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Neuroprotective Effect of Nevirapine on Cerebral Ischemic Stroke by Middle Cerebral Artery Occlusion in Wistar Rats

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ABSTRACT:

Objective: To evaluate the neuroprotective effect of Nevirapine on cerebral ischemia stroke by middle cerebral artery occlusion in wistar rats.
Methods: The rats were pre and post treated with Nevirapine (NVP) at selective doses (5, 10 mg/kg/g, p.o) for a period of 14 days followed by middle cerebral artery occlusion (MCAO). Neurobehavioral changes were evaluated by using Y-maze and open field habituation. Biochemical markers such as acetyl cholinesterase (AChE), glutamate, differential leukocyte count (DLC), lactate dehydrogenase (LDH), antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GP_X) and catalase were estimated.
Results: Obtain results revealed that 14 days of treatment with NVP was effective in averting neurotoxicity. NVP treatment significantly reduced AChE, glutamate, DLC, LDH and elevated levels of antioxidant parameters such as SOD, catalase and GP_X.
Conclusion: These results clearly revealed that Nevirapine exhibited cognitive improvement which is related to its antioxidant and neuroprotective activity. Further studies are suggested to evaluate molecular mechanism of involved in neuroprotection.

1. INTRODUCTION

Stroke is the sudden death of a portion of the brain cells due to lack of oxygen. A stroke occurs when blood flow to the brain is disrupted resulting is abnormal function of brain. It is caused by blockage or rupture of an artery of the brain. Cerebral ischemia occurs when the cerebral blood flow is insufficient in meeting the brains metabolic need (di Tullio et al., 1996). Stroke is ranked as the third major cause for clinical mortality in humans. About 4.7 million people in the world suffer from stroke each year. The central nervous system consumes 20% of the cardiac output for normal function. Under physiological conditions, the cerebral blood flow (CBF) is maintained around 50 to 60 ml/100gm/min. The brain critically depends on a continuous supply of oxygen and glucose, more so than any other organ. Cerebral ischemia triggers a complex series of biochemical and molecular mechanisms that impairs the neurologic functions through breakdown of cellular integrity mediated by excitotoxic glutamatergic signaling, ionic imbalance, free radical reactions, etc. if the hypoxia reaches a critical threshold, neuronal excitability and synaptic transmission disturbances occur, affecting multiple neural circuits. Interruption of neural circuits is usually attributed to intrinsic changes and postsynaptic membrane properties (Krause et al., 1988) and changes in the release mechanisms of mediators from synaptic vesicles (siesjo and siesjio 1996). Ischemia of cerebral tissue and cellular death underlie all forms of stroke, including focal ischemia (as in embolic occlusion of the middle cerebral artery (MCA), global ischemia (as in cardiac arrest)). Focal cerebral ischemia (FCI), commonly referred to as stroke, is the result of a highly localized reduction in CBF to a discrete area of the brain. It is most often caused by a thrombosis or

embolism. In contrast global cerebral ischemia (GCI) occurs when there is wide spread reduction in blood flow to all or most parts of the brain. Global ischemia and reperfusion is relevant to cardiac arrest, hypoxic-ischemia encephalopathy of the new born (birth asphyxia) occurs during severe intracranial hypertension caused by trauma or cerebral edema from infectious and metabolic causes.

A new class of antiviral agents acting on the reverse transcriptase enzyme through a different mechanism compared to nucleoside analogues Reverse Transcriptase (RT) inhibitors and classified as non-nucleoside RT inhibitors (NNRTIs) has been developed. NNRTIs bind directly to the HIV RT enzyme and blocks the RNA dependent and DNA-dependent DNA polymerase activities by causing disruption of the enzyme catalytic site. It was well demonstrated that NNRTIs, NVP can cross the Blood Brain Barrier (BBB) and enter the central nervous system (Gibbs et al., 2006; von Giesen et al., 2002; Treisman and kaplin, 2002; Wynn et al.,2002). These drugs may have little direct effect on the neurological features of HIV/ AIDS (kilby and eron, 2003). In addition, NVP can be detected in the cerebrospinal fluid in concentrations effective in suppressing HIV viral levels (Tashima et al., 1999), which might be a prerequisite for the proven CNS efficacy. Previous studies suggest that NNRTIs are of therapeutic importance in patients with Aids Dementia Complex (ADC) (Enting et al., 1998). Reduction in MDA levels in rats following administration of NVP is observed, which is one of the indicators of antioxidant and free radical scavenging activity of NVP. In the present study, the efficiency of NVP in treating cerebral ischemic injury, its effect on glutamate and subsequent memory loss has been studied.

2. METHODOLOGY

2.1 Drugs and chemicals

DTNB (5,5'-dithiobis(2-nitron benzoic acid)), Eserine, Acetylthiocholine iodide, Pyrogallol, Dexamethasone, Bovine serum albumin, Sodium carbonate, Sodium hydroxide, Copper sulphate, Sodium Potassium Tartarate, Folin Ciocalteau's reagent, Ninhydrin, Tris-HCl buffer, Hydrochloric acid, Tris-EDTA buffer, Pyrogallol, DETPA, Ammonium molybdate, Hydrogen peroxide, Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium azide, Reduced glutathione, TCA, Sodium citrate.

2.2 Experimental animals

Wistar rats of either sex, weighing 180-200g, at the age 3-4 weeks were used in the study. They were maintained on standard laboratory pellet chow diet Provimi limited (India), provided water ad libitum and were kept under standard conditions at 23-25 ^{0}c, 35 to 60% humidity and 12hr light /dark cycle. The rats were acclimatized to the laboratory conditions a week prior to experiment. Institutional animal ethics committee (IAEC) duly approved the experimental protocol and care of the animals was carried out as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA).
(Protocol no: I/IAEC/LCP/016/2013/WR/24 ♂ ♀).

2.3 Experimental design

For screening of cerebral ischemic study, the animals are randomly divided in to five groups of six rats per group as following:-
Group I: Vehicle treated orally (CMC)
Group II: MCAO ischemia induced.
Group III: Animals treated with Nevirapine 5mg/kg (p.o)
Group IV: Animals treated with Nevirapine 10mg/kg (p.o)
Group V: Animals treated as sham

2.4 Induction of cerebral ischemia

Cerebral ischemia was induced by obstructing cerebral blood flow by carotid artery ligation. Here animal was anesthetized with urethane. A longitudinal cut was made at thoracic region beside trachea and carotid artery was identified carefully and ligated with thread. The animal is kept aside with ligated artery for 40min. After 40min the ligated thread from carotid artery is removed and sutured to close the opening.

2.5 Behavioral Parameters

2.5.1 Open Field Habituation

In order to control possible effects on locomotor activity, animals were explored to a 40 cm×50 cm×60 cm open field whose brown linoleum floor was divided into 12 equal squares by white lines. In both sessions, the animals were placed in the rear left square and left to explore it freely for 5 min during which time the number of line crossings and nose poking were counted. (Maria et al., 2005)

2.5.2 Video Tracking Testing On Y-Maze Test for Learning and Memory

Y-Maze is a behavioral test used to assess memory function and the willingness of rodents to explore new environments. The Y-Maze is particularly useful in measuring cognitive deficit in rodents. Maze apparatus was made of black plastic with three arms (40cm×15cm×35cm) extending from a central platform at 120°. Each rat was placed at the end of one arm and allowed to move freely through the maze during a session lasting 8mins. Arm entry was defined as the entry of 4 paws into one arm. The sequence of arm entries was recorded visually. Alternation was defined as multiple entries into the 3 arms (A, B or C) on overlapping triplet sets. The percentage of spontaneous alternation was calculated as the ratio of the actual-to-

possible alternations (defined as the total number of arm entries minus 2), multiplied by 100: as shown in the following equation (Reddy, 1997).

Alternation % = [(no. of alternations) / (total arm entries-2)] ×100

2.6 Biochemical Parameters

2.6.1 Acetylcholinesterase (AChE) enzyme determination

20 mg of brain tissue per ml of Phosphate buffer (pH 8, 0.1 M) was homogenized in a Potter-Elvehjem homogenizer. A 0.4 ml aliquot of brain homogenate was added to a cuvette containing 2.6 ml of 0.1M phosphate buffer (pH 8). 100 µl of the DTNB reagent was added to the photocell. The absorbance was measured at 412 nm. 20 µl of the acetylthiocholine iodide was added. Changes in absorbance were recorded and the change in absorbance per minute was calculated. The enzyme activity is expressed as µmoles/minute/g tissue (Koladiya et al., 2008).

2.6.2 Estimation of Glutamate

The brain region is homogenized in 80% double distilled ethanol (for every 100mg of the brain tissue, 2ml of 80% alcohol is used). Homogenates are transferred to polypropylene tubes and centrifuged at 1200rpm for 10 min. 1ml of the supernatant is then transferred into small test tubes and evaporated to dryness at 70^{o}C in an oven. The residue is reconstituted in 100 ml distilled water and 10µl is used for spotting on Whatman No.1 Chromatography paper. Standard solution of glutamate at a concentration of 2 mM is also spotted using an Eppendorf micropipette; the spots are dried. The chromatograms are then stitched at the sides and placed in a chromatography chamber containing butanol: acetic acid: water (65: 15: 25, V/V) as solvent. When the solvent front reached the top of the papers, the papers are removed and dried. A second run is performed similarly, after which the papers are dried, sprayed with ninhydrin (0.25% in acetone with 1% pyridine) and placed in an oven at 100^{o}C for 4 min. The portions that carry glutamate spots corresponding with the standard are cut and eluted with 0.005% CuSO4 in 75% ethanol. Their absorbance is read against a blank at 515nm and the levels are expressed as mmoles/ gram wet weight tissue (Nair et al., 2004).

2.6.3 Differential Leukocyte Count

The haemocytometer and cover slip were cleaned and dried. The blood was collected from retro-orbital sinus puncture in a heparinized tube. The blood was withdrawn into a WBC pipette up to mark 0.5. Then it was made upto the mark 11 by diluting. Blood and diluting fluid were mixed thoroughly. The diluted blood was discarded through the lower stem of the pipette up to mark 1.0. A drop of blood was put on each counting platform (NEUBAUER CHAMBER) of haemocytometer holding pipette at 45°C. A cover slip was put on the two platforms. The haemocytometer was then placed under a microscope to count the number of cells.

2.6.4 Estimation of Lactate dehydrogenase

Lactate dehydrogenase estimation was done by using a standard kit obtained from Biosystems S.A. Costa Brava 30, Barcelona. Lactate dehydrogenase (LD or LDH) catalyzes the reduction of pyruvate by NADH, to form lactate and NAD^{+}. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm (Lorentz, 1993).

2.6.5 Superoxide dismutase

For monitoring pyrogallol auto-oxidation, 20 to 80 µl of pyrogallol was pipette out and the volume was made up to 100 µl with 0.01 N HCl. To this 600 µl of Tris –HCl buffer, 100 µl of DETPA, 100 µl of Tris EDTA, 100ul of distilled water was added. For the sample assay, 50 µl of pyrogallol and 50 µl of the sample were added and the reaction was initiated & measured at 420 nm at a constant temperature of 25ºC for 3min at 0.5 nm bandwidth. Superoxide dismutase activity was determined by the pyrogallol oxidation method. This is an indirect method that is based on the ability of the enzyme to inhibit the auto-oxidation of pyrogallol (Markland et al., 1974).

2.6.7 Glutathione peroxidase

0.4ml phosphate buffer, 0.1 ml sodium azide, 0.2 ml reduced glutathione, 0.1 ml hydrogen peroxide, 1 ml water is added to 0.1 ml enzyme (brain homogenate) and incubated for 0 minutes, $1_{1/2}$ min, 3min. 10% 1 ml trichloro acetic acid was added to the above solution and centrifuged for 15 min at a speed of 3000 rpm. To 1 ml of resultant supernatant, 4ml of phosphate solution, 0.5 ml DTNB was added and the absorbance was read at 412 nm (Lawrence and Burk., 1976).

2.6.8 Catalase

Add 1 mL of H_2O_2 to 100 µL of sample and mix well, after 60 seconds Add 1 mL ammonium molybdate. Read the absorbance at 405 nm (Goth, 1991).

2.6.9 Statistical analysis

All the values are expressed as mean ± S.E. Statistical significance between more than two groups was tested using one-way ANOVA followed by the tukey's test as appropriate using computer based fitting program (Graph pad prism). Differences were considered to be statistically significant when $P < 0.05$.

3. RESULTS

3.1 Nevirapine on Exploratory Behavior

In the open field habituation memory test the animals induced with ischemia by MCAO indicated a reduction in the line crossings significantly (P<0.05) when compared to the control animals. The treatment of Nevirapine in group IV protected the open field habituation memory by increasing the activity of line crossings with significant difference (P<0.05). All the results were presented in the table no.1 and fig no.1.

3.2 Nevirapine on Y-Maze Test

In the y-maze test, the group II animals indicated the impaired percentage alteration with significant (P<0.001) reduction on comparing with the control animals. In the treatment groups, Nevirapine at the doses of 5mg/kg and 10mg/kg improved the percentage alteration significantly (P<0.001). Moreover there was dose dependent increase in activity of the two doses of Nevirapine and all the results were shown in the table no.1 and fig no.1.

3.3 Nevirapine on Acetylcholinesterase Enzyme

The effect of Nevirapine on AChE was depicted in the table no.2 and fig no.1. In the negative control group (group II) induction of ischemia by MCAO elevated the level of AChE significantly (P<0.01) when compared to the control group (group I). In the treatment groups (group III&IV) of 5mg/kg and 10mg/kg of Nevirapine, there was a significant reduction in AChE enzyme with P<0.001 and P<0.001 respectively on comparison with negative control group.

3.4 Nevirapine on Glutamate

The table no.2 and fig no.1 shows the changes in glutamate levels of different animal groups. In the group II, the induction of ischemia increases the level of glutamate significantly (P<0.001) when compared to the control group. Nevirapine 5mg/kg and 10mg/kg treatment on the ischemia induced animals significantly (P<0.01) elevated the levels of glutamate when compared to the negative control.

3.5 Nevirapine on Monocytes

The effect of different groups of animals induced with ischemia and the changes in monocytes in the blood were denoted in the table no.3 and fig no.1. Induction of MCAO on group II, significantly (P<0.001) increased, the leukocyte count and the treatment of 5mg/mg and 10mg/kg nevirapine significantly (P<0.001) decreased the monocytes on comparison with negative control group.

3.6 Nevirapine on Lymphocytes

Table no.4 and fig no.2 indicated that group animals has a significant (P<0.001) increase in lymphocytes after ischemia when compared to normal control group. Upon treatment with nevirapine 10mg/kg it was noted that there was a decrease in lymphocytes significantly with P<0.01 when compared to the negative control group.

3.7 Nevirapine on Neutrophils

The neutrophils of all the treatment groups were mentioned in the table no.4 and fig no.2. The group II animals indicated the significant induction of ischemia after carotid artery ligation with remarkable increase in the neutrophils with P<0.001. After the treatment with two different doses (5mg/kg and 10mg/kg) of nevirapine the significant (P<0.001 & P<0.001) decrease in neutrophils respectively when compared to the ischemia induced group.

3.8 Nevirapine on Lactate dehydrogenase

The LDH of all the treatment groups were mentioned in the table no.3 and fig no.2. The group II animals indicated the significant induction of ischemia after carotid artery ligation with remarkable increase in the LDH enzyme with P<0.01. The decreased levels of LDH were escalated after the treatment with 5mg/kg and 10mg/kg Nevirapine indicates the significant (P<0.05 & p <0.01) decrease in LDH when compared to the only ischemia induced group.

3.9 Nevirapine on Catalase

The Catalase levels of all the groups were mentioned in the table no.5 and fig no.2. The group II animals indicated the significant induction of ischemia after carotid artery ligation with remarkable decrease in the catalase enzyme with P<0.001. The decreased levels of catalase were escalated after the treatment with 5mg/kg and 10mg/kg Nevirapine indicates the significant P<0.05 and p<0.001 reduction in catalase respectively when compared to the only ischemia induced group.

3.10 Nevirapine on SOD

The table no.5 and fig no.2 shows the changes in SOD levels of different animal groups. In the group II, the induction of ischemia reduce the level SOD significantly (P<0.001) when compared to the control group. Nevirapine 5mg/kg and 10mg/kg treatment on the ischemia induced animals significantly (P<0.001) elevated the levels of SOD when compared to the negative control and was merely equivalent to the normal control group.

3.11 Nevirapine on Enzyme GP_X

The effect of Nevirapine on GP_X was depicted in the table no.5 and fig no.2. In the negative control group (group II) induction of ischemia by MCAO decreased the level of GP_X significantly (P<0.001) when compared to the control group (group I). In the treatment groups (group III&IV) of 5mg/kg and 10mg/kg Nevirapine, there was a significant increase in GP_X enzyme with P<0.05 and P<0.01 respectively on comparison with negative control group.

Table No: 1 Effect of Nevirapine on Exploratory Behavior and Y-maze

S.NO	GROUP	Treatment	LINECROSSING	Y-MAZE
1	Group I	(vehicle)	92.18 ± 3.38	45.61 ± 1.93
2	Group II	(MCAO)	22.17 ±3.38***	19.21 ± 1.30***
3	Group III	(MCAO + NVP 5 mg/kg)	66.50 ± 7.43$^{\#}$	28.01 ± 2.14$^{\#\#\#}$
4	Group IV	(MCAO + NVP 10 mg/kg)	87.17 ± 8.62$^{\$}$	49.08 ± 1.84$^{\$\$\$}$
5	Group V	sham	55.83 ± 3.41	32.02 ± 1.738

***p<0.001 indicates comparison of negative control group with control group
$^{\#\#\#}$p<0.001 indicates comparison of low dose group with negative control group
$^{\$\$\$}$p<0.001, $^{\$\$}$p<0.01 indicates comparison of high dose with negative control group.

Table no: 2: Effect of Nevirapine on AChE and Glutamate

S.NO	GROUP	Treatment	ACHE	GLUTAMATE
1	Group I	(vehicle)	86.16 ± 32.13	395.1± 72.96
2	Group II	(MCAO)	156.8 ± 21.51**	726.3 ± 133.7**
3	Group III	(MCAO + NVP 5 mg/kg)	40.45 ± 4.84$^{\#\#\#}$	420.0± 127.5$^{\#\#}$
4	Group IV	(MCAO + NVP 10 mg/kg)	28.70 ± 13.95$^{\$\$\$}$	358.9± 99.25$^{\$\$}$
5	Group V	sham	13.68 ± 2.87	282.9± 18.51

$^{**}P$<0.01 indicates the significant difference on comparing Group II with Group I. $^{\#\#\#}$P<0.001$^{\#\#}P$<0.01 indicates comparison of low dose group with negative control group. $^{\$\$\$}$p<0.001, $^{\$\$}$p<0.01 indicates comparison of high dose with negative control group.

Table No: 3: Effect Of Nevirapine on Monocytes and Lymphocytes

S.NO	GROUP	Treatment	MONOCYTES	LYMPHOCYTES
1	Group I	(vehicle)	32.00 ± 2.15	23.50 ± 5.06
2	Group II	(MCAO)	64.25± 3.54***	44.25 ± 8.057***
3	Group III	(MCAO + NVP 5 mg/kg)	17.00 ± 1.44$^{\#\#\#}$	37.50 ± 2.64
4	Group IV	(MCAO + NVP 10 mg/kg)	14.25 ± 2.06$^{\$\$\$}$	26.00 ± 1.63$^{\$\$}$
5	Group V	sham	16.50 ± 0.70	47.00 ± 4.23

***P<0.001 indicates the comparison of Group II with Group I.
$^{\#\#\#}$P<0.001 indicates comparison of low dose group with negative control group. $^{\$\$\$}$p<0.001, $^{\$\$}$p<0.01 indicates comparison of high dose with negative control group.

Table No: 4 Effect Of Nevirapine on LDH and Neutrophils

S.NO	GROUP	Treatment	LDH	NEUTROPHILS
1	Group I	(vehicle)	448.0 ± 160.2	49.40 ± 4.65
2	Group II	(MCAO)	1224 ± 488.2**	64.60 ±3.29***
3	Group III	(MCAO + NVP 5 mg/kg)	610.7± 92.09$^{\#}$	49.60 ± 3.57$^{\#\#\#}$
4	Group IV	(MCAO + NVP 10 mg/kg)	455.6 ± 92.29$^{\$\$}$	36.60 ± 3.28$^{\$\$\$}$
5	Group V	sham	1056 ± 183.1	31.00 ± 4.23

***P<0.001 indicates the comparison of Group II with Group I.
$^{\#\#\#}$P<0.001 indicates comparison of low dose group with negative control group. $^{\$\$\$}$p<0.001, $^{\$\$}$p<0.01 indicates comparison of high dose with negative control group.

Table no: 5: Effect of Nevirapine on Catalase, SOD and GPx

S.NO	GROUP	Treatment	CATALASE	SOD	GPx
1	Group I	(vehicle)	3665 ± 155.8	2.545± 0.84	3.267 ± 10.2
2	Group II	(MCAO)	2265 ± 408.9 ***	0.8404± 0.35***	0.8104 ±0.28
3	Group III	(MCAO + NVP 5 mg/kg)	2899 ± 317.3 $^{\#}$	1.971± 0.48$^{\#}$	1.885 ±0.068
4	Group IV	(MCAO + NVP 10 mg/kg)	3779 ± 220.1 $^{\$\$\$}$	2.189 ± 0.33$^{\$\$}$	2.096 ± 0.26
5	Group V	Sham	2911 ± 335.3	0.6213±0.15	0.754 ± 0.14

***P<0.001 indicates the comparison of Group II with Group I.
$^{\#\#\#}$P<0.001 indicates comparison of low dose group with negative control group. $^{\$\$\$}$p<0.001, $^{\$\$}$p<0.01 indicates comparison of high dose with negative control group.
Values are expressed as mean ± SEM of 6 animals. Superscript letters represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.

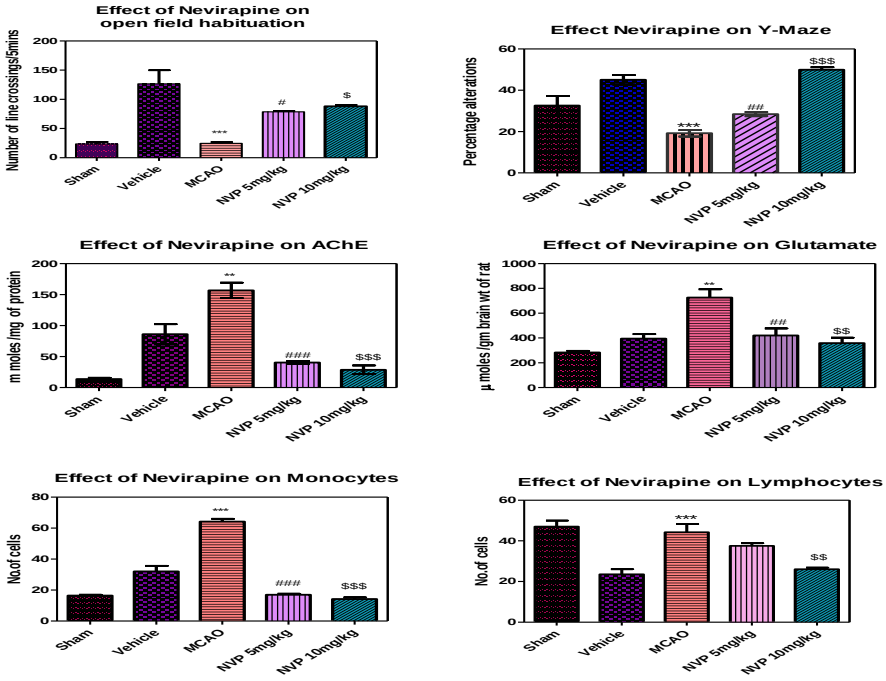

Fig: 1: Effect of Nevirapine on Open field habituation, Y-maze, AChE, Glutamate, Monocytes, Lymphocytes

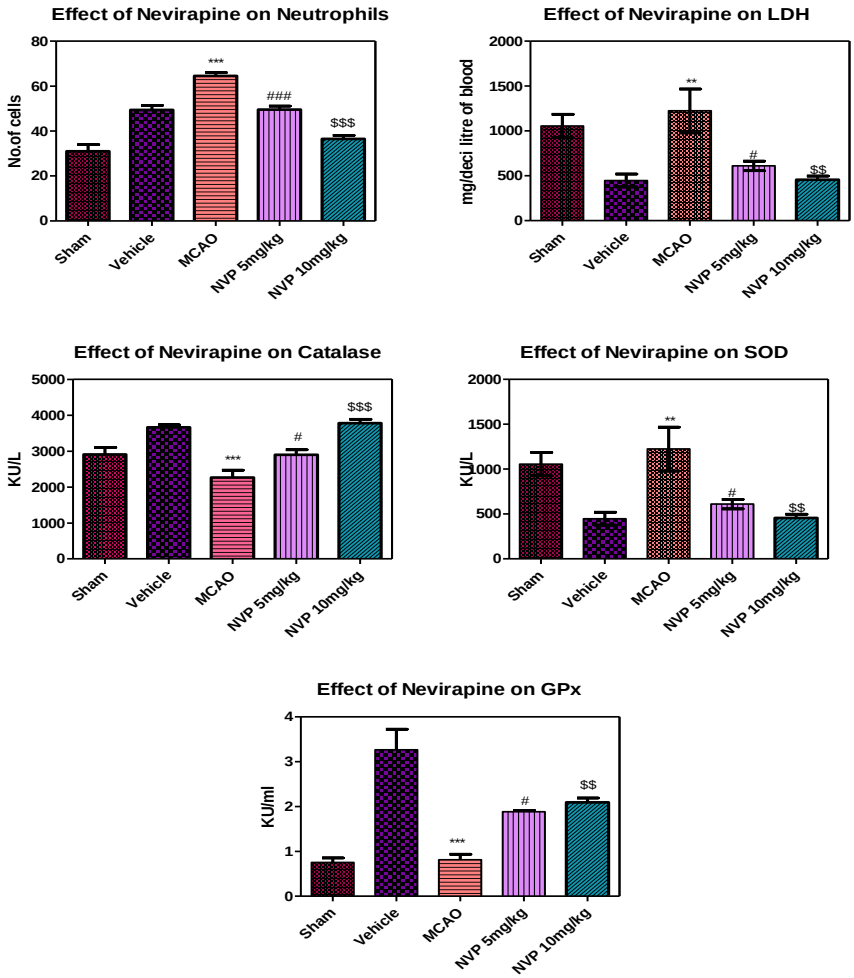

Fig: 2: Effect of Nevirapine on Neutrophils, LDH, Catalase, SOD, GPx

4. DISCUSSION

Stroke is the sudden death of a portion of the brain cells due to lack of oxygen. A stroke occurs when blood flow to the brain is disrupted resulting in abnormal function of brain. It is caused by blockage or rupture of an artery of the brain. Cerebral ischemia triggers a complex series of biochemical and molecular mechanisms that impairs the neurologic functions through breakdown of cellular integrity mediated by excitotoxic glutamatergic signaling, ionic imbalance, free radical reactions, etc. if the hypoxia reaches a critical threshold, neuronal excitability and synaptic transmission disturbances occurs, affecting multiple neural circuits. Interruption of neural circuits is usually attributed to intrinsic changes and postsynaptic membrane properties and changes in the release mechanisms of mediators from synaptic vesicles (siesjo and siesjio 1996). In the present study, neuro protective effect of Nevirapine was assed against various models. Open field test allows evaluating habituations memory through measurement of the exploratory behavior (crossing and head dips) (Natalia et al., 2013). The treatment of Nevirapine in group IV protected open field habituation memory by increasing the activity of line crossing with significant difference ($p \leq 0.05$). These results are consistent with the favorable effect on cognition in open field habituation memory.

Long-term potentiation (LTP) is a phenomenon responsible for the cellular mechanism of learning and memory processes (Natalia et al., 2013). Y maze is simple test for measuring spatial recognition memory that is based on discrimination of novelty versus familiarity in the three arms. The ability to alternate requires that the rat know which arm they have already visited (Wietrzych et al., 2005). In the study the group II animal's indicated impaired percentage alternation with significant reduction on comparing with the control animals, but in Nevirapine

treatment groups dose dependently improved the percentage alternation significantly ($p \leq 0.01$). Nevirapine improved percentage spontaneous alternation and indicates the improvement of LTP.

Evidence shows that the brain cholinergic system plays an important role in learning and memory and is vulnerable to oxidative damage and pathogenesis of AD (Satyabrata et al, 2004). In negative control group ischemia induced by MCAO oral administration in rats observed elevated levels of AChE in the hippocampus and leads to cognitive loss. In our study Nevirapine treated groups showing decreased levels of AChE and increased levels of acetylcholine. This suggests that Nevirapine shows AChE inhibitory activity and it may be due to the potential antioxidant effect by increasing the level of antioxidant defense system in the cholinergic neurons of the brain and thereby improves learning and memory defects.

Glutamate is excitatory neurotransmitter in the CNS, excessive levels of glutamate in the brain leads to neuronal toxicity (Sundaram et al., 2012). In negative control group (MCAO induced ischemia) observe elevated levels of glutamate compared to group I. In our study dose dependently group IV animals are showing decreased levels of glutamate compared to group III and negative control group. This suggests that Nevirapine prevents neuronal cell death in the brain.

Brain is almost exclusively dependent on the continuous steady flow of glucose and oxygen to undergo oxidative phosphorylation for energy production, because it has no stores of energy. The disruption of blood flow causes accumulation of lactic acid via anaerobic glycolysis, and the cellular ATP decreases. The reduction of cellular energy results in the impairment of vital cellular functions, such as the activity of ATPase, which contribute to Ca2+ overload. Then, the increase of intracellular Ca2+ concentration leads to cell damage and eventual death. Energy depletion and necrotic neuronal death can trigger the inflammatory injury. Evidence suggests that necrosis and apoptosis are the main characteristics of neuronal death following acute cerebral ischemia-reperfusion injury. In our investigation negative control group shows elevated levels of LDH compared to group I III and IV. NVP treated groups showing decreased the levels of LDH indicates the reduced the accumulation of lactate and improves energy production in the brain. In negative control group observe elevated levels compared to group I, III and IV. NVP treated groups observing decreased levels of leukocytes means increased incidence of neuroinflamation protective property. In our investigation improved levels of anti oxidant parameters like catalase, superoxide dismutase and GP_X. Thus from these pharmacological screening, it was found that the Nevirapine shows anti oxidant, anti-amnesic and neuroprotective effect to improve cognitive function.

5. CONCLUSION

In the present study, MCAO induces cerebral ischemia in male wistar rats which is confirmed by biochemical changes. Pre-treatment and post treatment with Nevirapine prevents cerebral ischemia by its antioxidant activity and also by its learning and memory enhancing property. This proved that Nevirapine can able to protect the rat's brain against ischemic damage. This finding might help to understand the beneficial effects of Nevirapine on neuroprotection against ischemic damage.

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