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# EVALUATION OF ERYTHROPOIETIN AS NEUROPROTECTANT IN ALCOHOL-INDUCED PERIPHERAL NEUROPATHY IN RATS

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**Keywords:**

Alcoholic peripheral neuropathy, Dysesthesias, Erythropoietin

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**ABSTRACT:** Alcoholic peripheral neuropathy is a potentially incapacitating complication of long-term excessive consumption of alcohol characterized by pain and dysesthesias, primarily in the lower extremities. The neurological effects of ethanol consumption are complex, encompassing both the central and peripheral nervous systems. It breaks into acetaldehyde which impairs and destroys the majority of neuronal functions. As a result, signals become intensified from the injured neurons and the nearby surrounding neurons, resulting in a painful condition. The study aims to evaluate the different doses of Erythropoietin, *i.e.*, 250U/kg and 500U/kg, in alcohol-induced peripheral neuropathy. The present study involved evaluating the neuroprotective activity of Epo in alcohol-induced PN in rats by assessing behavioral, biochemical and electrophysiological, liver function tests, parameters. This study concludes that the two doses of Epo improved sciatic nerve conduction, behavioral studies and antioxidant activity in sciatic nerve homogenate. Among the two different doses of Epo, 500U/kg was most effective besides showing neuroprotective properties against alcohol-induced PN.

**INTRODUCTION:** Peripheral neuropathy (PN) is damage to or disease affecting nerve, which may impair the sensation, movement, gland or organ function, or other aspects of health, depending on the type of nerve affected. Alcohol abuse, Anti-HIV drugs, Diabetes, Low vitamin $B_6$ levels, Infections that affect the nerves like leprosy, Lyme disease, Spinal cord injuries. Alcohol consumption produces painful PN for which there is no reliable, successful therapy, mainly due to a lack of understanding of its pathobiology.

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Long-term excessive alcohol drinking causes nerve damage and is characterized by spontaneous burning pain, hyperalgesia and allodynia. Ethanol diminishes thiamine absorption in the intestine, reduces hepatic stores of thiamine and affects the phosphorylation of thiamine, which converts it to its active form. In addition to thiamine deficiency, recent studies have indicated a direct neurotoxic effect of ethanol or its metabolites.

Current treatment options for PN include antidepressants, anticonvulsants, tramadol, and capsaicin. These agents are modestly effective for symptomatic relief, but they do not affect the underlying pathology, nor do they slow the progression of the disease. Currently, no effective treatment is available to prevent or treat peripheral neuropathy; hence an attempt was made to select the drug for prevention or treatment of peripheral

neuropathy based on the concept of repositioning and repurposing of an existing drug. Erythropoietin (EPO) was known for erythropoiesis, supporting survival, proliferation and differentiation of erythroid progenitor cells. It is now evident that Epo is a multifunctional trophic factor. EPO has potent neurotrophic activity on various neural cells and includes Epo-induced prevention of cell death, induction of neuronal differentiation. EPO is also synthesized locally in the brain, primarily by astrocytes but also by neurons.

Like the hematopoietic mechanism, neural Epo synthesis is regulated by an oxygen-sensing mechanism such that Epo production is dramatically up-regulated in astrocytes and neurons in hypoxic environments [1].

Epo is a low molecular weight glycoprotein hormone stimulator of erythropoiesis. Epo is a 30.4Da glycoprotein that consists of four glycosylated chains, including three N-linked and one O-linked acidic oligosaccharide side chains.

The glycosylated chains are important for the biological activity of Epo and protect Epo from oxygen-free radical degradation. Epo is primarily produced and secreted in the kidney.

Secondary sites of Epo production and secretion involve the liver, uterus, hepatocytes, and peripheral endothelial cells.

EPO regulates bone marrow erythroid cell proliferation, differentiation, and survival by binding to an erythroid progenitor cell surface Epo receptor (EpoR). The EpoR is expressed in numerous non-erythroid bloodlines that include neurons, microglia, astrocytes [2].

All the above data suggest that Epo has an activity on the neuronal damage, so that Epo is used in evaluating its Neuroprotectant activity in alcohol-induced neuropathy.

## MATERIALS AND METHODS:

**Requirements:** Experimental animals: Healthy Wistar rats, weighing about 160-200g at the start of the experiment, were used as experimental animals in the present study.

The use of animals in these experiments was authorized by IAEC (Institutional Animal Ethical Committee) IAEC approval no: AACP/IAEC/Jan2015/01. Throughout the experiment, rats were conducted by the CPCSEA guidelines.

**TABLE 1: LIST OF CHEMICALS AND MATERIALS**

| S. no. | Chemical | Source |
|---|---|---|
| 1 | Alcohol | Changshu yangyuan chemical China |
| 2 | Erythropoietin | Micro labs Ltd. |
| 3 | Alanine transaminase assay kit | Span diagnostics limited |
| 4 | Aspartate transaminase assay kit | Span diagnostics limited |
| 5 | Triglycerides assay kit | Span diagnostics limited |
| 6 | 5,5 dithiobis 2-nitrobenzoic acid | Sigma Aldrich |
| 7 | Trichloro barbituric acid | Sigma Aldrich |
| 8 | Trichloro acetic acid | S.D fine. chem. Ltd |
| 9 | Hydrogen peroxide | S.D fine. chem. Ltd |
| 10 | EDTA | Rankem |
| 11 | di-Potassium hydrogen orthophosphate | Titan biotech |
| 12 | N-(1-napthyl)-ethylenediamine dihydrochloride | S.D fine. chem. Ltd |
| 13 | Orthophosphoric acid | S.D fine. chem. Ltd |
| 14 | Sulphanilamide | S.D fine. chem. Ltd |
| 15 | Sodium carbonate | S.D fine. chem. Ltd |
| 16 | Tris HCl | Titan biotech |
| 17 | Sodium nitrate | Reachem laboratory chemicals |
| 18 | Sodium hydroxide | Merck |
| 19 | Potassium sodium tartrate tetrahydrate | Merck |
| 20 | Potassium dihydrogen orthophosphate | Thomas Baker chemicals |
| 21 | Copper sulphate | Rankem |
| 22 | Sodium dihydrogen orthophosphate | Thomas Baker chemicals |
| 23 | Sodium citrate | Qualigens fine chemicals |
| 24 | Folin-Ciocalteau reagent | S.D fine. chem. Ltd |

**TABLE 2: LIST OF EQUIPMENT AND INSTRUMENTS**

| Sl. no. | Equipment's and instruments | Model/Manufacturer |
|---|---|---|
| 1 | UV-Visible spectrophotometer | Thermo electron – 100 |
| 2 | Homogenizer | Servewell instruments |
| 3 | Semi auto analyser | Artos |
| 4 | Analytical balance | Acculab |
| 5 | Cold centrifuge | Remi |
| 6 | Incubator | Servewell instruments |
| 7 | NCV | AD Instruments |
| 8 | Rota rod | Roberston |
| 9 | Actophotometer | Techno |
| 10 | Eppendrof tubes | Tarsons |
| 11 | Capillary tubes | Tarsons |
| 12 | Microtips | Tarsons |

**Grouping for Protective Studies:**

**Group 1:** Negative control Normal rats (Distilled water, p.o., for 10 weeks).

**Group 2:** Positive control 35% v/v Ethanol (10 g/kg, twice a day p.o; for 10 weeks)

**Group 3:** Positive control + Erythropoietin (250U/kg i.p) twice a week for 10 weeks.

**Group 4:** Positive control + Erythropoietin (500U/kg i.p) twice a week for 10 weeks.

**Materials and Methods:** The absolute alcohol of 100% was diluted to 35% using distilled water. The diluted 35% (10g/kg twice a day p.o) of alcohol was given to the rats for 10 weeks to induce the neuropathy. The EPO of 250U/kg and 500U/kg was given twice a week i.p to the rats for10 weeks. The degree of protection was determined by measuring behavioural parameters like motor coordination, thermal hyperalgesia, electrophysiological properties of sciatic nerve like nerve conduction velocity, Biochemical estimation like AST, ALT, TG, Antioxidants properties like catalase and reduced glutathione in the sciatic nerve and liver homogenates, and histopathology of sciatic nerve and liver.

**Parameter Studied:**

**Behavioral Parameters:**

**1. Neuromuscular Coordination Assessment:** Rotarod test was performed to investigate the effect of acute SD (Sleep deprivation) on the sensorimotor coordination of rats. The apparatus (Rotamex-5, Columbus instruments) is composed of a horizontal metal rod and four partition plates, placed at the height of 44.5 cm with a diameter of 7 cm and a length of 9.5 cm, and is attached to a motor with variable speed. The height discourages the animals from jumping. All the four group animals (n=6–7 in each group), *i.e.*, Vehicle-undisturbed sleep (VUD), (2) Vehicle sleep-deprived (VSD), (3) B-TCE fed sleep-deprived (TSD), and (4) B-TCE fed undisturbed sleep (TUD) were subjected to rotarod at a speed of 10 rpm for 5 min. The amount of time spent on the rotating rod and the number of falls were recorded for each animal and then averaged for the respective group [3].

**2. Thermal Hyperalgesia:** Hot and cold hyperalgesia are assessed using the tail-immersion test in water maintained at low (40C) or high (46 °C) temperature. The duration of tail immersion is recorded, and a cut-off time of 15 s is taken [4].

**3. Locomotor Activity Assessment:** The locomotor activity can easily be measured using an actophotometer that operates on photoelectric cells connected in a circuit with a counter. A count is recorded when the beam of light falling on a photocell is cut off by an animal. The rats are placed, and the counts are taken for 10 min [5].

**4. Mechanical Hyperalgesia:** The nociceptive flexion reflex was quantified using the fabricated Randall Selitto paw pressure device which applies a linearly increasing mechanical forcing to the dorsum of the rat's hind paw Nociceptive threshold, expressed in grams, was applied by increasing pressure to the hind paw until a squeak (vocalization threshold) was elicited.

As this test involves animal handling, the experiment gets the rat used to being handled as follows: 3 days before the experiment, Rats were handled without scaping from the end of the experimenter for 20s, 2 or 3times depending on their capacity to be quiet. On the day of the experiment, rats were again handled 2–3 times for 20 s. No rats should show aversive reaction during handling. Then, the paw of the rat was placed under the tip, and the progressive pressure was applied until their at vocalized. The vocalization threshold was measured 3 or 4 times to obtain two consecutive values that differed no more than 10%

and respect an interval of at least 10 min between two measures [6].

**Mechanical Allodynia:** Sensitivity to noxious mechanical stimuli was determined by qualifying the withdrawal threshold of the hind paw in response to mechanical stimulation using a fabricated Von-frey an esthesiometer. The mechanical stimulation is expressed in grams. The rats were placed in individual plexiglass boxes on a stainless steel mesh floor and were allowed to acclimatize for at least 20 min. A 0.5 mm diameter polypropylene rigid tip was used to apply a force to the plantar surface of the recorded by the anaesthesiometer. The test was repeated four-five times at 5 min intervals, and mean values were taken [7].

**Electrophysiological Study:**

**Measurement of Nerve Conduction Velocity:** A nerve conduction velocity test (NCV) is an electrical test used to determine the adequacy of the conduction of the nerve impulse as it courses down a nerve. This test is used to detect signs of nerve injury [8]. The nerve is electrically stimulated in this test, and the electrical impulse 'downstream' from the stimulus is measured. One electrode stimulates the nerve with a very mild electrical impulse. The other electrodes record the resulting electrical activity. The distance between stimulating and recording electrodes and the time it takes for electrical impulses to travel between electrodes are used to calculate the speed of impulse transmission (nerve conduction velocity). The variation in conduction velocity in the various fibres in the nerve is an important factor in determining the shape of the Compound Action Potential (CAP), since the conduction velocity of each fiber determines the latency of its contribution to the CAP. The conduction velocity of the nerve is normally reported in meters per second. It is more easily recorded as millimeters per millisecond.

**Procedure:** Animals are anesthetized. The nerve conduction velocity is measured using AD instrument. The left limb is connected to the positive and negative electrodes, and the right to the earthing electrode is connected using the tape. The setup is connected to the system having LabChart 7. The stimulator is taken, the stimulation is given at the left limb's proximal and distal parts, and the graphs are recorded in the LabChart 7. The distance between the proximal and distal parts is measured. And latency between the proximal and distal parts is calculated.

The nerve conduction velocity is calculated using the formula:

Velocity = Distance (distal-proximal) / Latency (distal-proximal)

**Biochemical Estimations:** All the biochemical estimations were carried out using fasting serum samples obtained from rats and analyzed using Artos semi-autoanalyzer.

**Collection of Blood:** The blood for the estimations was removed from the retro-orbital cavity. The animal under light ether anaesthesia was held gently but firmly by the scruff of the neck on a solid surface so that the eye protruded. This helps occlude the venous return from the head and neck. Care must be taken not to obstruct breathing. The orbital sinus was then penetrated with a heparinised capillary tube. This was pushed through the conjunctiva laterally (outer side), dorsally (above) or medially (inner side), to the back wall of the orbit where it punctures the venous sinus and so fills with blood, collected in an Eppendorf tube for serum and in a heparinised Eppendorf tube for blood. The scruff of the neck was released momentarily before the withdrawal of the capillary tube to minimize haemorrhage from the puncture site. Care was taken not to abrade the cornea whilst putting pressure on the globe to limit haemorrhage after the sample had been obtained. The orbital region was cleaned gently using cotton balls.

**Collection of Serum:** The blood sample collected in the Eppendorf tube was allowed to clot for half an hour. The sample was then centrifuged using cold centrifuge at 8000 rpm for 10 min. The clear supernatant was carefully separated and transferred to the estimation tubes.

**Lipid Profile Estimation:**

**Triglycerides Assay:**

**Principle:** Triglycerides are hydrolyzed by lipoprotein lipase (LPL) to produce glycerol and free fatty acid (FFA). In the presence of glycerol kinase (GK), adenosine triphosphate (ATP) phosphorylates glycerol to produce glycerol 3-

phosphate and adenosine diphosphate (ADP). Glycerol 3-phosphate is further oxidized by glycerol 3-phosphate oxidase (GPO) to produce dihydroxyacetone phosphate (DAP) and $H_2O_2$.

In the presence of peroxidase (POD), hydrogen peroxide couples with 4-aminoantipyrine (4-AAP) and 4-chlorophenol to produce red quinoeimine dye. The absorbance of coloured dye is measured at 505 nm and is proportional to triglycerides concentration in the sample, which is estimated as mg/dL.

**Triglycerides:**

Lipoprotein lipase $\xrightarrow{\text{Lipoprotein lipase}}$ glycerol + free fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol kinas}}$ Glycerol 3-Phosphate + ADP

Glycerol 3-Phosphate + $\xrightarrow{O2 \quad GPO}$ DAP + $H_2O_2$

2 $H_2O_2$ + 4-Chlorophenol + 4-AAP $\xrightarrow{POD}$ Quinoeimine dye + 4 $H_2O$

**TABLE 3: REAGENT COMPOSITION**

| Reagent no | Reagent | Composition | Concentration |
|---|---|---|---|
| 1 | Triglyceride mono Reagent | Pipes buffer | 50 mmol/L |
| | | 4-Chlorophenol | 5 mmol/L |
| | | Magnesium ion | 5 mmol/L |
| | | ATP | 1 mmol/L |
| | | Lipase | 5000 U/L |
| | | Peroxidase | 1000 U/L |
| | | Glycerol kinase | 400 U/L |
| | | 4-Aminoantipyrine | 0.4 mmol/L |
| | | Glycerol 3-phosphate oxidase | 4000 U/L |
| | | Detergents | Qs |
| | | Preservatives | Qs |
| | | Stabilizers | Qs |
| 2 | Triglyceride Standard | Triglyceride | 200 mg/dL |
| | | Stabilizer | Qs |
| | | Preservative | Qs |

**TABLE 4: ASSAY PARAMETERS**

| Mode | End point |
|---|---|
| Wavelength | 505 nm |
| Flow-cell temperature | 370 C |
| Optical path length | 1 cm |
| Blanking | Reagent blank |
| Sample volume | 10 µL |
| Reagent volume | 1000 µL |
| Incubation time | 10 minutes |
| Concentration of standard | 200 mg/dL |
| Stability of final colour | 1 hour |
| Permissible reagent blank absorbance | < 0.3 AU |
| Linearity | 1000 mg/dL |
| Units | mg/dL |

**PROCEDURE:**

| Pipette into tube marked | Blank | Standard | Test |
|---|---|---|---|
| Serum/Plasma | - | - | 10 µL |
| Reagent 2 | - | 10 µL | - |
| Reagent 1 | 1000 µL | 1000 µL | 1000 µL |

## Calculation:

Triglycerides (mg / dl) = (Absorbance of test) / (Absorbance of standard) × 200

## Conversion Factor:

Triglycerides concentration in mmol / l = Triglycerides in mg / dl × 0.0113

**Liver Function Tests:** Estimation of serum levels of aspartate transaminase and alanine transaminase is an indicator of liver functioning. Elevation in the levels of these enzymes indicates hepatic damage.

### A) ALT (SGPT):

**Principle:** Alanine aminotransferase (ALT) catalyzes the transamination of L-alanine and α-ketoglutarate to form pyruvate and L-glutamate In a subsequent reaction, Lactate Dehydrogenase(LD) reduces pyruvate to lactate with simultaneous oxidation of nicotinamide adenine dinucleotide [reduced] (NADH) to nicotinamide adenine dinucleotide (NAD).

The rate of oxidation of NADH is measured kinetically by monitoring the decrease in absorbance at 340nm. LD rapidly and completely reduces endogenous sample pyruvate during the

initial incubation period, so that it does not interfere with the assay. Serum ALT values are represented as activity in nkat/L.

L-Alanine +α-Ketoglutarate $\xrightarrow{\text{ALT}}$ Pyruvate +L-glutamate

Pyruvate + NADH + H+ $\xrightarrow{\text{LD}}$ Lactate+NAD

**Reagent Procedure:**

| Reagent no | Reagent | Composition | Concentration |
|---|---|---|---|
| 1 | Buffer | Tris buffer (pH 7.5) | 100 mmol/L |
| | | l- alanine | 500 mmol/L |
| | | LD | >1200 U/L |
| 2 | Substrate | α-ketoglutarate | 15 mmol/L |
| | | NADH | 0.18 mmol/L |

**Working Reagent Preparation:** Add reagent 2 to reagent 1 in the 1:4 ratio.

**Procedure:**

1. Prepare the test solution by adding 1000 µL of working reagent to 100 µL of serum, mix them well and aspirate immediately for measurement.
2. Program the analyzer as per assay parameters.
3. Blank the analyzer with purified water.
4. Read absorbance after 60 seconds. Repeat after every 30 seconds upto 120 seconds at nm wavelength
5. Determine the mean absorbance change per minute (ΔA/ minute).

ALT activity (IU/L) = ΔA/ minute X kinetic factor

Where, ΔA/ minute= change in absorbance per minute.

Kinetic factor = 1768 (Kinetic factor is calculated by using the formula):

$$K = 1 / M \times TV/SV \times 1/P \times 106$$

M = molar extinction coefficient of NADH and is equal to 6.22 X 103 lit/mol/cm at 340 nm. TV= sample volume + reagent volume SV= sample volume. P= optical path length.

**Conversion Factor:**

ALT activity in nkat / L = ALT activity in IU/L X16.67

**AST (SGOT):**

**Principle:** Aspartate aminotransferase (AST) catalyzes the transamination of L-aspartate and α-ketoglutarate to form L-glutamate and oxaloacetate. In a subsequent reaction, Malate Dehydrogenase (MDH) reduces oxaloacetate to malate with simultaneous oxidation of nicotinamide adenine dinucleotide (NAD). The rate of oxidation of NADH is measured kinetically by monitoring the decrease in absorbance at 340nm and is directly proportional to AST activity in the sample. Lactate dehydrogenase (LD) is added to the enzyme system to prevent endogenous pyruvate interference, normally present in the serum. Serum AST values are represented as activity in nkat/L.

L-Aspartate + α-Ketoglutarate $\xrightarrow{\text{AST}}$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H+ $\xrightarrow{\text{MDH}}$ L-Malate + NAD

**Procedure:**

1. Prepare the test solution by adding 1000 µL of working reagent to 100 µL of serum, mix them well and aspirate immediately for measurement.
2. Program the analyzer as per assay parameters.
3. Blank the analyzer with purified water.
4. Read absorbance after 60 seconds. Repeat after every 30 seconds upto 120 seconds at 340nm wavelength.
5. Determine the mean absorbance change per minute (ΔA/ minute).

**Calculation:**

AST activity (IU / L) = ΔA / minute X kinetic factor

Where, ΔA/ minute= change in absorbance per minute.

Kinetic factor = 1768 (Kinetic factor is calculated by using the formula):

$$K = 1/M \times TV/SV \times 1/P \times 106$$

M= molar extinction coefficient of NADH and is equal to 6.22 X 103 lit/mol/cm at 340 nm.

TV= sample volume + reagent volume SV= sample volume, P= optical path length.

**Conversion Factor:**

AST activity in nkat / L = AST activity in IU/L X16.67

**Biochemical Estimation:**

**Estimation of Reduced Glutathione:**

**Principle:** Glutathione present in RBC consist of some sulfhydryl groups. 5, 5 dithiobis 2-nitrobenzoic acid (DTNB), a disulphide compound, gets readily attacked; by these sulfhydryl groups and forms a yellow colored anion which measured colorimetrically at 412 nm.

**Procedure:** Reduced glutathione levels will be quantified by the method of Moron *et al.* (1979). The tissue homogenate (1 ml) is treated with 1 ml of 5% TCA in 1 mM EDTA and centrifuged at 2000 rpm for 10 min. After that 1 ml of the filtrate is mixed with 5 ml of 0.1 M phosphate buffer (pH 8.0) and 0.4 ml of 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB). The absorbance of the solutions is estimated at 412 nm against blank. Different concentrations (10-50 µg) of standard glutathione will be taken and processed as above for the standard graph. The amount of reduced glutathione is expressed as µg of GSH/mg protein.

**Estimation of Catalase:**

**Principle:** Catalase exerts a dual function because it catalyzes the following reaction.

$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$

$H_2O_2 + AH_2 \rightarrow 2H_2O + A$

In the ultra-violate range, $H_2O_2$ shows a continued increase in absorption with decreasing wavelength. The decomposition of $H_2O_2$ can be followed directly by the decrease in absorbance at 240 nm. The difference in the absorbance per unit time is a measure of the catalase activity.

**Procedure:** Catalase activity is measured in a reaction mixture (3 ml) containing 100 mM phosphate buffer pH 6.8 (2 ml), 30 mM $H_2O_2$ (0.5 ml) and 0.5 ml enzyme extract. The decrease in absorbance due to hydrogen peroxide depletion is recorded at 240 nm by UV-VIS spectrophotometer for one min. Catalase activity is calculated by using the extinction coefficient of 0.043 L mmol-1 cm-1 and expressed as µmol L-1 min-1 mg-1 of protein.

**Serum Nitric Oxide:**

**Principle:** This assay determines nitric oxide concentrations based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Griess Reaction. The Griess Reaction is based on the two step diazotization reaction in which acidified $NO_2$ produces a nitrosating agent, which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to N-(1-naphthyl) ethylenediamine to form the chromophoric azo-derivative which absorbs light at 540-570 nm [8].

**Procedure:** The assay mixture consists of medium (100 µL) and Griess reagent (100 µL) placed in round-bottom 96-well tissue culture plates (incubation time 30 min), and absorption is measured at 570 nm on a Microplate Reader. The amount of nitrite will be determined by comparison of unknowns with a $NaNO_2$ standard curve. The nitrite detection limit is 0.20 nM.

**Statistical Analysis:** All data were expressed as mean ± SEM and analyzed with a one-way analysis of variance between the groups. The Dunnett Multiple Comparison Test was used to assess differences between the groups. Probability values *p<0.05, **p<0.01,***p<0.001 were considered significant.

**RESULT AND DISCUSSION:**

**Percentage of Body Weight:**

**Effect of Erythropoietin on Body Weight in Alcohol-Induced Neuropathy in Male Rats:** Effects of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on body weight have a % variation of -2.138±1.100. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having an activity of 28.747±2.924.

Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on body weight having % variation was significantly higher on comparing with Positive control 35% v/v Ethanol (10g/kg, p.o.) having a % variation of 33.293±4.616& 29.845±2.993.

**TABLE 5: EFFECT OF ERYTHROPOIETIN ON BODY WEIGHT IN ALCOHOL-INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | % variation of body weight |
|---|---|---|
| Group 1 | Normal control | 28.747±2.924*** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | -2.138±1.100 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 33.293±4.616*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 29.845±2.993*** |

**Behavioral Studies:**

**Effect of Erythropoietin on Neuro Muscular Co-ordination in Alcohol-Induced Neuropathy in Male Rats:** Effects of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on neuromuscular coordination have a latency of 45.6678±5.162sec. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to normal control having a latency of169.500±4.193sec. Effect of treatment with Epo of two different doses Epo (250U/kgi.p) & Epo (500U/kg i.p) on neuromuscular coordination was significantly higher on compared with Positive control 35% v/v Ethanol (10g/kg, p.o.) having a latency of 93.333±3.997sec & 131.833±8.845sec respectively.

**TABLE 6: EFFECT OF ERYTHROPOIETIN NEUROMUSCULAR COORDINATION ON IN ALCOHOL-INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Latency in sec |
|---|---|---|
| Group 1 | Normal control | 169.500±4.193 *** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg, p.o.) | 45.6678±5.162 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 93.333±3.997 *** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 131.833±8.845 *** |

**Effect of Erythropoietin on Locomotor Activity in Alcohol-Induced Neuropathy in Male Rats:** Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) in males rats on locomotor activity have a counts/10 min of 229.833±4.473 counts/10min. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to normal control counts/10 min of 259.133±7.113 counts/10min. Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on locomotor activity was significantly higher on compared with Positive control 35% v/v Ethanol (10g/kg, p.o.) having a counts/10 min 253.720±3.169 counts/10min &277.287±6.048 counts/10min respectively.

**TABLE 7: EFFECT OF ERYTHROPOIETIN ON LOCOMOTOR ACTIVITY IN ALCOHOL-INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Fall of time/10 min |
|---|---|---|
| Group 1 | Normal control | 259.133±7.113 ** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 229.833±4.473 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 253.720±3.169 * |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 277.287±6.048 *** |

**Effect of Erythropoietin on Cold Hyperalgesiain Alcohol Induced Neuropathy in Male Rats:** Effects of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on cold hyperalgesia have a latency of 61.167±4.028sec. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having a latency of 76.500±1.2043sec. Effect of treatment with Epo of two different doses Epo (250U/kgi.p) & Epo (500U/kg i.p) on cold hyperalgesia was significantly higher on comparing with Positive control 35% v/v Ethanol (10g/kg, p.o.) having a latency of 79.667±2.824 sec & 79.167±2.056 sec respectively.

**TABLE 8: EFFECT OF ERYTHROPOIETIN ON COLD HYPERALGESIA IN ALCOHOL-INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Latency in sec |
|---|---|---|
| Group 1 | Normal control | 76.500±1.204** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 61.167±4.028 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 79.667±2.824*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 79.167±2.056*** |

**Effect of Erythropoietin on Hot Hyperalgesia in Alcohol-Induced Neuropathy in Male Rats:** Effects of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on hot hyperalgesia have a latency of 9.667±1.116 8sec. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having a latency of 19.000±1.183 sec. Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on hot hyperalgesia was significantly higher on compared with Positive control 35% v/v Ethanol (10g/kg, p.o.) having a latency of 25.500±1.432 sec & 22.667±1.054 sec respectively.

**TABLE 9: EFFECT OF ERYTHROPOIETIN ON HOT HYPERALGESIA IN ALCOHOL-INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Latency in sec |
|---|---|---|
| Group 1 | Normal control | 19.000±1.183*** |
| Group 2 | Positive control 35% v/v Ethanol | 9.667±1.116 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 25.500±1.432*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 22.667±1.054*** |

**Effect of Erythropoietin on Mechanical Hyperalgesiain Alcohol Induced Neuropathy in Male Rats:** An effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on mechanical hyperalgesia has a force of 76.000±4.219 g. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having a force of 133.000±6.083 g. Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on mechanical hyperalgesia was significantly higher on compared with Positive control 35% v/v Ethanol (10g/kg, p.o.) having a force of 120.333±3.528 g & 131.800±2.818 g respectively**.**

**TABLE 10: EFFECT OF ERYTHROPOIETIN ON MECHANICAL HYPERALGESIA IN ALCOHOL-INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Force in g |
|---|---|---|
| Group 1 | Normal control | 133.000±6.083*** |
| Group 2 | Positive control 35% v/v Ethanol | 76.000±4.219 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 120.333±3.528*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 131.800±2.818*** |

**Effect of Erythropoietin on Mechanical Allodyniain Alcohol Induced Neuropathy in Male Rats:** Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) in males rats on mechanical allodynia has a force of 4.722±0.154 g. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to normal control having a force of 8.887±0.201 g. Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on mechanical allodynia was significantly higher on compared with Positive control 35% v/v Ethanol (10g/kg, p.o.) having a force of 8.698±0.268 g & 8.445±0.183 g respectively.

**TABLE 11: EFFECT OF ERYTHROPOIETIN ON MECHANICAL ALLODYNIA IN ALCOHOL-INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Force in g |
|---|---|---|
| Group 1 | Normal control | 8.887±0.201*** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 4.722±0.154 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 8.698±0.268*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 8.445±0.183*** |

p<0.001) when compared with positive control by using one way ANOVA followed by Dunnett multiple comparison test.

**Liver Function test:**

**Effect of Erythropoietin on Aspartate Amino-transferase in Alcohol-Induced Neuropathy in Male Rats:** Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on serum aspartate aminotransferase have an activity of 4402.325±314.021 nkat/L. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having an activity of 2431.264±140.216 nkat/L. Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg

i.p) on serum aspartate aminotransferase was significantly higher on comparing with Positive control 35% v/v Ethanol (10g/kg, p.o.) having a activity of 3824.326 ± 182.21nkat/L & 4195.755 ± 172.540 nkat/L respectively.

**TABLE 12: EFFECT OF ERYTHROPOIETIN ON ASPARTATE AMINOTRANSFERASE IN ALCOHOL INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Serum AST(nkt/L) |
|---|---|---|
| Group 1 | Normal control | 4402.325±314.021*** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 2431.264±140.216 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 3824.326±182.216*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 4195.755±172.540*** |

**Effect of Erythropoietin on Alanine Amino-transferase in Alcohol-Induced Neuropathy in Male Rats:** Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on serum alanine aminotransferase have an activity of 967.443±31.372 nkat/L. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having an activity of 3103.871±136.487 nkat/L. Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on serum alanine aminotransferase was significantly higher on compared with Positive control 35% v/v Ethanol (10g/kg, p.o.) having an activity of 3543.958±54.594nkat/L & 4094.763±122.689 nkat/L respectively.

**TABLE 13: EFFECT OF ERYTHROPOIETIN ON ALANINE AMINOTRANSFERASE IN ALCOHOL INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Serum ALT (nkat/L) |
|---|---|---|
| Group 1 | Normal control | 3103.871±136.487*** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 967.443±31.372 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 3543.958±54.594*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 4094.763±122.689*** |

**Lipid Profile Estimation:**

**Effect of Erythropoietin on Triglyceride in Alcohol-Induced Neuropathy in Male Rats:** Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on serum triglycerides have a concentration of 0.656±0.028 mmol/L. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having a concentrationof0.871±0.034 mmol/L. Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on serum triglycerides was significantly higher on compared with Positive control 35% v/v Ethanol (10g/kg, p.o.) having a concentration of 0.922±0.021 mmol/L &1.097±0.059 mmol/L respectively.

**TABLE 14: EFFECT OF ERYTHROPOIETIN ON TRIGLYCERIDE IN ALCOHOL INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Conc.(mmol/L) |
|---|---|---|
| Group 1 | Normal control | 0.871±0.034*** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 0.656±0.028 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 0.922±0.021*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 1.097±0.059*** |

**Electrophysiological Estimation:**

**Effect of Erythropoietin on Nerve Conduction Velocity in Alcohol-Induced Neuropathy in Male Rats:** Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on nerve conduction Velocity have a conduction velocity of 23.167±1.302 m/sec.

Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having a conduction velocity of 48.333±2.028 m/sec. Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on serum triglycerides was significantly higher on compared with Positive control 35% v/v Ethanol (10g/kg, p.o.) having a conduction velocity of 32.667±1.606 m/sec & 43.333±1.520 m/sec respectively.

**TABLE 15: EFFECT OF ERYTHROPOIETIN ON NERVE CONDUCTION VELOCITY IN ALCOHOL INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Nerve Conduction Velocity(m/sec) |
|---|---|---|
| Group 1 | Normal control | 48.333±2.028*** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 23.167±1.302 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 32.667±1.606** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 43.333±1.520*** |

**Biochemical Estimation:**

**Effect of Erythropoietin on Reduced Glutathione in Sciatic Nerve Homogenate in Alcohol-Induced Neuropathy in Male Rats:** Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on reduced glutathione in sciatic nerve homogenate have an activity of 34.993±1.742 µg of GSH/mg protein.

Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having an activity of 45.655 ± 1.081µg of GSH/mg protein Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on reduced glutathione in sciatic nerve homogenate was significantly higher on comparing with Positive control 35% v/v.

Ethanol (10g/kg, p.o.) has an activity of 58.536±2.014µg of GSH/mg protein & 62.606±1.213µg of GSH/mg protein, respectively.

**TABLE 16: EFFECT OF ERYTHROPOIETIN ON REDUCED GLUTATHIONE IN SCIATIC NERVE HOMOGENATE IN ALCOHOL-INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Reduced glutathione (µg of GSH/mg protein) |
|---|---|---|
| Group 1 | Normal control | 45.655±1.081*** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 34.993±1.742 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg,p.o.)+Erythropoietin (250U/kg i.p) | 58.536±2.014*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 62.606±1.213*** |

**Effect of Erythropoietin on Reduced Glutathione in Liver Homogenate in Alcohol-Induced Neuropathy in Male Rats:** Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on reduced glutathione in liver homogenate have an activity of 133.321±6.672 µg of GSH/mg protein. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having an activity of 299.491 ±20.825µg of GSH/mg protein. Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on reduced glutathione in the liver was significantly higher on compared with Positive control 35% v/v Ethanol (10g/kg, p.o.) having an activity of 377.007± 34.929µg of GSH/mg protein& 335.682±22.393µg of GSH/mg protein respectively.

**TABLE 17: EFFECT OF ERYTHROPOIETIN ON REDUCED GLUTATHIONE IN LIVER HOMOGENATE IN ALCOHOL-INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Reduced glutathione(µg of GSH/mg protein) |
|---|---|---|
| Group 1 | Normal control | 299.491±20.825*** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 133.321±6.672 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 377.007±34.929*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 335.682±22.393*** |

**Effect of Erythropoietin on Catalase in Sciatic Nerve Homogenate in Alcohol-Induced Neuropathy in Male Rats:** Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on catalase in sciatic nerve homogenate have an activity of 66.194±1.353 µmol/l/min/mg of protein. Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to

Normal control having an activity of88.904±1.379 µmol/l/min/mg of protein effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo(500U/kg i.p)on catalase in sciatic nerve homogenate was significantly higher on comparing with Positive control 35% v/v Ethanol (10g/kg, p.o.) having an activity of 79.256 ± 0.914 µmol/l/ min/mg of protein & 87.014±1.161 µmol/l/ min/mg of protein respectively.

**TABLE 18: EFFECT OF ERYTHROPOIETIN ON CATALASE IN SCIATIC NERVE HOMOGENATE IN ALCOHOL-INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Activity in µmol/l/min/mg of protein |
|---|---|---|
| Group 1 | Normal control | 88.904±1.379*** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 66.194±1.353 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 79.256±0.914*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 87.014±1.161*** |

**Effect of Erythropoietin on Catalase in Liver Homogenate in Alcohol-Induced Neuropathy in Male Rats:** Effects of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on catalase in sciatic nerve homogenate have an activity of 64.901±1.410 µmol/l/min/mg of protein.

Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having an activity of102.824±2.328 µmol/l/min/mg of protein Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on catalase in liver homogenate was significantly higher on comparing with Positive control 35% v/v Ethanol (10g/kg, p.o.) having an activity of 74.910±0.880 µmol/l/min/mg of protein & 85.680±1.290 µmol/l/min/mg of protein respectively.

**TABLE 19: EFFECT OF ERYTHROPOIETIN ON CATALASE IN LIVER HOMOGENATE IN ALCOHOL-INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Activity in µmol/l/min/mg of protein |
|---|---|---|
| Group 1 | Normal control | 102.824±2.328*** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 64.901±1.410 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (250U/kg i.p) | 74.910±0.880*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoietin (500U/kg i.p) | 85.680±1.290*** |

**Effect of Erythropoietin on Nitric Oxide in Sciatic Nerve Homogenate in Alcohol-Induced Neuropathy in Male Rats:** Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) in male rats on nitric oxide in sciatic nerve homogenate have a concentration of 28.446 ±1.313 µg/ml.

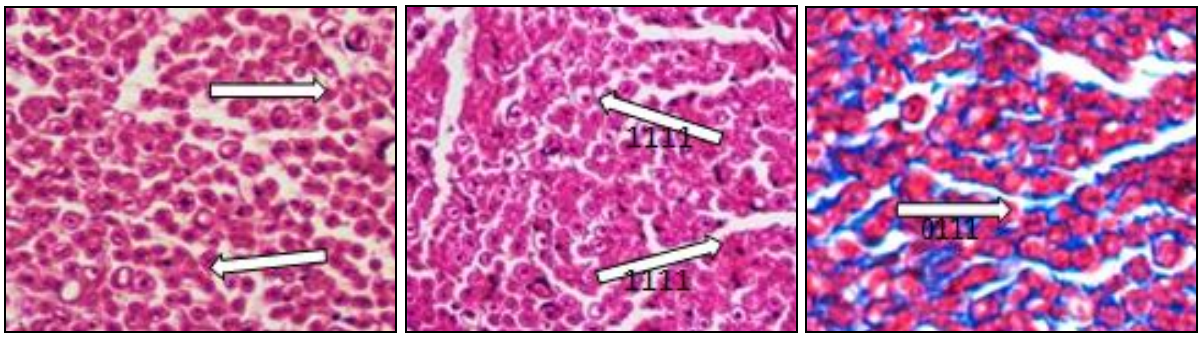
Effect of Positive control 35% v/v Ethanol (10g/kg, p.o.) was significantly lower when compared to Normal control having an activity of 59.167 ± 1.254µg/ml. Effect of treatment with Epo of two different doses Epo (250U/kg i.p) & Epo (500U/kg i.p) on nitric oxide in sciatic nerve homogenate was significantly higher on compared with Positive control 35% v/v Ethanol (10g/kg, p.o.) having an activity of 51.342 ± 2.575µg/ml & 61.511±1.371µg/ml respectively.

**TABLE 20: EFFECT OF ERYTHROPOIETIN ON NITRIC OXIDE IN SCIATIC NERVE HOMOGENATE IN ALCOHOL INDUCED NEUROPATHY IN MALE RATS**

| Groups | Treatment | Nitric conc.(µg/ml) |
|---|---|---|
| Group 1 | Normal control | 59.167±1.254*** |
| Group 2 | Positive control 35% v/v Ethanol (10g/kg,p.o.) | 28.446±1.313 |
| Group 3 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoiet in (250U/kg i.p) | 51.342±2.575*** |
| Group 4 | Positive control 35% v/v Ethanol (10g/kg, p.o.)+Erythropoiet in (500U/kg i.p) | 61.511±1.371*** |

**Histopathology of Sciatic Nerve:**

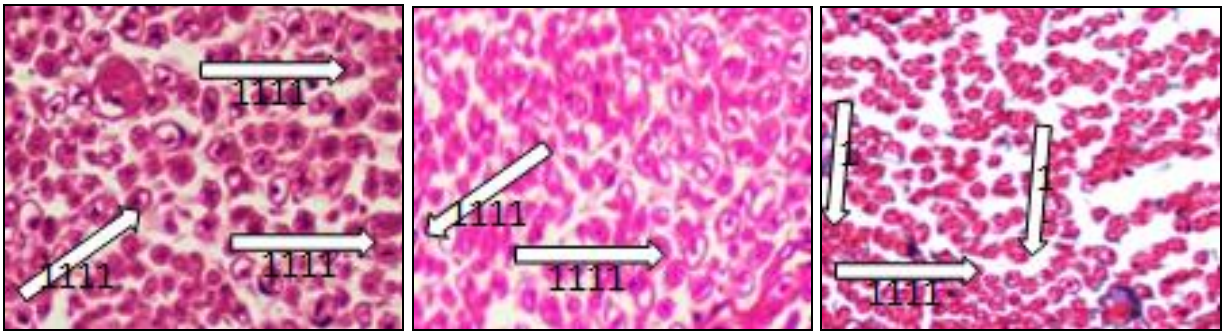
**Group: 01 (Normal control):**



**A. HEMATOXYLIN AND EOSIN 80X B. HEMATOXYLIN AND EOSIN 80X C. MASSON'S TRICHROME 160X**

**FIG. 1: HISTOLOGY OF RAT SCIATIC NERVE OF NORMAL CONTROL GROUP: (A) LIGHT MICROSCOPY TRANSVERSE SECTION SHOWING CLOSELY PACKED NERVE FIBERS AND AN OCCASIONAL ENDONEURIAL BLOOD VESSEL. (B) LIGHT MICROSCOPY TRANSVERSE SECTION SHOWING INDIVIDUAL NERVE FIBERS AND A CENTRAL AXON SURROUNDED BY A SHEATH OF MYELIN. (C) TRANSVERSE SECTION OF SPECIAL STAIN FOR COLLAGEN HIGHLIGHTS THE ENDONEURIAL MATRIX SEPARATING THE NERVE FIBERS, AND COLLAGENOUS COMPONENT IS STAINED BLUE**

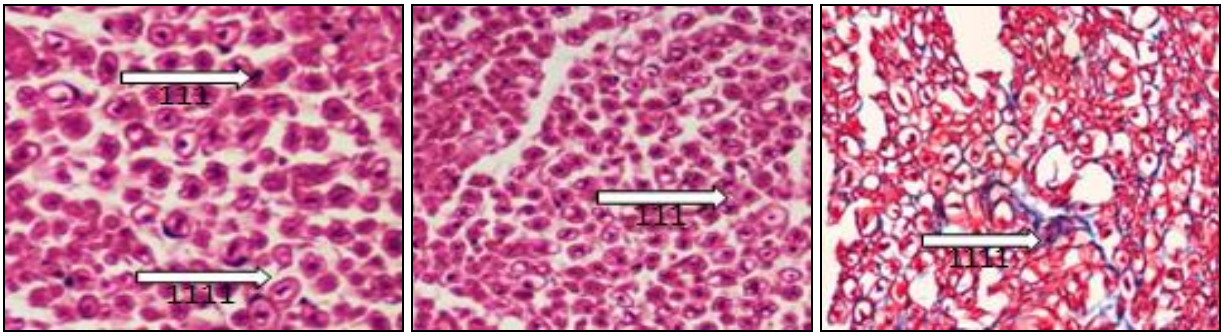
**Group: 02 (Positive control):**



**A. HEMATOXYLIN AND EOSIN 80X B. HEMATOXYLIN AND EOSIN C. MASSON'S TRICHROME160X**

**FIG. 2: HISTOLOGY OF RAT SCIATIC NERVE OF POSITIVE CONTROL GROUP: (A) LIGHT MICROSCOPY OF TRANSVERSE SECTION SHOWING SMALL NERVE FUNICLES WITH REDUCED FIBER DENSITY,(B) SCATTERED FIBERS WITH AXONAL SWELLING AND DEGENERATION (ARROWS). (C) TRANSVERSE SECTION OF SPECIAL STAIN FOR COLLAGEN HIGHLIGHTS AXONAL DEGENERATION**

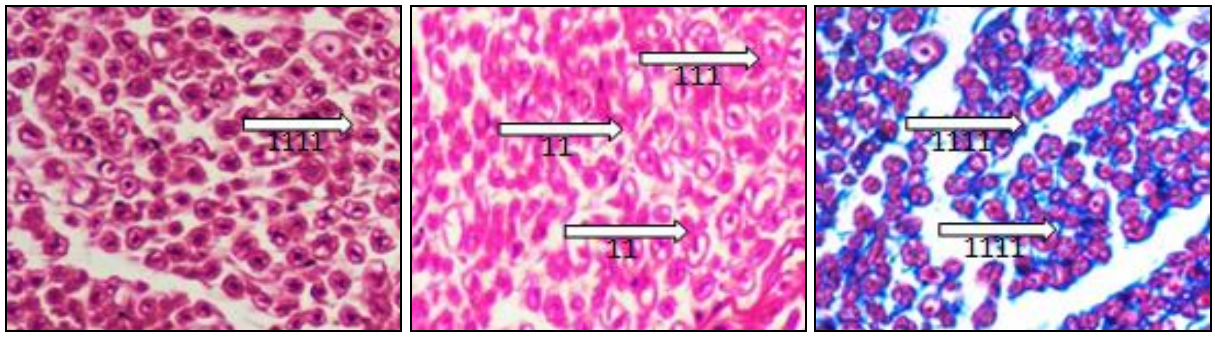
**Group: 03 (Epo 250):**



**A. HEMATOXYLIN AND EOSIN 80X B. HEMATOXYLIN AND EOSIN 80X C. MASSON'S TRICHROME 160X**

**FIG. 3: HISTOLOGY OF RAT SCIATIC NERVE OF POSITIVE CONTROL TREATED WITH EPO (250U/KG) GROUP: (A) LIGHT MICROSCOPY OF TRANSVERSE SECTION SHOWING NORMAL DENSITY OF MYELINATED FIBERS. (B) LIGHT MICROSCOPY TRANSVERSE SECTION SHOWING INDIVIDUAL NERVE FIBERS AND A CENTRAL AXON SURROUNDED BY A MYELIN SHEATH. (C) LIGHT MICROSCOPY OF TRANSVERSE SECTION SHOWING NORMAL COLLAGEN**

**Group: 04 (Epo 500):**



**A. HEMATOXLYIN AND EOSIN 80X B. HEMATOXLYIN AND EOSIN 80X C. MASSON'STRICHROME 160X**

**FIG. 4: HISTOLOGY OF RAT SCIATIC NERVE OF POSITIVE CONTROL TREATED WITH EPO (500U/KG) GROUP: (A) LIGHT MICROSCOPY OF TRANSVERSE SECTION SHOWING NORMAL DENSITY OF MYELINATED FIBERS. (B) LIGHT MICROSCOPY TRANSVERSE SECTION SHOWING INDIVIDUAL NERVE FIBERS AND A CENTRAL AXON SURROUNDED BY A SHEATH OF MYELIN. (C) LIGHT MICROSCOPY OF TRANSVERSE SECTION SHOWING NORMAL COLLAGEN**

**CONCLUSION:** The present study involved evaluation of the neuroprotective activity of Epo in alcohol-induced PN in rats by assessing behavioral, biochemical, and electrophysiological liver function test parameters.

This study concludes that the two doses of Epo improved sciatic nerve conduction, behavioral studies, and antioxidant activity in sciatic nerve homogenate. Among the two different doses of Epo 500U/kg was most effective besides showing neuroprotective properties against alcohol-induced PN.

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**Ethical Approval:** Approval was taken from the institutional animal ethics committee.

**CONFLICTS OF INTEREST:** None declared.

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