There was significantly (P<0.05) reduced GR activity by 39.24% in cerebellum due to ethanol administration. ME-Ap (50mg/kg, 100mg/kg, 150mg/kg) and Vit E (25mg/kg) supplementation to ethanol treated groups showed a significant rise (P<0.05) in GR activity by 82.09%, 115.59%, 128.72% and 147.26% respectively (Fig. 16).

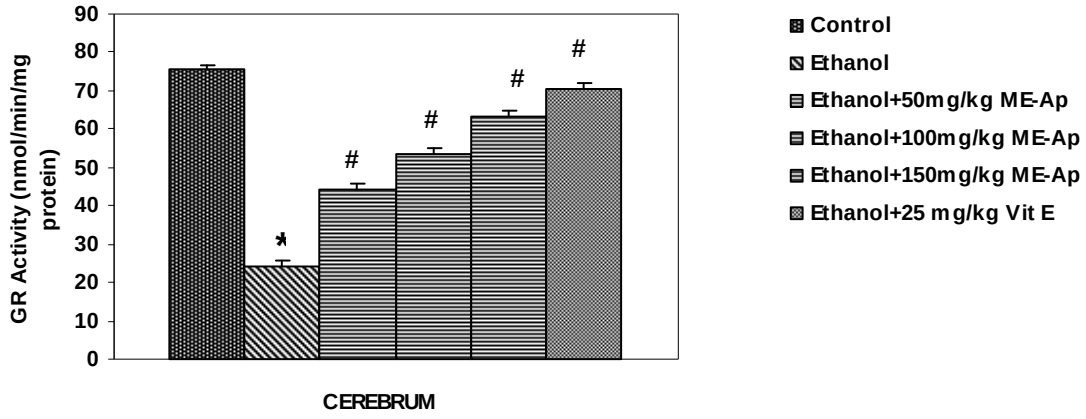

Fig. - 15 : Effect of Methanol extract of Andrographis paniculata on Glutathione reductase activity in Cerebrum of Mice Treated with Chronic dose of Ethanol.
*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

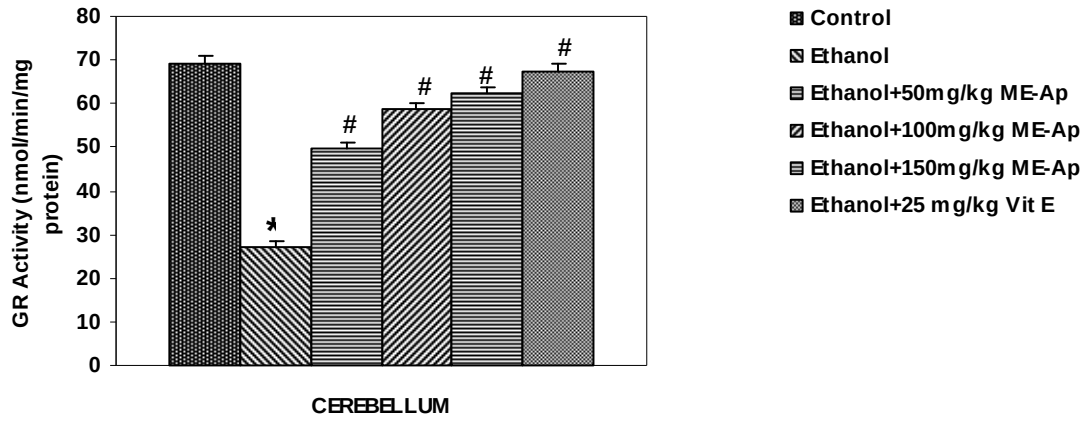

Fig. - 16 : Effect of Methanol extract of Andrographis paniculata on Glutathione reductase activity in Cerebellum of Mice Treated with Chronic dose of Ethanol.
*** P<0.05 Compared to control, # P<0.05 Compared to ethanol**

4. DISCUSSION

The deleterious effects of ethanol on nervous system could result either from a direct toxic effect of ethanol or from an indirect effect involving free radical generation through it metabolism. In the central nervous system, astrocytes represent the major cellular localisation of ethanol metabolism. Three metabolic pathways of ethanol have been described in the human body so far. They involve the following enzymes: alcohol dehydrogenase, microsomal ethanol oxidation system (MEOS) and catalase. Each of these pathways could produce radicals which affect the antioxidant system of body and oxidative stress [43].Various *in vitro*, as well as *in vivo*, data suggest that prenatal and postnatal ethanol induces elevated level of oxidative stress either by generation of free radicals (ROS/RNS) or disruption of antioxidative defense mechanisms and, thereby, promotes cell death in the cerebellum of rodent brains and cortical neurons[44,45].

In the present investigation, we found that ethanol treatment significantly increases the MDA and CD content in the experimental tissues. Researchers found that lipid peroxides were increased in the rat brain through acute and chronic ethanol administration [16,46,47,48].Lipid peroxidation is one of the main manifestations of oxidative damage initiated by ROS. Enhanced lipid peroxidation associated with cellular antioxidants depletion in tissue, may yield variety of neurodegenerative disorders [13].In this study, elevated levels of MDA and CD were restored due to the free radical scavenging property of methanol extract of *Adrographis paniculate* and vitamin E. In our previous report, we have demonstrated that the active principle of this plant has a hepato-renal protective activity against ethanol toxicity [49].

This study showed that chronic ethanol administration caused significant depletion of SOD activities as compared with those of the control mice. The decreased activity of SOD was may be due to dispose off the superoxide anions, produced by ethanol toxicity. A significant elevation of superoxide anion generation was observed in brain sub-mitochondrial particles isolated from rats having received an aqueous ethanol solution (10% v/v) as their sole drinking fluid for 4 weeks [50].The increase in brain mitochondrial superoxide anion generation following a moderate alcohol intoxication model suggests that these mitochondria represent an early target for free radical attack. Since, CytP450 IIE1 is induced in the brain by chronic ethanol treatment [16] an increased free radical generation may also occur at the microsomal level. Another disturbance that may contribute to an enhanced generation of aggressive prooxidant radicals is the increase in low-molecular weight iron species that was apparent in cerebellar cytosolic fraction after long term ethanol administration [51]. The administration of ME-Ap and alpha tocopherol increases in SOD activity, a fact that has been confirmed in the pretreatment carrier out in our investigation.

Beside this, on ethanol treatment, hydrogen peroxide produced by dismutation of superoxide anion, converted to O_2 and water by catalase and the activity of this enzyme showed a marked reduction. The depletion of these antioxidant enzyme activities may be due to inactivation of enzyme protein by ethanol induced ROS generation, down regulation of transcription and translation processes. Decreased SOD and CAT activities reflect the susceptibility of brain to ethanol induced oxidative stress. Administration of methanol extract before ethanol treatment significantly increases the activity of CAT. Bondarenko L B., et al. (2014) observed decrease of brain catalase activity in rats

chronically exposed to 15 % ethanol solution [52]. Other authors suggest that catalase could be a more major contributor to ethanol oxidation in the brain [53]. Inhibition of brain catalase activity in mice inhibits many of the pharmacologic effects of ethanol and changes formation of acetaldehyde in brain homogenates [53]. In our present investigation, significantly decreased activity of catalase activities were returned to the control level, were may be due to either activation of enzyme protein or scavenging of free radicals by the plant extract and vit E.

Reduced glutathione (GSH) is an endogenous nonenzymatic antioxidant of cell that protects biomolecules against free radicals, peroxides and the detoxification of drugs. It is oxidized to glutathione disulfide (GSSG) by glutathione peroxidase (GPx). In this study the significant fall of GSH levels, GSH/GSSG ratio and GPx activities were observed after ethanol treatment and may indicate the more utilization of GSH for scavenging of ethanol-induced H_2O_2 and inhibition of endogenous synthesis of GSH. A decrease in GSH/GSSG ratio [16] in brain and decreased GSH levels in both striatum and cerebellum [54] after chronic ethanol treatment suggested that these changes could be the consequence of increased generation of prooxidant free radicals, leading to oxidative attack in the brain tissue. Pretreatment with ME-Ap and vit E restored the GSH level, and GSH/GSSG ratio. It was also observed that the GSSG levels, after ME-Ap and vit. E administration, diminished.

Chronic ethanol administration to vitamin E-deficient rats elevated GR activity in the cerebral cortex, cerebellum and brain stem and GPx activity in the cerebral cortex and cerebellum [55]. In this investigation, the significant fall GR activity was observed after ethanol treatment and pretreatment with plant extract and vit E restored GR activity as compared to the ethanol treated group. In this study, pretreatment with ME-Ap and vit.E were able to compensate the antioxidant status, more or less to the control level. One possible reason behind this findings is may be the antioxidant property of plant extract.

5. CONCLUSION

Chronic ethanol administration altered the antioxidant defense system of brain. In this investigation, supplementation of mice with ME –Ap and vit E were able to compensate antioxidant enzyme status, i.e., significantly and dose dependently prevent the oxidative damage of brain. One possible reason behind this findings is may be antioxidant property of plant extract. However, further studies are required to establish the active principle of plant extract as a neuroprotective agent.

6. Acknowledgement: Financial support from the Vice chancellor, Vidyasagar University is acknowledged.

7. REFERENCES:

[1] Himwich h. E. and Callison D. A. The effects of alcohol on evoked potentials of various parts of the central nervous system of the cat. In, The Biology of alcoholism. Vol. 2, Physiology and behavior. (Kissin, B., and Begleiter, H., eds.) Plenum Press, New York, 67-84, (1972).

[2] Harper C. G., Kril J. J. and Holloway, R. Brain shrinkage in chronic alcoholics: a pathological study, *Br Med J,* **290**: 501-504,(1985).

[3] de la Monte S. M. Disproportionate atrophy of cerebral white matter in alcoholics. *Arch Neurol*, **45**: 990-992,(1988).

[4] Victor M., Adams, R. D. and Mancall, E. L. A restricted form of cerebellar cortical degeneration occuring in alcoholic patients. *Arch neurol*, **1**: 579-688, (1959).

[5] Torvic A. and Torp, S. The prevalence of alcoholic cerebellar atrophy. A morphometric and histological study of an autopsy material. *J Neurol Sci*, **75**: 43-51, (1986).

[6] Kubota M, Nakazaki S, Hirai S, et al. Alcohol consumption and frontal lobe shrinkage: Study of 1432 non-alcoholic subjects. *J. Neurology, Neurosurgery, and Psychiatry*. **71**:104–106, (2001).

[7] Fadda F. and Rossetti Z. L. Chronic ethanol consumption: from neuro-adaption to neurodegeneration. *Prog. Neurobiol*. 56: 385-431, (1998).

[8] Skaper SD, Floreani M, Ceccon M, Facci L, Giusti P. Excitotoxicity, oxidative stress, and the neuroprotective potential of melatonin. *Ann NY Acad Sci* ,890: 107-18, (1999).

[9] Rouach H., Houze P., Orfanelli M. T., Gentil M., Bourdon P., and Nordmann R Effect of acute ethanol administration on the subcellular distribution in rat liver and cerebellum. *Biochem. Pharmacol*, 39. 1095-110, (1990).

[10] Gonthier B., Jeunet a., and Burret L., Electron spin resonance study of free radical produced from ethanol and acetaldehyde after exposure to a Fenton system or to brain and liver microsomes. *Alcohol,* **8**:369-375, (1991).

[11] Warner, M., and Gustafsson, J.A. Effect of ethanol on cytochrome P450 in the rat brain. Proceedings of the National *Academy of Sciences of the United States of America* **91**:1019–1023, (1994).

[12] Hansson, T., Tindberg, N., Ingelman-Sundberg, M., Kohler, C. Regional distribution of ethanol-inducible cytochrome P450 IIE1 in the rat central nervous system. *Neuroscience* **34,** 451–463, (1990).

[13] Sun, A. Y., Sun, G. Y. Ethanol and oxidative mechanisms in the brain. *J. Biomed. Sci.* **8,** 37–43, (2001).

[14] Qin, L., Crews F. T. NADPH oxidase and reactive oxygen species contribute to alcohol-induced microglial activation and neurodegeneration . *J Neuroinflammation,* **9:** 1-19, (2012).

[15] Uysal M, Katalp G, Ozdemirler G, Aykac G. Ethanol-induced changes in lipid peroxidation and glutathione content in rat brain. *Drug Alcohol Depen*. 23:227–230, (1989).

[16] Montoliu C, Valles S, Renau-Piqueras J, Guerri C. Ethanol-induced oxygen radical formation and lipid peroxidation in rat brain: effect of chronic alcohol consumption. *J Neurochem*. **63**:1855–1862, (1994).

[17] Reddy SK, Husain K, Schlorff EC, Scott RB, Somani SM. Dose response of ethanol ingestion on antioxidant defense system in rat brain subcellular fractions. *NeuroTox*. **20**:977–988, (1999).

[18] Heaton MB, Mitchell JJ, Paiva M. Amelioration of ethanol-induced neurotoxicity in the neonatal rat central nervous system by antioxidant therapy. *Alcohol Clin Exp Res*. **24**:512–518, (2000).

[19] Shirpoor A, Salami S, Khadem-Ansari MH, Minassian S, Yegiazarian M. Protective effect of vitamin E against ethanol-induced hyperhomocysteinemia, DNA damage, and atrophy in the developing male rat brain. *Alcohol Clin Exp Res*. **33**:1181–1186, (2009).

[20] Sridevi K. Anticancer and immunostimulatory compounds from Andrographis paniculata. *J Ethnopharmacol*, **92**(2-3): 291-295, (2004).

[21] Puri A. Immunostimulant agents from Andrographis paniculata. *J Nat Prod*, **56**(7): 995–999. (1993).

[22]Amroyan E. Inhibitory effect of andrographolide from A. paniculata on PAF-induced platelet aggregation. *Phytomed* **6**(1): 27–31, (1996)

[23] Mandal SC, Dhara AK, Maiti BC. Studies on psychopharmacological activity of Andrographis paniculata extract. *Phytother Res* **15**(3): 253-256, (2001).

[24] Handa SS, Sharma A. Hepatoprotective activity of andrographolide against galactosamine & paracetamol intoxication in rats. *Indian J Med Res* **92**: 284–292, (1990).

[25] Handa S S, Sharma A. Hepatoprotective activity of andrographolide from Andrdographis paniculata against carbontetrachloride. *Indian J Med Res* **92**: 276–283 (1990).

[26]Rana AC, Avadhoot Y. Hepatoprotective effects of Andrographis paniculata against carbon tetrachloride-induced liver damage. *Arch Pharm Res* **14**(1): 93–95. (1991).

[27] Zhang C, Kuroyangi M, Tan BK. Cardiovascular activity of 14-deoxy-11,12-didehydroandrographolide in the anaesthetised rat and isolated right atria. *Pharmacol Res*., **38**(6): 413-417,(1998).

[28]Borhanuddin M. Hypoglycaemic effects of Andrographis paniculata Nees on nondiabetic rabbits. *Bangladesh Med Res Counc Bull*. **20**(1):.24-229, (1994) .

[29]Amroyan E. Inhibitory effect of andrographolide from Andrographis paniculata on PAFinduced platelet aggregation. *Phytomed*.**6**(1):.27–31, (1999).

[30]Zhao HY, Fang WY, Protective effect of Andrographis paniculata nees on post infarction myocardium in experimental dogs. *J Tongji Med Univ.***10**(4):212-217, (1990).

[31] Zhao HY. Antithrombotic effects of Andrographis paniculata nees in preventing myocardial infarction. *Chin Med J* (Engl.) **104**(9): 770-775, (1991).

[32] Singha P K, Roy and Dey S. Antimicrobial activity of Andrographis paniculata. Fitoterapia. **74**(7-8): 692-694. (2003).

[33] Sivananthan M. and Imanina C W. Synergistic activity of chloroform and methanol exreact of Andrographis paniculata with erythromycin against streptococcus agalatiae. International *J. Pharm Tech Res*. **5**: 1393-1398, (2013).

[34] Ohkawa, H., Ohishi, N., and Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, **95** : 351-358, (1979).

[35] Slater, T. I. Overview of methods used for detecting lipid peroxidation. *Methods Enzymol*, **105** : 283-293, (1980).

[36] Grifith, O. W. Determination of glutathione and glutathione sulfide using glutathione reductase and 2-Vinyl pyridine. *Anal Biochem*, **106** : 207, (1980).

[37] Marklund, S. and Marklund, G. Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay of superoxide dismutase. *Eur J Biochem*, **47**: 469-474, (1974).

[38] Beers, R.F. and Sizer, I.W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem*, **195**: 133-140, (1952).

[39] Paglia, D. E., and Valentine, W.N. Studies on qualitative and quantitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* , **70**:158-169, (1967).

[40] Miwa, S. Hematology, In: Modern medical techonology. **3** : 306-310, (1972).

[41] Lowry, O.H., Rosenbrough, N. J., Farr, A.L., and Randall, R. J. Protein measurement with the Folin Phenol Reagent. *J Biol Chem*, **193** : 255-275, (1951).

[42] Das, D and Das, A Statistics in Biology and Psychology. Academic Publishers, Calcutta. (1998).

[43] Zima T., Fialova L., Mestek O., Janebova M., Crkovska J., Malbohan I., Stipek S., Mikulikova L., and Popov P. Oxidative stress, metabolism of ethanol and alcohol-related diseases. *J Biomed Sci*. **8(1)**: 59-70, (2001).

[44] Heaton, M.B.; Paiva, M.; Mayer, J.; Miller, R. Ethanol-mediated generation of reactive oxygen species in developing rat cerebellum. *Neurosci. Lett.*, **334**, 83–86, (2002).

[45] Ramachandran, V.; Watts, L.T.; Maffi, S.K.; Chen, J.; Schenker, S.; Henderson, G. Ethanol-induced oxidative stress precedes mitochondrially mediated apoptotic death of cultured fetal cortical neurons. *J. Neurosci. Res.* **74,** 577–588, (2003).

[46] Nordmann, R. Oxidative stress from alcohol in the brain. *Alcohol Alcohol* supp. **1**:75–82, (1987)

[47] Rouach, H.; Park, M.K.; Orfanelli, M.T.; Janvier, B.; and Nordmann, R. Ethanol-induced oxidative stress in the rat cerebellum. *Alcohol Alcohol*, **22** (supp. 1):207–211, (1987).

[48] Gonthier, B.; Signorini-Allibe, N.; Soubeyran, A.; Eysseric, H.; Lamarche, F. & Barret, L. (2004). Ethanol can modify the effects of certain free radical-generating systems on astrocytes. *Alcoholism*, **28**: 526-533. (2004).

[49] Singha, P.K.,Roy S. and Dey S. Protective activity of andrographolide and arabinogalactan protein from *Andrographis paniculata* Nees against ethanol induced toxicity in mice. *J. Ethnopharmacol*. **111**; 13-21, (2007).

[50] Ribiere C., Hininger I, Saffar-Boccara C, Sabourault D, and Nordmann R. Mitochondrial respiratory activity and superoxide radical generation in liver, brain and heart after ethanol intake. *Biochem. Pharmacol*. **47**(10);1827-33. (1994).

[51] Nordmann R., Rouach H., Houze P. Alcohol,Iron and oxidative stress. B*ul Acad Nat Med* **174**(1). 95-1102, (1990).

[52] Bondarenko L B., Shayakhmetoval G M.,Kovalenkol V M., Kharchenko O L., Bohun L., and Omelchenko O. Y. Chronic Alcoholism-mediated Metabolic Violations in Albino Rats Brain. *International Journal of Biochemistry Research & Review* **4**(3): 269-283, (2014)

[53] Wang J, Du H, Jiang L, Ma X, de Graaf RA, Behar KL, Mason GF. Oxidation of ethanol in the rat brain and effects associated with chronic ethanol exposure. *PNAS.* **110**(35):14444-9, (2013)

[54] Bondy S. C. and Guo S. X. Regional selectivity in ethanol-induced pro-oxidant events within the brain. *Biochem. Pharmacol*. **49**(1);69-72. (1995).

[55] Marcus S R, Chandrakala M V, and Nadiger H A. Interaction between vit. E and glutathione in rat brain: Effect of chronic ethanol administration. *Asia Pac J Clin Nutr*. **7**(3/4): 201-205, (1998).

Received: June 20, 2014, Revised: June 25, 2014, Accepted: June 30, 2014.

Corresponding Author: *Dr. Somenath Roy, Professor, Immunology and Microbiology Laboratory, Department of Human Physiology with Community Health, Vidyasagar University, Midnapore-721 102, West Bengal, India,
Email: sroy.vu@mail.vidyasagar.ac.in