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In vivo effect of *Andrographis paniculata* against ethanol induced oxidative stress

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Abstract:

Ethanol intoxication is associated with increased risk of many diseases including cancer of liver. Alcohol- induced generation of reactive oxygen species plays the development of these diseases in our body. The present study was under taken to explore the influence of aqueous extract of *Andrographis paniculata* (AE-AP) against ethanol induced oxidative damage of liver and kidney of mice. Lipid peroxidation in ethanol treated group (4 gm/kg body weight, i.p. for 21 days) was significantly increased by a marked elevation in the level of malondialdehyde (MDA) and diene conjugates. Reduced glutathione (GSH) level was decreased and oxidized glutathione (GSSG) level was increased in ethanol treated group as compared to control group. Aqueous extract of *Andrographis paniculata* supplementation (200 mg/kg bodyweight/ day) significantly reduced the lipid peroxidation and restored both GSH and GSSG level. The decreased activities of antioxidant enzymes in ethanol treated mice were also significantly increased after supplementation with AE-AP. The findings of this study demonstrated that aqueous extract may have some property to diminished ethanol induced oxidative damage of liver and kidney.

Keywords: Ethanol, *Andrographis paniculata*, oxidative stress, ROS, Andrographolide

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1. INTRODUCTION

Ethanol is a commonly abused substance in our society. Long term ingestion of alcohol can led to different types of disorders like gastritis, pancreatitis, gastric ulcer, fatty liver, cirrhosis[1]. Alcohol has shown to produced oxidative stress in liver and other organs. Superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$) and alcohol-derived radicals arise from microsomal, mitochondrial, peroxisomal and cytosolic sources *Andrographis paniculata*[2-4]. These radicals are main candidates for oxidative stress. So, the liver is highly susceptible to the oxidative damage due to ethanol consumption [5]. The kidney may also be affected by ethanol induced alpha-hydroxyethyl radical oxidant species. Chronic ethanol consumption has shown to increase lipid peroxidation in kidney [6]. Ethanol or its metabolites can stress the balance in our body toward auto-oxidation, either reducing the antioxidant cell defense or action as a prooxidants [7].

The protective action of antioxidants would be probably due to an inhibition of free-radical-induced chain reactions, with the resulting prevention of peroxidative deterioration of structural lipid in cell membranes. Antioxidants such as vitamin E have been suggested as therapeutic option in acute and chronic liver diseases [8]. Another study demonstrated that ethanol-induced liver damage is associated with oxidative stress and co-administration of N-acetylcysteine which attenuates this damage effectively in rat model [9]. So these interventions have been put forward to counteract the vulnerability of the different tissues to oxidative challenges during ethanol intoxication by reinforcing the endogenous antioxidant system.

Last 100 years, rural people of Bengal use the water extract of *Andrographis paniculata* for gastric and liver disorder. But we have not found any report to use this extract to combat alcohol related diseases. This experiment was been adopted to elucidate possible protective roles of *Andrographis paniculata* on ethanol-induced damage of liver and kidney of mice. In this study aqueous extract was administered following ethanol intoxication in order to determine whether it could prevent ethanol induced oxidative damage of liver and kidney of mice..

2. MATERIALS AND METHODS

2.1 **Animals:**

Male Swiss mice aged 7-12 weeks were obtained 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 $^{\circ}$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. A voucher specimen (IIT- VU/ AP-1) was deposited in the herbarium of Botanical Survey of India, Sibhpur, Howrah, WB, 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 wwere purchased from Sisco research laboratories PVT,Ltd. (Mumbai 400 099, India)

2.4 Preparation of aqueous extract:

The fresh aerial parts of *Adrographis paniculata* was blended and extracted with distilled water (10:1). The mixture was filtered with Whatman filter paper (No.1) and concentrated at 38 °C by a rotary evaporator, then allowed to stand at room temperature over night. The filtration and concentration processes were repeated to yield an aqueous solution. This solution was then centrifuged at 2000 × g for 10 minutes and supernatant was freeze dried to obtain the crude water extract (Zang, C.Y. et al) [10]

2.5 Experimental Design:

The experimental animals were divided into 6 individual groups. The group I, II, III and IV were injected (intraperitonially) saline, ethanol (4gm/ kg BW), AE-AP extract at the dose of 100 mg/kg BW and 200 mg/kg BW respectively, whereas group V, and VI were treated both ethanol (4gm/ kg BW) and AE-AP extract at the dose of 100 and 200 mg/kg BW respectively. All the experimental animals were maintained at standard animal house for 21 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 liver and kidney 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). [11] 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. [12] 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 testicular 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). [13] 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 µM/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). [14] 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). [15] Specific activity of catalase is defined in terms of µMol of H_2O_2 consumption / mg of protein / minute.

2.8.3 Reduced Glutathione (GSH):

GSH was measured according to the modified method of Griffith, 1980. [13] 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). [16] 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). [17] 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 $protein^{-1}$.

2.8.6 Glutathione S-Transferase activity (GST): The GST activity was measured according to the method of Habig *et al.*, (1974). [18] The tubes for enzyme assay were incubated at 25ºC and contained 2.85 ml of 0.1 M potassium phosphate (pH 6.5) containing 1mM of GSH, 0.05 ml of 60mM 1-chloro-2-4-dinitrobenzene (CDNB) and 0.1ml of tissues homogenate. The activity of this enzyme was determined by monitoring the decrease in absorbance at 340nm. The enzyme activity of sample was calculated using the extinction coefficient of 9.6 mM^{-1}/cm^{-1} and expressed as nM of product formed per min per mg of protein.

2.9 Protein Estimation:

The total plasma and tissues protein was measured according to the method of Lowry *et al.*, (1951)[19] using bovine serum as standard.

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).[20] 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:

Changes in body weight of mice 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 AE-AP100 and AE-AP 200 (Table1).There was significant increased in liver weight after ethanol administration with respect to vehicle controls. In contrast, aqueous extract exposed ethanol treated mice exhibited a significant recovery towards the control level in liver, kidney weight (Table 1).

3.2 Content of malonaldehyde (MDA) and Conjugated Dines (CD)

These are the lipid peroxidation products by reactive oxygen species (ROS). MDA and CD levels were significantly ($P<0.05$) increased in liver, kidney of mice due to ethanol intoxication as compared to the control group (Table 2,3). Supplementation with AE-AP (100mg / kg, 200mg/kg) shows significant diminution of MDA and CD content in above tissues as compared to treated group (Fig1,2,3 and 4).

3.3 Content of GSH and GSSG

Ethanol treatment led to a significant diminution in GSH content (Table 4 & 5) and significant elevation of GSSG content of different tissues (Table 4 & 5) when compared to vehicle treated controls. In contrast, post exposure of AE-AP100 followed by ethanol treatment results significant elevation of GSH level in liver except other experimental

tissue. Ethanol exposure followed by AE-AP200 results a significant recovery of GSH and GSSG level in liver and kidney (Fig 5,6 and 7, 8).

3.4 Antioxidant enzyme activities

For the study of antioxidant status of these tissues, superoxide dismutase (SOD), catalase, GPx, GR, and GST were measured. The activities of SOD, catalase, GPx, GR, and GST of liver and kidney were significantly reduced in ethanol treated group in relation to vehicle treated controls (Table 4 & 5). Post exposure of AE-AP 200 followed by ethanol results a significant recovery of the activities of SOD and catalase in all the experimental tissues when compare to only ethanol treated mice (Fig 9-12). It was further observed that the activities of GPx, GR, GST were significantly increased in all tested organs when supplementation of ethanol treated mice with AE-AP 100 and AE-AP 200 extracts (Fig 13-18).

Table 1: Effect of aqueous extract of A. paniculata on body weight and different tissue weight in response to ethanol.

Condition	Body weight (gm)		Tissue weight (mg)	
	Initial	Final	Liver	Kidney
VEHICLE CONTROL	20.6±0.42	24.0±0.51	1006.6±22.80	292.1±8.66
ETHANOL (4gm/ kg BW)	21.3±0.80	24.6±0.66	1708±7.304 [a]	395.0±7.18[a]
AE-AP 100 (100 mg/kg BW)	22.3±0.58	23.3±0.84	1016.6±19.45	277.8±9.69
AE-AP 100 + ETHANOL	21.6±0.31	23.5±0.50	1122.8±73.41	330.6±5.96
AE-AP 200 (200 mg/kg BW)	23.0±0.31	26.6±0.76	1021.6±31.57	290.0±10.56
AE-AP 200 + ETHANOL	21.6±0.61	22.5±0.34	1200.16±65.72 [b]	310.3±18.68 [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 2: Effect of aqueous extract of *A. paniculata* on Malondialdehyde (MDA) level in different tissues of male albino mice in response to ethanol

Groups	MDA level (nmol/mg tissue)	
	Liver	Kidney
Control	11.33±0.402	20.59±0.262
Ethanol (4gm/ kg BW)	25.16±1.135 [a]	43.16±1.812 [a]
AE-AP 100 (100 mg/kg BW)	12.08±0.926	22.22±0.65
AE-AP 100 + Ethanol	16.53±0.75 [b]	32.08±1.122 [b]
AE-AP 200 (200 mg/kg BW)	13.88±1.336	20.68±1.686
AE-AP 200 + Ethanol	13.03±1.323 [b]	27.91±0.907 [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: Effect of aqueous extract of *A. paniculata* on conjugated dienes level in different tissues of male albino mice in response to ethanol

Groups	Conjugated Dienes level (n mol/mg tissue)	
	Liver	Kidney
Control	409.41±13.377	642.85±17.180
Ethanol (4gm/ kg BW)	763.88±11.914 [a]	1554.55±10.692 [a]
AE-AP 100 (100 mg/kg BW)	467.45±20.63	668.97±22.24
AE-AP 100 + Ethanol	556.26±15.43 [b]	934.55±15.238 [b]
AE-AP 200 (200 mg/kg BW)	413.85±27.37	675.03±22.798
AE-AP 200 + Ethanol	425.83±15.414 [b]	816.61±37.114 [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 4: Effect of aqueous extract of *A. paniculata* on glutathione level and activities of antioxidant enzymes in liver of ethanol induced male albino mice

Parameters	Control	Ethanol	AE-AP100	AE-AP100+ Ethanol	AE-AP200	AE-AP200+ Ethanol
GSH level (micromol/gm tissue)	7.53±0.612	4.4±0.29 [a]	8.25±0.42	6.71±0.42 [b]	7.3±0.62	7.1±0.85 [b]

GSSG level (micromol/gm tissue)	0.55 ±0.019	0.85±0.01 [a]	0.54±0.03	0.79±0.017	0.52±0.078	0.69±0.012 [b]
SOD activity (unit/mg protein)	8.82±0.35	3.92±0.13 [a]	8.03±0.26	4.25±0.40 [b]	8.45±0.49	7.15±0.39 [b]
Catalase acivity (micromol/min/mg protein)	38.33±0.44	22.47±0.32 [a]	39.95±0.74	25.11±0.18	39.92±0.96	29.79±0.54 [b]
GPx acivity (nmol/min/mg protein)	56.28±1.98	26.7±1.86 [a]	58.73±1.83	33.64±1.22 [b]	57.26±2.09	40.92±2.19 [b]
GR acivity (nmol/min/mg protein)	66.64±1.93	28.32±1.53 [a]	65.63±2.55	31.59±2.04 [b]	65.48±2.55	50.47±2.76 [b]
GST acivity (nmol/min/mg protein)	410.3±10.21	255.5±11.52 [a]	422.6±12.74	319.8±14.71 [b]	442.7±10.81	372.7±13.72 [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 5: Effect of aqueous extract of *A. paniculata* on glutathione level and activities of antioxidant enzymes in kidney of ethanol induced male albino mice

Parameters	**Control**	**Ethanol**	**AE-AP100**	**AE-AP100+ Ethanol**	**AE-AP200**	**AE-AP200+ Ethanol**
GSH level (micromol/gm tissue)	11.46±0.73	6.01±0.71 [a]	11.1±0.80	6.98±0.34	10.5±0.83	9.26±0.84 [b]
GSSG level (micromol/gm tissue)	0.40 ±0.02	0.62±0.03 [a]	0.41±0.01	0.53±0.01 [b]	0.42±0.02	0.51±0.01 [b]
SOD activity (unit/mg protein)	10.77±0.57	3.04±0.14 [a]	9.33±0.17	5.74±0.23 [b]	9.11±0.11	6.28±0.10 [b]
Catalase acivity (micromol/min/mg protein)	31.51±0.32	15.61±0.51 [a]	32.51±0.63	19.11±1.29 [b]	30.61±2.63	22.72±1.50 [b]
GPx acivity (nmol/min/mg protein)	66.29±1.57	33.13±0.51 [a]	65.31±0.74	43.71±0.89 [b]	67.21±1.21	56.75±1.56 [b]
GR acivity (nmol/min/mg protein)	71.3±1.29	37.52±1.36 [a]	70.72±1.57	43.19±1.12 [b]	69.66±1.21	55.69±1.31 [b]
GST acivity (nmol/min/mg protein)	388.6±9.73	248.7±8.83 [a]	390.9±7.01	296.5±12.2 [b]	391.6±12.0	352.9±9.60 [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)

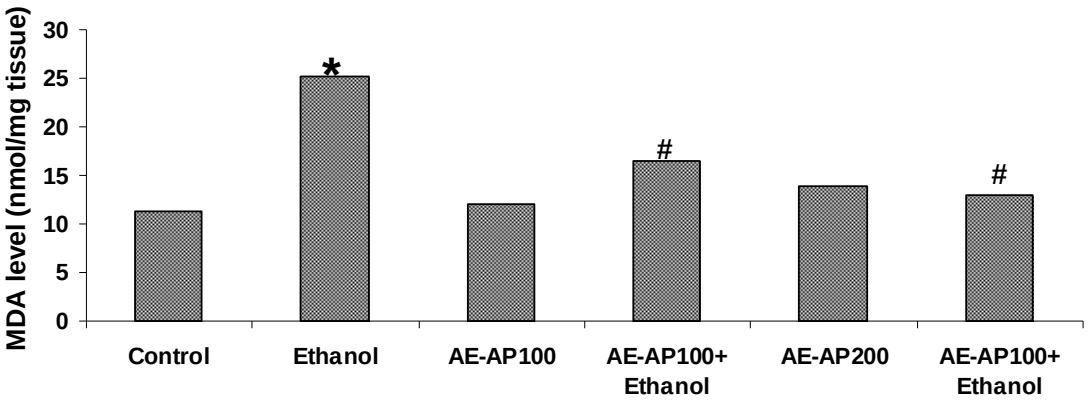

Fig. - 1 : Effect of Aqueous extract of *Andrographis paniculata* on MDA level in Liver of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

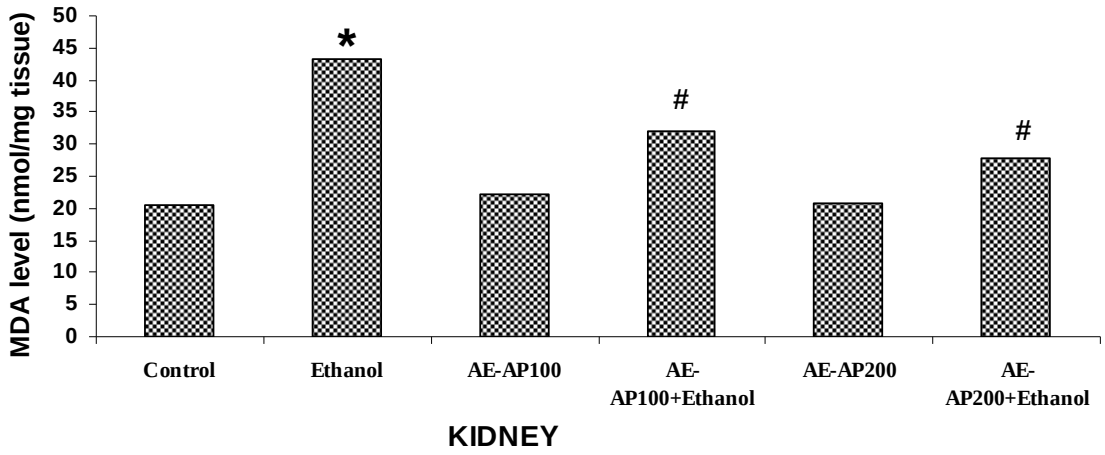

Fig. - 2 : Effect of Aqueous extract of *Andrographis paniculata* on MDA level in kidney of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

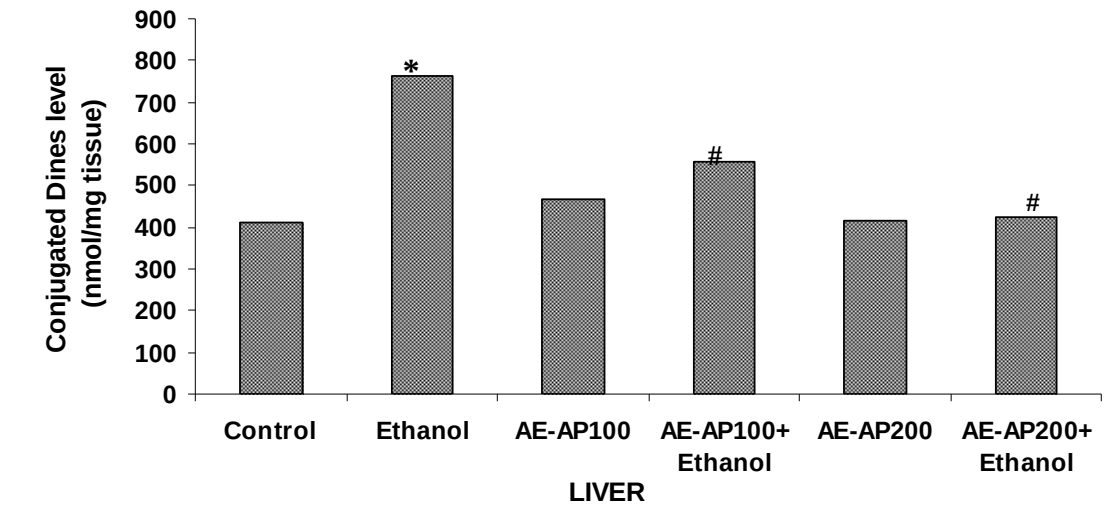

Fig. - 3 : Effect of Aqueous extract of *Andrographis paniculata* on CD level in liver of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

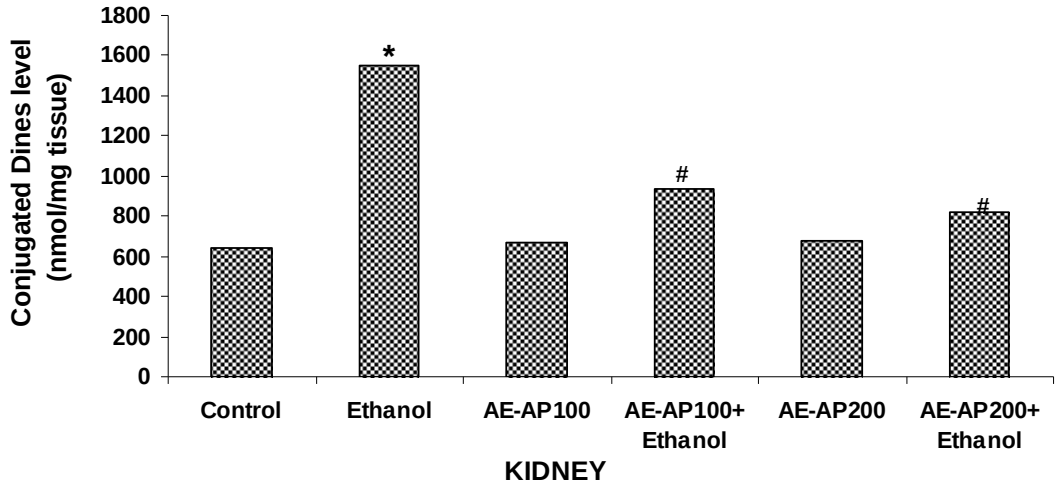

Fig. - 4 : Effect of Aqueous extract of *Andrographis paniculata* on CD level in kidney of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

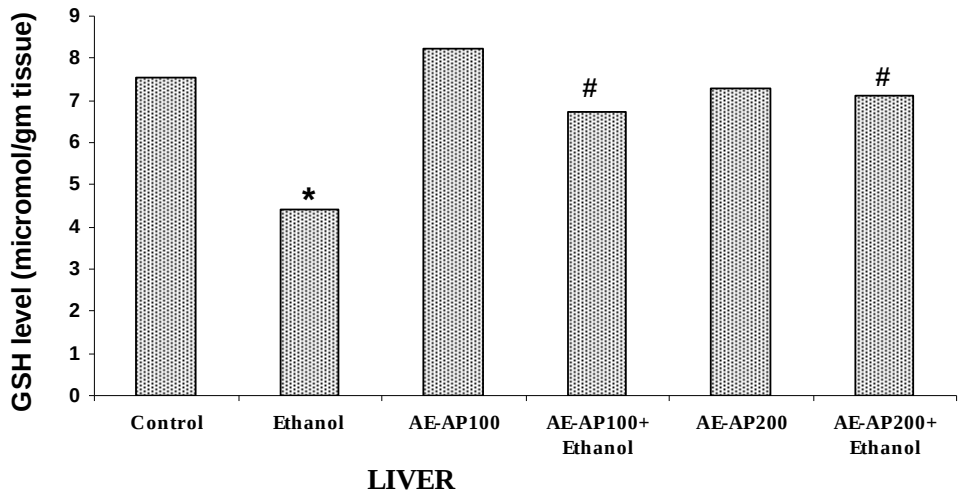

Fig. - 5 : Effect of Aqueous extract of *Andrographis paniculata* on GSH level in liver of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

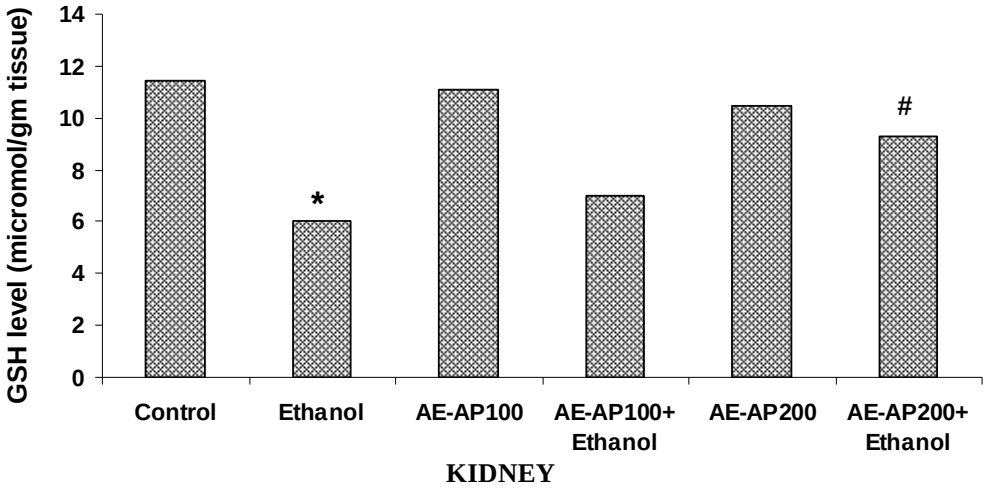

Fig. - 6 : Effect of Aqueous extract of *Andrographis paniculata* on GSH level in kidney of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

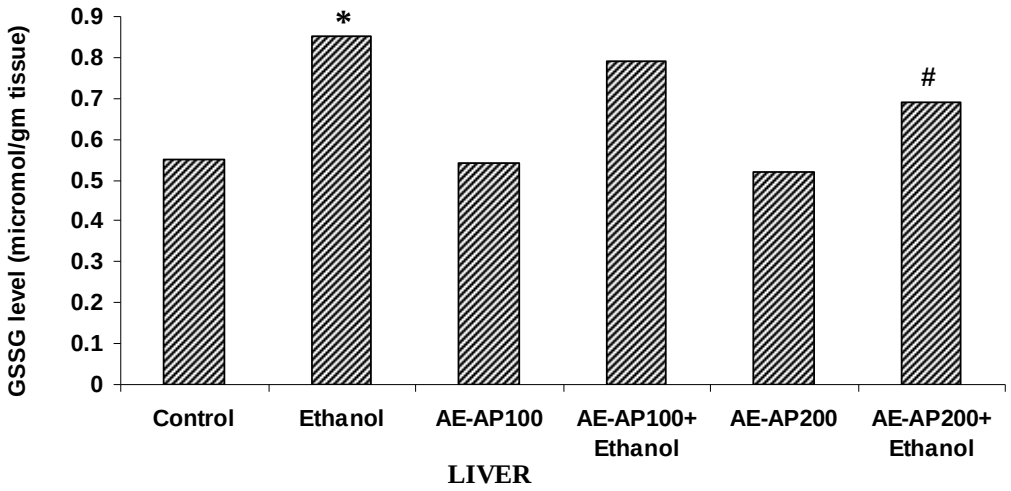

Fig. - 7 : Effect of Aqueous extract of *Andrographis paniculata* on GSSG level in liver of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

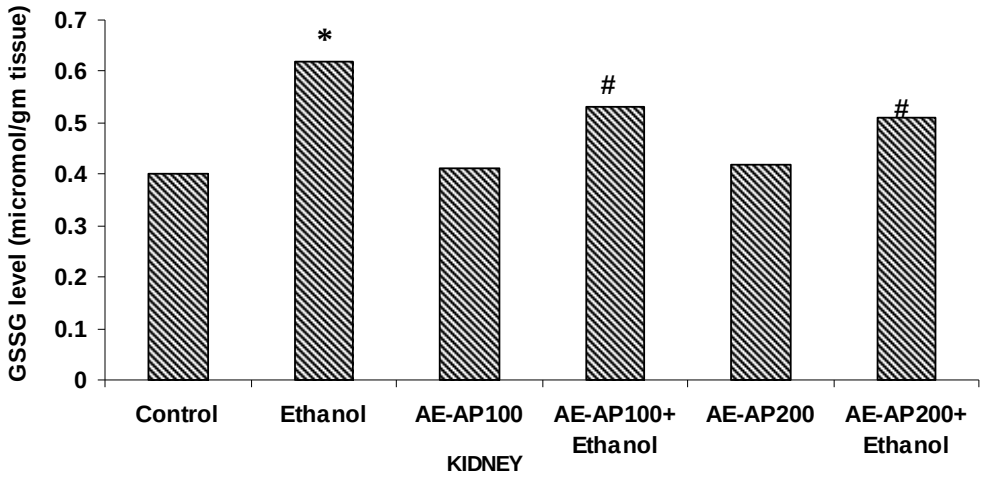

Fig. - 8: Effect of Aqueous extract of *Andrographis paniculata* on GSSG level in kidney of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

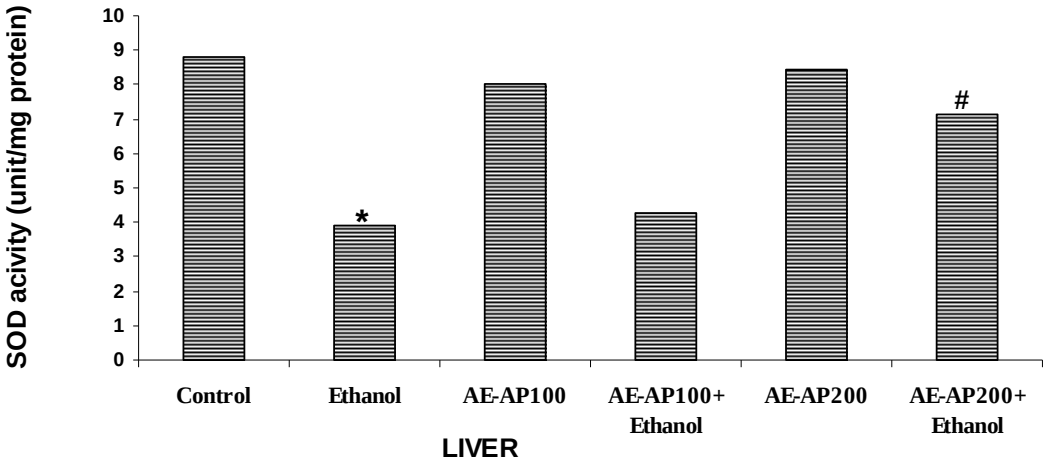

Fig. -9 : Effect of Aqueous extract of *Andrographis paniculata* on SOD activity in liver of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

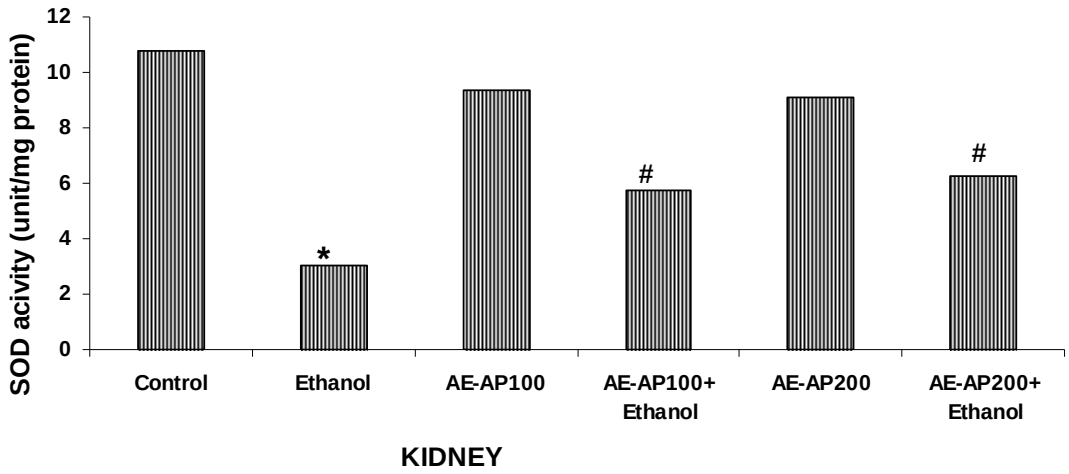

Fig. -10 : Effect of Aqueous extract of *Andrographis paniculata* on SOD activity in kidney of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

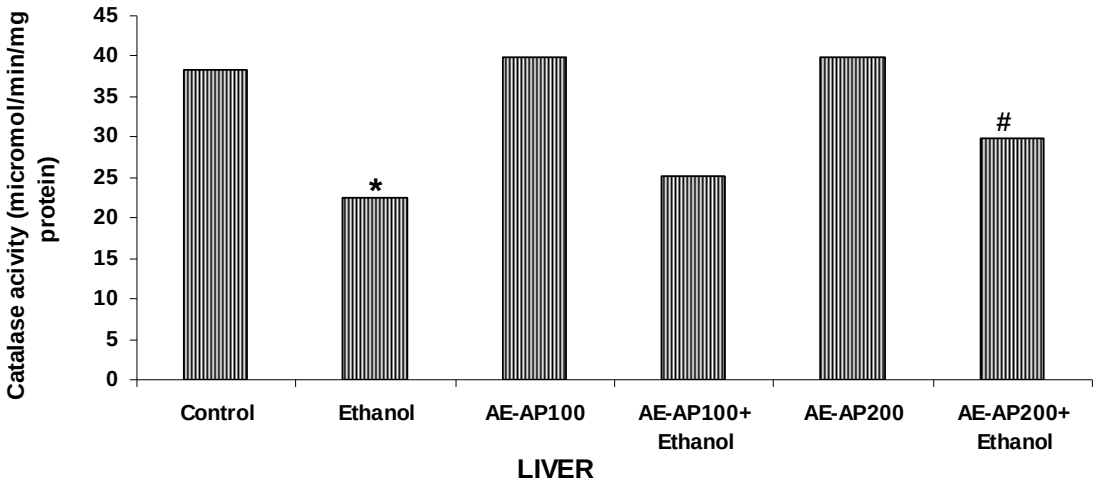

Fig. -11 : Effect of Aqueous extract of *Andrographis paniculata* on catalase activity in liver of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

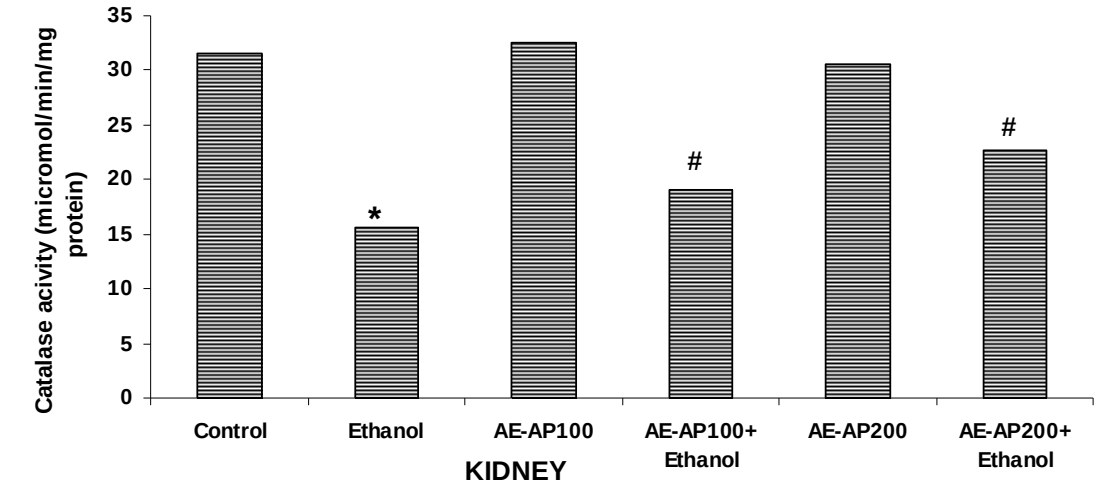

Fig. -12 : Effect of Aqueous extract of *Andrographis paniculata* on catalase activity in liver of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

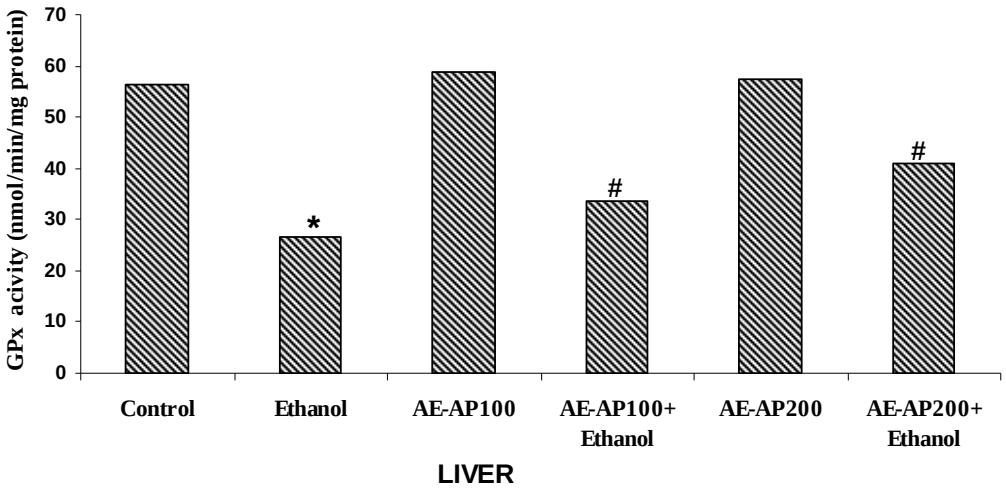

Fig. -13 : Effect of Aqueous extract of *Andrographis paniculata* on glutathione peroxidase ctivity in liver of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

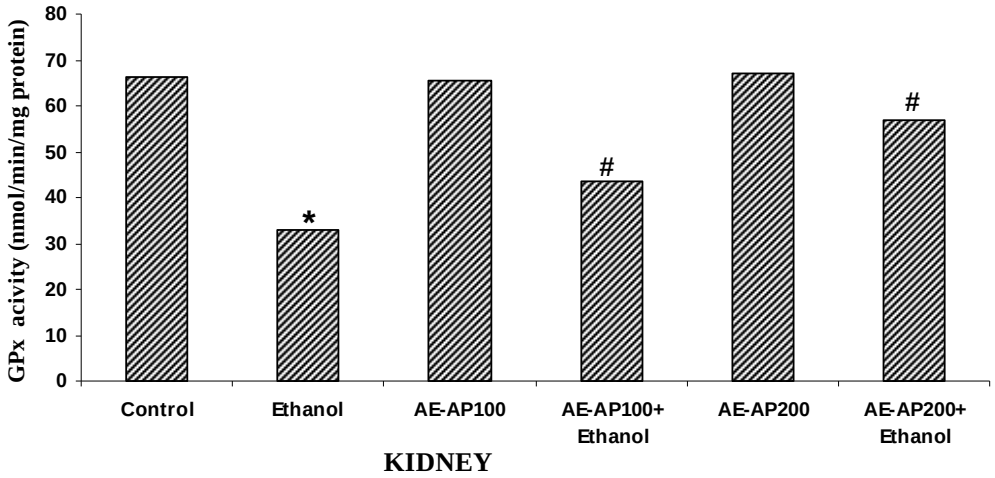

Fig. -14 : Effect of Aqueous extract of *Andrographis paniculata* on glutathione peroxidase activity in kidney of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

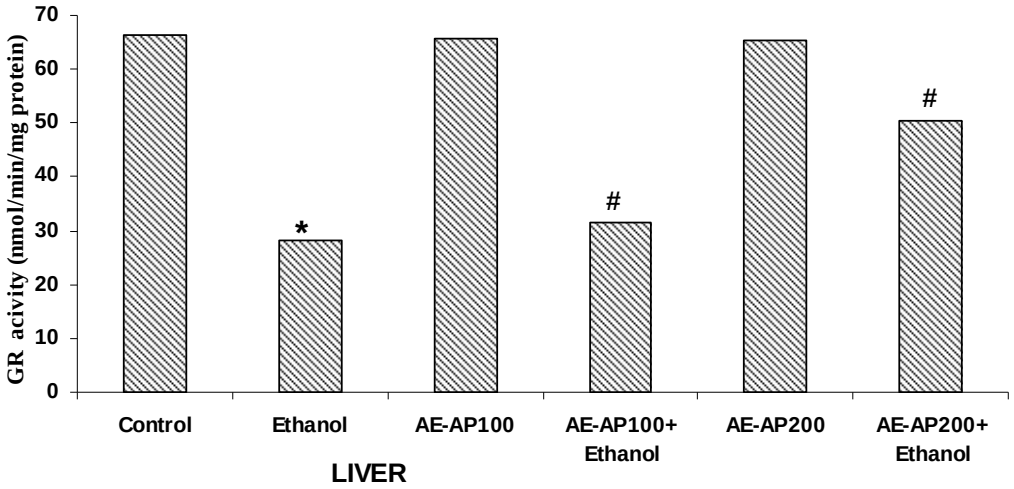

Fig. -15 : Effect of Aqueous extract of *Andrographis paniculata* on glutathione reductase ctivity in liver of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

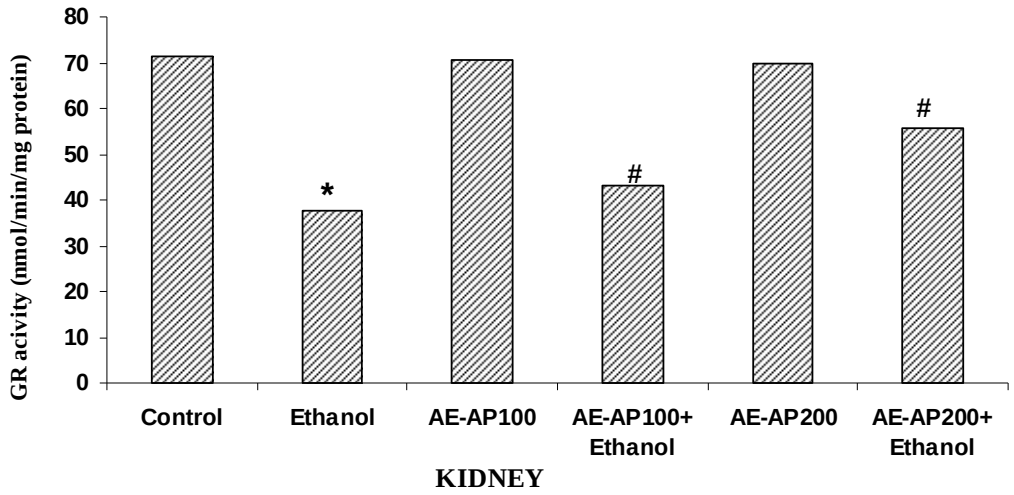

Fig. -16 : Effect of Aqueous extract of *Andrographis paniculata* on glutathione redutase activity in kidney of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

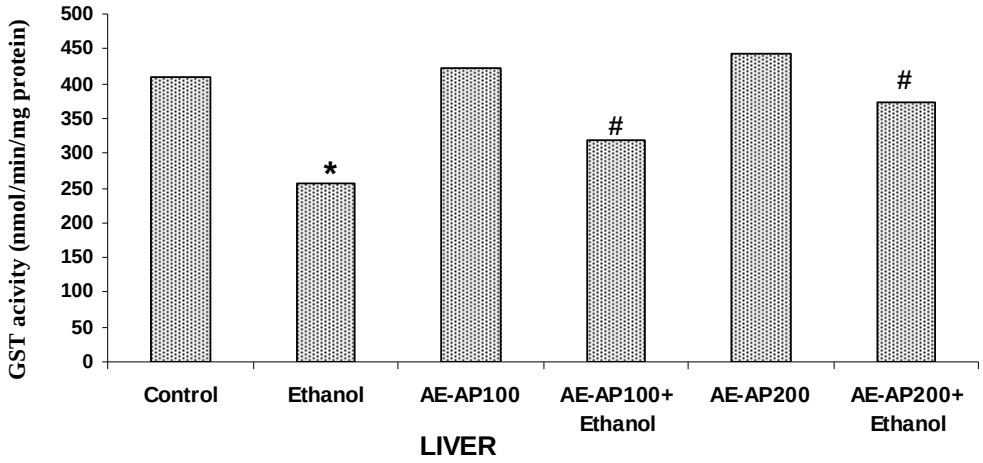

Fig. -17 : Effect of Aqueous extract of *Andrographis paniculata* on glutathione S-transferase ctivity in liver of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

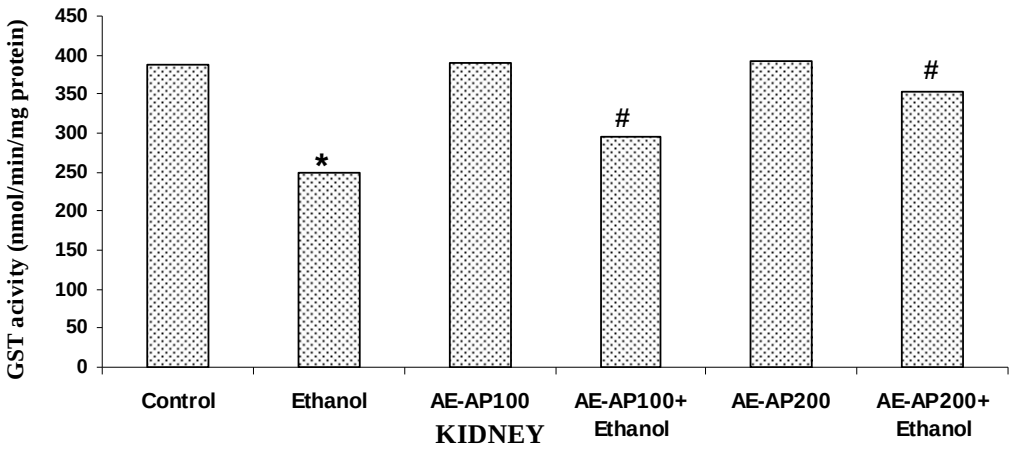

Fig. -18 : Effect of Aqueous extract of *Andrographis paniculata* on glutathione S-transferase activity in kidney of male swiss mice. * P<0.05 Compared to control, # P<0.05 Compared to ethanol

4. DISCUSSION

Oxidative stress is characterized by increased lipid peroxidation or altered nonenzymatic and enzymatic antioxidant system. Ethanol has been shown to produce oxidative stress in vital tissues of the body [21,22].On the other hand, diminished levels of antioxidant substances have been also associated to heavy ethanol consumption [23]. The removal of the toxic metabolites is believed to be the vital initial step in providing cell survival during ethanol intoxication [22]. Although many pathways have been suggested to

explain how ethanol induces oxidative stress, the exact mechanism remains an enigma. The present study was conducted to study the effect of crude aqueous extract of *A. paniculata* (AE-AP) to combat ethanol induced toxicity in liver and kidney.

We have found that ethanol treatment caused significant elevation of MDA, CD, GSSG levels and significant fall in SOD, catalase, GPx, GR, GST activities and GSH level. The depletion of antioxidant enzyme activity reflects the susceptibility of liver to ethanol induced oxidative stress. thus this study has demonstrated that chronic ethanol treatment decreases the activity of antioxidant defenses in liver and kidney of murines as it has been reported by several investigators [24,25].The leaf extract of *Andrographis paniculata* and andrographolide provides protective action against carbontetrachloride-induced hepatic toxicity in rats. It was reported that aqueous extract of *Andrographis paniculata* has significant hepatoprotective activity against BHC [26,27]. Halliwell and Gutteridge[28] suggested that MDA and CD are the marker of the extent of lipid peroxidation and consequent tissue damages. In the present study, elevation of MDA and CD levels in ethanol treated group (Fig 1-4) indicates a clear manifestation of excessive formation of free radicals. Significant depletion in the concentration of MDA and CD in liver and kidney of AE-AP supplemented mice indicates the free radicals scavenging properties of the herbal product of this plant.

Membrane damage caused by lipid peroxidation is an early event in cellular injury, which is followed by oxidation of sulphydryl enzymes. The onset of these mechanisms is presumably triggered by imbalances between production of reducing equivalents and cellular demands of the same. The low molecular weight thiol, glutathione (GSH) and "reactive" protein sulphydryls (exposed cysteines in many proteins) are primary participants in cellular antioxidant systems. Glutathione is abundant (3-10 mM) in cytoplasm, nuclei and mitochondria and is the major soluble antioxidant in these cell compartments. Reactive proteins sulphydryls are abundant in both soluble proteins and in membrane bound proteins. GSH is endogenous antioxidant of different tissues. Significantly decreased level of GSH in liver was observed by Rajagopal S et al.,[29] on ethanol treated rats as compared with those of control. In the present study, it was also observed that GSH level was decreased in liver, kidney on ethanol treated mice as compared to vehicle treated control (Fig 5-6). The observed impaired hepatic and renal glutathione redox status represents increased utilization of GSH due to ethanol induced oxidative tissue damage. Administration of AE-AP (200 mg/kg) significantly increases the GSH level in liver and kidney. Moreover, the GSH/GSSG ratio is an index of tissue oxidative stress. In the present investigation, this ratio decreased after ethanol intoxication (4 g/kg) in liver, kidney. After aqueous extract supplementation (200 mg/kg) this ratio increased. This results may suggests that herbal extract of this plant attenuates oxidative stress in experimental tissues.

Superoxide dismutase is a very important enzyme that functions as a cellular antioxidant. It catalyzes the dismutation of superoxide anion. Rajagopal S et al.,[29] observed significant lowered activity of SOD of liver on alcohol treated rat as compared with control rat. In present study, SOD activity was decreased in liver, kidney of ethanol treated mice (Fig9,10). Therefore, depletion of SOD activity may result in a number of deleterious effects due to the accumulation of super oxide anion radicals.

Catalase is an antioxidising enzyme which catalyses the decomposition of H_2O_2. It was found that catalase activity was significantly reduced on alcohol treated rats as compared

with control rats. This study also demonstrates that ethanol treatment significantly decreased the catalase activity in liver, kidney (Fig 11,12). On the other hand co-administration of *Andrographis paniculata* restored the catalase activity. This study may suggested that ethanol induced oxidative stress could be protected or minimized by administration with AE-AP. The CAT/SOD ratio is of significant importance in that it can indicate a tissue's ability to cope with oxidative stress. This ratio decreased in ethanol intoxicated mice in experimental tissues whereas, it increased in aqueous extract supplemented mice after ethanol insult. Di Luzio[30] was demonstrated that ethanol could affect the antioxidant balance of the hepatic cell.

Glutathione peroxidase is another enzyme involve in the removal of H_2O_2. It is found in the cytoplasm and mitochondria of the cells through out the body as well as in plasma. Chronic ethanol treatment (5g/kg) caused significant decreased in GPx activity as compared with those of the normal mice [26]. We also found that aqueous extract of *A. paniculata* increases the activity of GPx (Fig 13, 14). Decreased GPx/SOD ratio indicates inability of tissue to cope with ethanol induced oxidative stress. On the contrary, increased GPx/SOD ratio indicates resistance to oxidative damage in response to chemical stress [5]. This ratio decreased in ethanol intoxicated mice whereas, it increased in aqueous extract supplemented mice after ethanol insult. Glutathione reductase is another antioxidant enzyme that involve in the conversion of GSSG to GSH. Chronic ethanol treatment (5g/kg) caused significant decreased in GR activity as compared with those of the normal mice [26] We also found that aqueous extract of *Andrographis paniculata* increases the activity of GR (Fig 15,16).The GR/GPx ratio represents the efficiency of conversion of GSSG to GSH (Husain [5] In the present investigation this ratio decreased in ethanol intoxicated mice whereas, it increased in aqueous extract supplemented mice after ethanol intoxication.

Glutathione S-transferase is an phase II defensive enzyme which involves in the detoxification product of oxidative stress. It is found in the cytoplasm and mitochondria of the cells through out the body as well as in plasma. In present investigation, glutathione S-transferase activity was significantly decreased in liver, kidney of chronic ethanol (4g/kg) treated mince(Fig 17, 18). Treatment with aqueous extract of *Andrographis paniculata* (200mg/kg) recovered the GST activity when compared to the ethanol treated groups. The depletion in the activities of these enzymes may result in the involvement of deleterious oxidative changes due to the accumulation of toxic product. So the rise of hepatic and renal antioxidant levels indicate the protective effect of *Andrographis paniculata* against ethanol induced oxidative stress.

5. CONCLUSION

Present investigation suggested that ethanol induced oxidative stress could be protected or minimized through the administration with aqueous extracts of *A. paniculata*, Natural antioxidants strengthen the endogenous antioxidant defences from ROS and restored the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. In this context, *Andrographis paniculata* may rightly be mentioned as a plant with antioxidant activity. The protective action of this plant may be due to presence of andrographolide. It may suppress the ethanol induced ROS generation and ROS mediated oxidative stress in tissues of mice. This finding may explain that aqueous extract may have some important

components, having the antioxidant property to diminish or prevent ethanol induced oxidative stress in liver and kidney of mice.

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