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Research Article

Neuroprotective Effect Of Morin Hydrate And Gemigliptin Against Parkinson's Disease

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ABSTRACT

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The objective of the study was to evaluate the neuroprotective outcome of morin hydrate and Gemigliptin combination in reserpine and rotenone induced parkinsonism. Reserpine (1 mg/kg, s.c.) was used to induce orofacial dyskinesia and rotenone (1.5 mg/kg, i.p.) was used to induce parkinsonism in rats. Injection of reserpine for 3 days on alternate days significantly increased the vacuous chewing movements (VCM), tongue protrusions (TP) and reduced the locomotor activity in rats. Treatment with Morin hydrate (50 and 100 mg/kg, p.o. for 5 days) and Gemigliptin (50 and 100 mg/kg, p.o. for 5 days) showed dose dependent significant reduction in VCM and TP. Morin hydrate and Gemigliptin also showed significant increase in locomotor activity. Administration of rotenone for 10 days in rats exhibited significant reduction in latency to fall off from rotarod

and decrease in nose pokes in hole board test. Treatment with similar doses of morin hydrate and Gemigliptin showed significant boost in latency to fall off from rotarod and increase in nose pokes. Reserpine and rotenone considerably increased lipid peroxidation and declined the levels of self-protective antioxidant enzymes like catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD) in rat brain. Morin hydrate and Gemigliptin combination upturned these effects of reserpine and rotenone on oxidative stress indices; indicating amelioration of oxidative stress in rat brains. Current study predetermined that Morin hydrate in combination with Gemigliptin has a protective effect against reserpine induced orofacial dyskinesia and rotenone induced parkinsonism. Thus, use of these drugs could form a strategic therapeutic potential against parkinsonism.

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INTRODUCTION:

Parkinson's disease (PD) is chronic and the most ordinary neurodegenerative disorder. After Alzheimer's disease, PD affects ~1% of the population over age 60. Every year, around 60,000 patients in the U.S. are diagnosed with PD. Worldwide, an approximate 7 to 10 million people live with PD. Men are 1.5 times more vulnerable to PD as compared to women. Parkinson's disease is mainly characterized by the impaired motor functions, manifesting in rigidity, tremor, bradykinesia and postural instability. Nonmotor symptoms may also be associated including olfactory and/or autonomic dysfunction, cognitive impairment, psychiatric symptoms, sleep disorders, pain and fatigue.^[1,2]

Bradykinesia is the majority characteristic clinical feature of PD that manifests by troubles in initiation, execution and seize of movement. Rigidity, increased resistance to muscles stretch and relaxation due to tightness and stiffness of muscles and may occur proximally e.g. neck, shoulders, hips and distally e.g. wrists, ankles or both. Tremor at rest, mainly in distal part of the extremities, is in addition one of the most identifiable symptoms of PD. The later stage of PD is followed by freezing, akinesia. Particularly in patients with postural instability and flexed truncal posture, festination of gait (involuntary quickening of gait) may also occur^[3]. The accurate etiology of PD is yet unclear and the exact mechanisms that cause this disease yet to be identified.^[4] At the cellular level, PD is related to surplus production of reactive oxygen species (ROS), alterations in catecholamine metabolism, modifications in mitochondrial electron transport chain (METC) function, intensification of iron deposition in the substantia nigra pars compacta (SNpc). The stoppage of normal cellular processes that take place in relation to the aging process are also supposed to contribute to the amplified vulnerability of dopaminergic neurons.^[5] Familial forms of PD involve mutations in number of genes,^[6] mitochondrial dysfunction, neuroinflammation and environmental factors are increasingly appreciated as key determinants of dopaminergic neuronal susceptibility in PD and these are the features of both familial and sporadic forms of the disease.^[7]

Morin is a plant derived flavonoid widely available from *Moraceae* family. It is reported that morin exerts antioxidant, antidiabetic and neuroprotective effects by modulating the activities of several enzymes. Morin has been reported to inhibit ROS formation, apoptosis, caspase-3 activation in several animal models. This mechanism is also involved in the pathology of PD. Therefore, morin can act as a protective therapeutic agent against PD.^[8] Gemigliptin is a novel DPP-4 inhibitor that was approved in patients with type 2 diabetes mellitus. Some studies were designed to determine the anti-apoptotic and anti-inflammatory effects of DPP-4 inhibitor in cardiomyocytes. Gemigliptin has been reported to reduce endoplasmic reticulum stress and apoptosis signaling molecules.^[9]

In the present investigation the combined neuroprotective role of morin hydrate and Gemigliptin was evaluated in reserpine induced orofacial dyskinesia and rotenone induced neurobehavioral and biochemical changes in rats. The effect of the combination was studied on oxidative stress indices in rat brains. Epidemiological studies suggest that exposure to environmental agents, such as pesticides, may increase PD risk.^[10] Mitochondrial dysfunction has also been linked to PD. Specifically; there are systemic reductions in the activity of complex I of the mitochondrial electron transfer chain (ETC) in PD brain, muscle, and platelets.^[11]

MATERIALS AND METHODS

Experimental Animals

Wistar strain rats (200-220 g) of either sex was used for the study. Animals were procured and housed in polypropylene cages and maintained under the standard laboratory environmental conditions; temperature $25 \pm 2^\circ\text{C}$, 12: 12 h L: D cycle and $50 \pm 5\%$ RH with free access to food and water *ad libitum*. Animals were acclimatized to laboratory conditions before the test. All the experimental work carried out during the light period (08:00-16:00 h). The study carried out in harmony with the guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India). The Institutional Animal Ethical Committee

of M.V.P.S College of Pharmacy, Nashik approved the protocol of the study (IAEC/2019/01).

Drugs used

Morin hydrate (SIGMA ALDRICH, Bengluru, India), Gemigliptin (ZEMIGLO 50mg, SANOFI, Goa, India), Reserpine (RESEARCH LAB, Mumbai, India), Rotenone (Sigma Aldrich, Mumbai, India), Vitamin E (Merck Ltd., Goa, India), Levodopa-Carbidopa (SUN PHARMA Lab. Ltd).

Chemical Used

Nitrobluetetrazolium Chloride (ALFA AESAR, A Johnson Matthey Company, Chennai, India). 2-Thiobarbituric acid (RESEARCH LAB, Fine Chem., Mumbai, India), Ethylenediamine tetra-acetic acid (Research Lab, Fine Chem., Mumbai, India), 5,5'-Dithiobis (2-nitrobenzoic acid) (ALFA AESAR, A Johnson Matthey Company, Chennai, India)

Experimental design for reserpine-induced orofacial dyskinesia in rats

Animals were divided into eight groups. Group I treated with 0.2% PEG in distilled water, p.o., for 5 days and 0.1% acetic acid solution for 3 days, every other day. Group II with reserpine (1 mg/kg, s.c.) in 0.1% acetic acid for 3 days, every other day. Group III treated with reserpine (1 mg/kg, s.c.) for 3 days, every other day and morin hydrate (50 mg/kg, p.o.) for 5 days. Group IV with reserpine (1 mg/kg, s.c.) for 3 days, every other day and morin hydrate (100 mg/kg, p.o.) for 5 days. Group V with reserpine (1 mg/kg, s.c.) for 3 days, every other day and Gemigliptin (50 mg/kg, p.o.) for 5 days. Group VI with reserpine (1 mg/kg, s.c.) for 3 days, every other day and Gemigliptin (100 mg/kg, p.o.) for 5 days. Group VII with reserpine + morin hydrate (1 mg/kg, s.c. + 50 mg/kg, p.o.). Group VIII treated with reserpine (1 mg/kg, s.c.) for 3 days, every other day and Vitamin E (10 mg/kg, p.o.) for 5 days.

Procedure

Animals were divided into 8 groups, each group containing 6 animals weighing 200-220g. Standard laboratory diet and drinking water were freely accessible to all the animals of each group. Body weight of each animal was measured before and at the end of the treatment. Average food and water intake were recorded. Group I (Control group) animals were received 0.2% PEG in distilled water, p.o., for 5

days and 0.1% acetic acid for 3 days, every other day. Group II animals received reserpine (1 mg/kg, s.c.) in 0.1% acetic acid for 3 days, every other day. Animals in Group III and IV were received morin hydrate at a dose 50 and 100 mg/kg, p.o. respectively for 5 days and reserpine for 3 days, every other day. Rats in Group V and group VI were received Gemigliptin at a dose of 50 and 100 mg/kg, p.o. respectively for 5 days along with reserpine, every other day. Group VII animals were received combination dose of morin hydrate and Gemigliptin (50 mg/kg, p.o. + 50 mg/kg, p.o. respectively) along with reserpine, every other day. Group VIII animals received standard drug Vitamin E (10 mg/kg, p.o.) for 5 days and reserpine for 3 days, every other day. The first injection of acetic acid was given 24 h after the administration of PEG. A time interval of 30 minutes was maintained in between administration of test/standard drug and Reserpine. On the fifth day, 24 h after the second reserpine or vehicle injection, all the rats had been observed for the quantification of orofacial dyskinesia. After 1 h of behavioural measurements all animals were sacrificed for evaluation of biochemical parameters.

Behavioural testing;

Quantification of Dyskinesia

To quantify the event of oral dyskinesia on the test day, rats positioned individually into a small Plexiglas observation cage (30 × 20 × 20 cm) to keep count vacuous chewing movements (VCM) and tongue protrusion frequencies. Animals allowed for 10 min accustoming to the observation cage before behavioural assessments performed. Mirrors placed under the floor and behind the back wall of the cage to allow observation of oral dyskinesia when the animal faced away from the observer. The VCM and tongue protrusion were defined as a single mouth opening in the vertical plane not directed towards physical material and visible extension of the tongue outside of the mouth respectively. If VCM or tongue protrusion occurred during a period of grooming, they were not considered. The behavioural parameters of oral dyskinesia measured constantly for a period of 15 min. In all the experiments, the observer was blind to the identity of the animals. ^[12]

Assessment of total locomotor activity by digital photo-actophotometer

Locomotor activity is an index of awareness of mental activity as most of the drugs acting on CNS influence locomotor activity. It is measured with the help of photo-actophotometer which operates on photoelectric cells that relate to circuit with counter. Interruption of light beams as a measure of movements of rats in a cage has been used by many authors. When a beam of light falling on photocell is cut-off by the animal, a count is recorded. Locomotion was measured up to 10 min for each rat, 24 h after administration of last dose of treatment. ^[13]

Experimental design for Rotenone-induced neurodegeneration in rats

Group I treated with WFI, p.o. for 10 days. Group II with rotenone (1.5 mg/kg, i.p.) in PEG: DMSO for 10 consecutive days. Group III with rotenone (1.5 mg/kg, i.p.) and morin hydrate (50 mg/kg, p.o.) for 10 days. Group IV treated with rotenone (1.5 mg/kg, i.p.) and morin hydrate (100mg/kg, p.o.) for 10 days. Group V with rotenone (1.5 mg/kg, i.p.) and Gemigliptin (50 mg/kg, p.o.) for 10 days. Group VI treated with rotenone (1.5 mg/kg, i.p.) and Gemigliptin (100 mg/kg, p.o.) for 10 days. Group VII with rotenone (1.5 mg/kg, i.p.) and morin hydrate + Gemigliptin (50 mg/kg, p.o.+ 50 mg/kg, p.o.) 10 days. Group VIII treated with rotenone (1.5 mg/kg, i.p.) and Levodopa-Carbidopa (30 mg/kg, p.o.) for 10 days.

Procedure

As per experimental design, animals were divided into 8 groups, each group containing 6 animals weighing 200-220 g. Standard laboratory diet and drinking water were freely accessible to all the animals of each group. Body weight of each animal was measured before and at the end of treatment. Average food and water intake were recorded. Group I (Control group) animals received WFI, p.o., for 10 days and Group II animals received rotenone 1.5 mg/kg, i.p. in PEG and DMSO in ratio 1:1 for 10 days. Animals in Group III and IV received morin hydrate at a dose 50 and 100 mg/kg, p.o. respectively along with rotenone 1.5 mg/kg, for 10 days. Rats in Group V and VI received Gemigliptin at a dose of 50 and 100 mg/kg, p.o. respectively along with rotenone for 10

days. Group VII animals received combination dose of morin hydrate + Gemigliptin (50 mg/kg, p.o.+ 50 mg/kg, p.o.) along with rotenone for 10 days. Group VIII animals received standard drug Levodopa-Carbidopa (30 mg/kg, p.o.) and rotenone (1.5 mg/kg, i.p.) for 10 days. On the 10th day, 24 h after the rotenone or vehicle injection, all the rats were observed for the behavioural tests. Animals were sacrificed about 1 h after behavioural measurements for further evaluation of biochemical parameters. ^[14,15]

Behavioural testing;

Rotarod test

The apparatus consists of a horizontal wooden pole or metal rod coated with rubber with 3 cm diameter attached to a motor with the speed adjusted to 2 rpm. The rod was 75 cm in length and was divided into 6 sections by plastic discs, thereby permitting the simultaneous testing of 6 mice or rats. The rod was in a height of about 50 cm above the tabletop in order to discourage the animals from jumping off the roller. Cages below the sections serve to restrict the movements of the animals when they fall from the roller. Rats undergo a pre-test on the apparatus. Only those animals which had confirmed their ability to remain on the revolving rod for at least 1 minute were used for the test. The test compounds were administered intraperitoneal or per orally. Thirty minutes after intraperitoneal or 60 min after oral administration the rats were placed for 1 min on the rotating rod. The number of animals falling from the roller during this time was counted. The rota rod test was used to assess motor coordination and balance. Rats must maintain their balance on a rotating rod. The rod was rotated at 4 rpm and gradually increased to 20 rpm. The latency time required for the rat to fall off the rod rotating at different speeds or under continuous acceleration (e.g., from 4 to 20 rpm) was recorded. ^[13]

Hole and Board test

The hole-board has a size of 40 × 40 cm. 16 holes with a diameter of 3 cm each are distributed evenly on the floor. The animal's tendency to insert its head into the holes was observed and quantified. The board was elevated so that the rats poking its nose into the hole, does not see the bottom. Vehicle-treated

control and rotenone-treated animals could explore the holes in the board every day for 5 min, and the total number of pokes per 5 min was recorded for each rat. Nose-poking was thought to point out curiosity and was measured by visual inspection in the old description and counted by electronic devices in more recent modifications. Moreover, in the newer modification's motility was measured in addition by counting interruption of light beams. Thirty minutes after administration of the test compounds and standard the animal was placed on the hole-board and counting was done for 5 min. ^[16]

Estimation of various antioxidant enzymes in rat brain

Dissection and homogenization:

On the last day, 1 h after all behavioural assessments, the animals were sacrificed by using euthanasia chamber provided with CO₂ and brains were removed. The separated brains were rinsed with isotonic saline solution and weighed. 0.1M phosphate buffer (pH 7.4) was used to prepare tissue homogenate (10% w/v). Post nuclear fraction for catalase assay: Centrifugation of the homogenate (10% w/v) at 1000 × g for 20 min at 4 °C (Remi - C30, Remi Industries Ltd. Mumbai, India), Post nuclear fraction for other enzyme assays: Centrifugation of homogenate at 12000 × g for 60 min at 4°C. Elico BL 200 bio spectrophotometer was used for succeeding assays. ^[17]

Estimation of catalase:

In this method, breakdown of H₂O₂ is measured at 240 nm as per Luck (1971) method. Assay mixture required was consists of 3ml of 0.01 M H₂O₂-phosphate buffer (pH 7) and 0.05 ml of supernatant of tissue homogenate (10% w/v). After 1 min, the change in absorbance was measured at 240nm. The millimolar extinction coefficient of H₂O₂ (0.071) was used to calculate enzyme activity. The results were expressed as μM of H₂O₂ decomposed per minute per milligram of protein. ^[18]

Estimation of reduced glutathione:

Ellman (1959) method was used. A 0.75 ml sample of homogenate was used which was precipitated with 0.75 ml of 4 % sulphosalicylic acid. The samples were centrifuged at 1200 × g for 15 min at 4°C. The assay

mixture was consisting of 0.5 ml supernatant and 4.5 ml of 0.01 M DTNB [5, 5'- dithiobis (2-nitrobenzoic acid)] in 0.1 M phosphate buffer (pH 8.0). The yellow colour was developed, and it was read immediately at 412nm. The results were expressed as μM of GSH per milligram of protein. ^[19]

Estimation of superoxide dismutase:

In this estimation procedure, the reduction of nitrobluetetrazolium chloride (NBT) was inhibited by the superoxide dismutase and measured at 560nm spectrophotometrically according to Kono (1978) method. The reaction was initiated by the addition of 0.1 ml of 1mM hydroxylamine hydrochloride to the reaction mixture containing 0.1 ml of 0.1 m Methylenediamine tetra-acetic acid (EDTA), 0.1 ml of 24 μM NBT, 0.1 ml of 0.03% v/v Triton X100 reagent and post nuclear fraction of brain homogenate to make 1 ml final volume. The mixture was incubated at 37°C for 20 min and absorbance was measured at 560 nm. Results were expressed as percentage inhibition of reduction of NBT. ^[20]

Lipid peroxidation assay:

Wills (1966) method was used for the quantitative assessment of lipid peroxidation in rat brains. In this method, the amount of malondialdehyde (MDA) formed was measured by reaction with thiobarbituric acid at 532 nm. The reaction mixture contains 0.1 ml tissue homogenate, 0.2 ml of 8% sodium lauryl sulphate (SLS), 1.5 ml of 20% acetic acid, and 1.5 ml of 0.8% thiobarbituric acid (TBA) solution. This mixture was then heated at 95 °C for 1 h on water bath. Then, 5 ml mixture of n-butanol and pyridine (15:1 ratio) was added to it. Mixture was shaken vigorously and centrifuged at 2200 × g for 5 min. The absorbance of upper layer (organic layer) was measured at 532 nm. The results were expressed as nM of MDA per milligram of protein using the molar extension coefficient of chromophore ($1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$). ^[21]

Histopathological evaluation:

Animal were sacrificed and brains were separated for histopathological evaluation. The separated brains were excised and rapidly fixed in 10% buffered formalin. The cerebral cortex from the brain was used for sectioning, which was embedded in paraffin after being dehydrated in alcohol. Sections were fixed in

paraffin blocks and by using microtome, four to five micrometer thick serial histological sections were obtained. Staining of sections was done by using hematoxylin and eosin (H and E). After this, under light microscope stained sections were placed and examined. Photomicrographs of sections were taken for evaluation.

Statistical analysis:

All results were expressed as mean \pm SEM. All the data of groups were analysed by applying one-way analysis of variance followed by Dunnett's test using Graph Pad Prism 5.0 software (Graph-Pad®, San Diego, CA, USA).

RESULTS:

Reserpine induced orofacial dyskinesia.

Effect of Morin hydrate and Gemigliptin on total locomotor activity: Group II showed significant ($p < 0.001$) decrease in total locomotor activity compared to group I. Group IV, VI, VII, showed significant ($p < 0.001$) increase in total locomotor activity as compared to group II. However, group III and V showed non-significant increase in total locomotor activity compared to group II. Group VIII also showed significant ($p < 0.001$) increase in total locomotor activity compared to Group II.

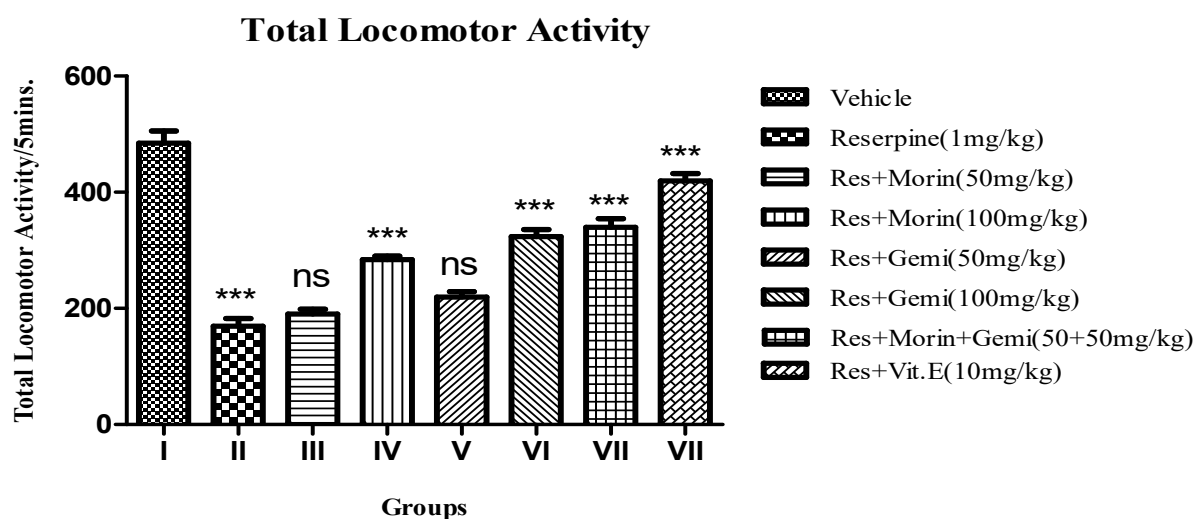


Fig.1 Effect of morin hydrate and Gemigliptin on total locomotor activity.

Values expressed as mean \pm S.E.M. Group II compared with group I, Groups III, IV, V, VI, VII and VIII compared with group II. One-Way ANOVA followed by Dunnett's test, ns – Non significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Effect of morin hydrate and Gemigliptin on vacuous chewing movements: Group II showed

significant ($p < 0.001$) increase in vacuous chewing movement compared to group I. Group IV, V, VI, VII showed significant ($p < 0.001$) decrease in vacuous chewing movements. Group III showed non-significant decrease in vacuous chewing movement as compared to group II. Group VIII also showed significant ($p < 0.001$) decrease in vacuous chewing movements compared to Group II.

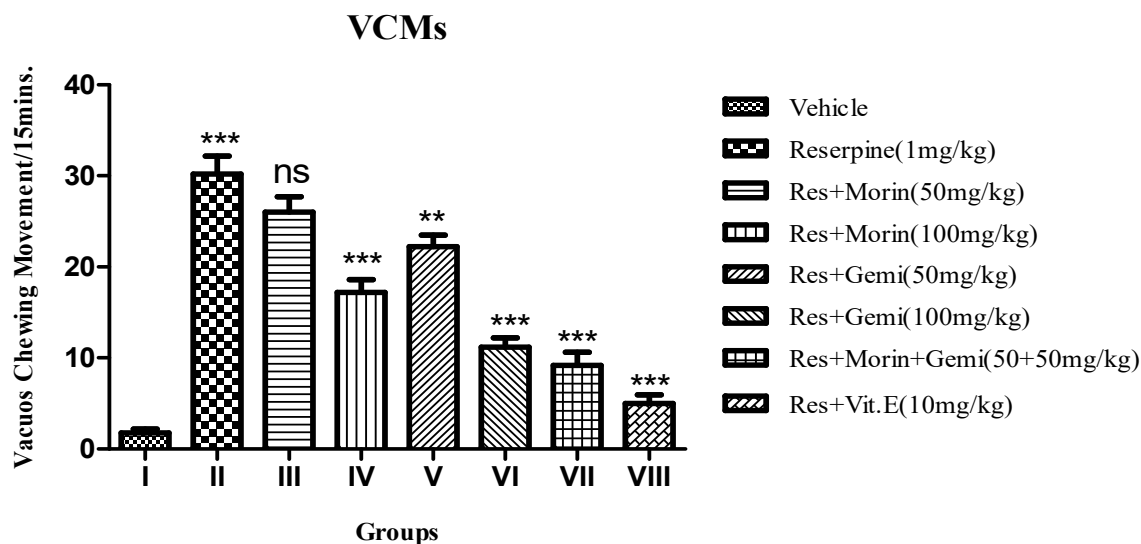


Fig.2 Effect of morin hydrate and Gemigliptin on vacuous chewing movement.

Values expressed as mean \pm S.E.M. Group II compared with group I. Groups III, IV, V, VI, VII and VIII compared with group II. One-Way ANOVA followed by Dunnett's test. ns – Non significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Effect of morin hydrate and Gemigliptin on tongue protrusions: Group II showed significant ($p < 0.001$)

increase in Tongue protrusion frequency compared to group I. Group III, IV, V, VI, VII showed significant ($p < 0.001$) decrease in Tongue protrusion frequency compared to Group II. Group VIII also showed significant ($p < 0.001$) decrease in tongue protrusion compared to Group II.

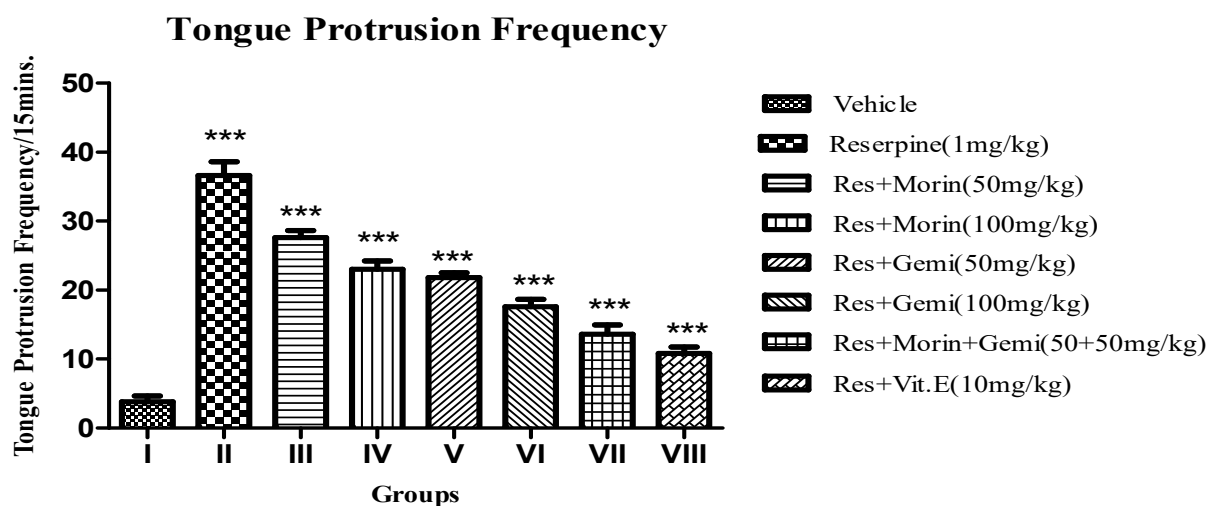


Fig.3 Effect of morin hydrate and Gemigliptin on tongue protrusion frequency.

Values expressed as mean \pm S.E.M. Group II compared with group I. Groups III, IV, V, VI, VII and VIII compared with group II. One-Way ANOVA followed by Dunnett's test. ns – Non significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Biochemical parameters:

Effect of morin hydrate and Gemigliptin on catalase (CAT) level in rat brain: Group II showed significant ($p < 0.001$) decrease in CAT level as

compared to Group I. Group IV, VII ($p < 0.001$) and Group VI ($p < 0.05$) showed significant increase in CAT level as compared to group II. Group III and group V showed non-significant increase in CAT level

as compared to Group II. Group VIII also showed significant ($p < 0.001$) increase in CAT level as compared to Group II.

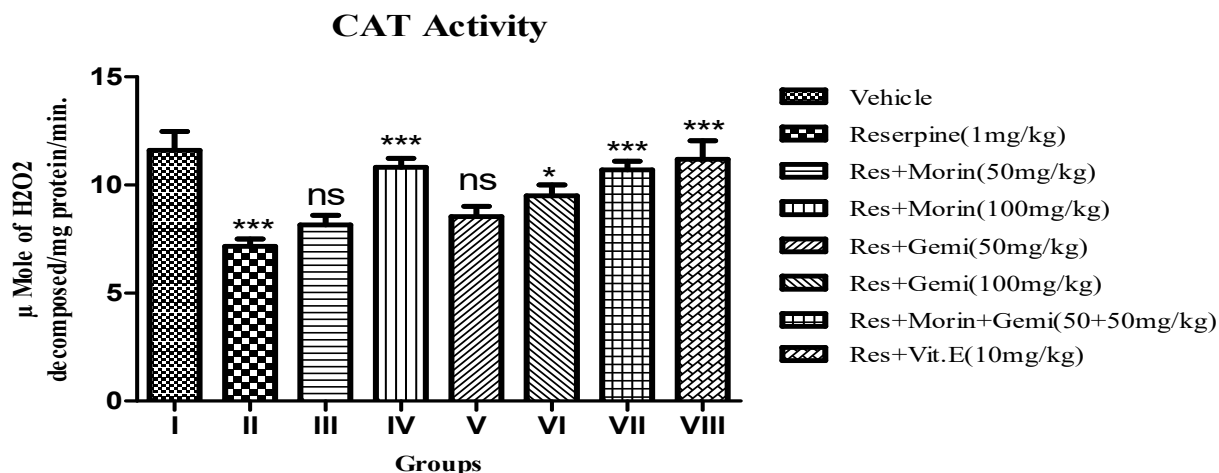


Fig.4 Effect of morin hydrate and Gemigliptin on catalase (CAT) level in rat brain.

Values expressed as mean \pm S.E.M. Group II compared with group I. Groups III, IV, V, VI, VII and VIII compared with group II. One-Way ANOVA followed by Dunnett's test. ns – Non significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Effect of morin hydrate and Gemigliptin on reduced glutathione (GSH) level in rat brain:

Group II showed significant ($p < 0.001$) decrease in GSH level as compared to Group I. Group III, IV, V, VI, VII showed significant ($p < 0.001$) increase in GSH level compared to Group II. Group VIII also showed significant ($p < 0.001$) increase in GSH level compared to Group II.

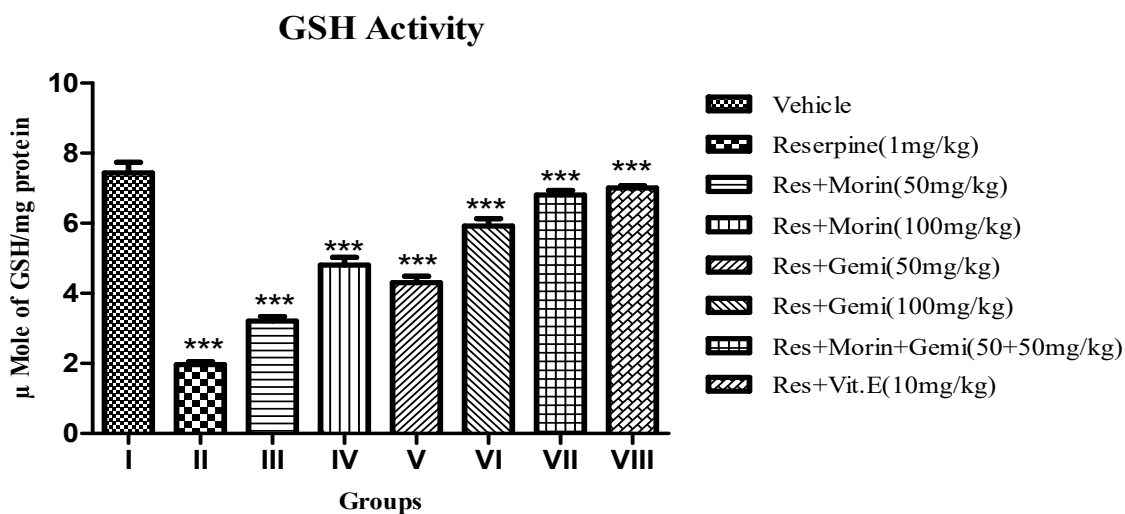


Fig. 5 Effect of morin hydrate and Gemigliptin on reduced glutathione (GSH) level in rat brain.

Values expressed as mean \pm S.E.M. Group II is compared with group I. Groups III, IV, V, VI, VII and VIII compared with group II. One-Way ANOVA

followed by Dunnett's test. ns – Non-significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Effect of morin hydrate and Gemigliptin on superoxide dismutase (SOD) level in rat brain:

Group II showed significant ($p < 0.001$) decrease in SOD level as compared to Group I. Group V, VI, VII ($p < 0.001$) and Group IV ($p < 0.05$) showed significant

increase in SOD level compared to Group II. Group III showed non-significant increase in SOD level as compared to Group I. Group VIII also showed significant ($p < 0.001$) increase in SOD level compared to Group II.

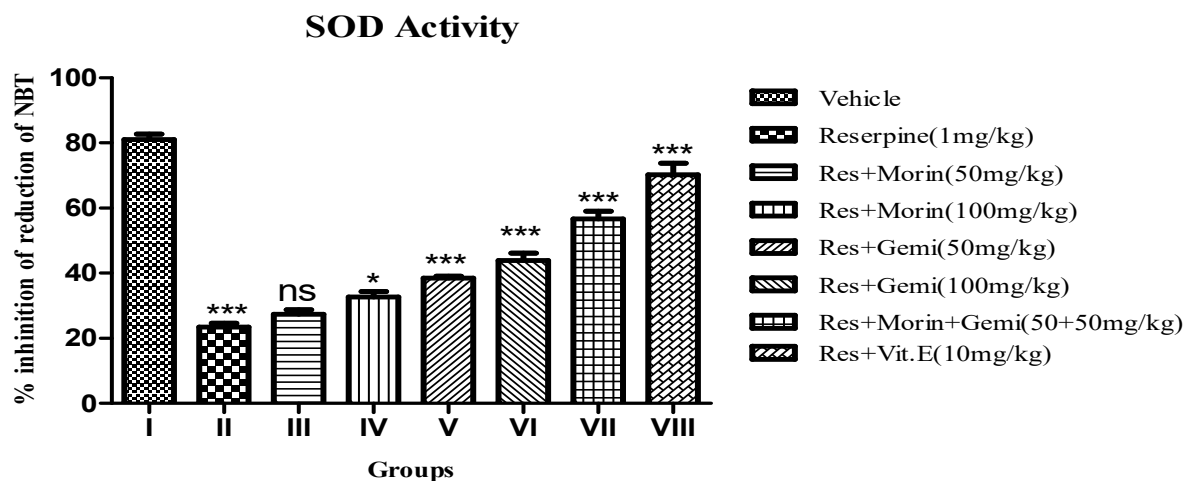


Fig.6 Effect of morin hydrate and Gemigliptin on superoxide dismutase (SOD) level in rat brain.

Values expressed as mean \pm S.E.M. Group II is compared with group I. Groups III, IV, V, VI, VII and VIII compared with group II. One-Way ANOVA followed by Dunnett's test. ns – Non-significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Effect of morin hydrate and Gemigliptin on Lipid peroxidation (LPO) level in rat brain: Group II

showed significant ($p < 0.001$) increase in LPO level as compared to Group I. Group IV, VI, VII ($p < 0.001$) and Group V ($p < 0.05$) showed significant decrease in LPO level as compared to Group II. Group III showed non-significant decrease in LPO level as compared to Group II. Group VIII also showed significant ($p < 0.001$) decrease in LPO level as compared to Group II.

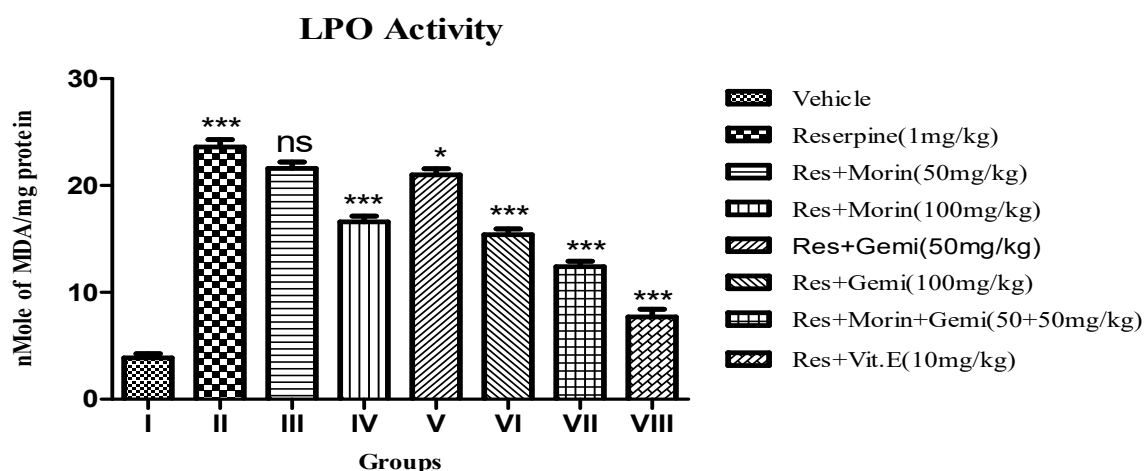
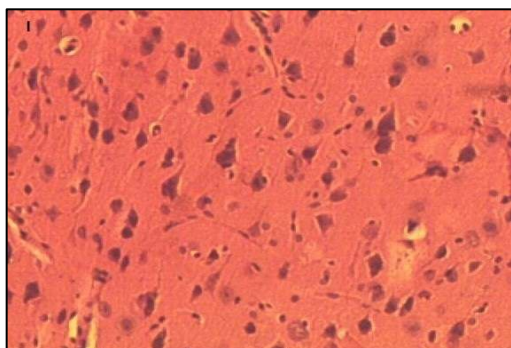


Fig.7 Effect of morin hydrate and Gemigliptin on lipid peroxidation (LPO) level in rat brain.

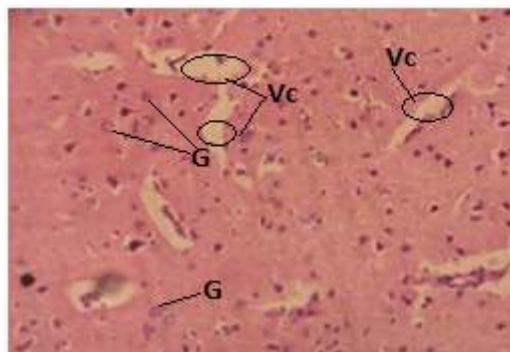
Values expressed as mean \pm S.E.M. Group II compared with group I. Groups III, IV, V, VI, VII and

VIII compared with group II. One-Way ANOVA followed by Dunnett's test. ns – Non-significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

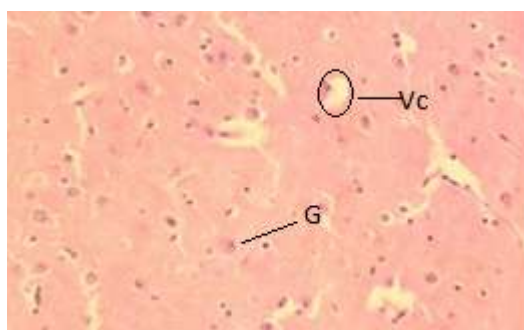
Histopathological Analysis



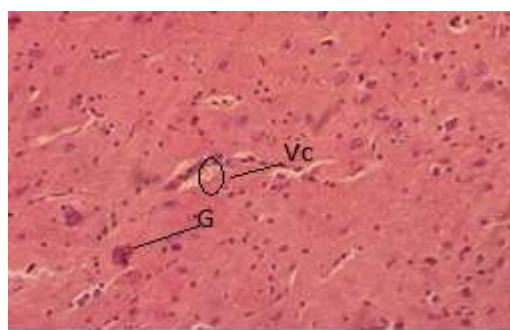
Group I



Group II



Group III



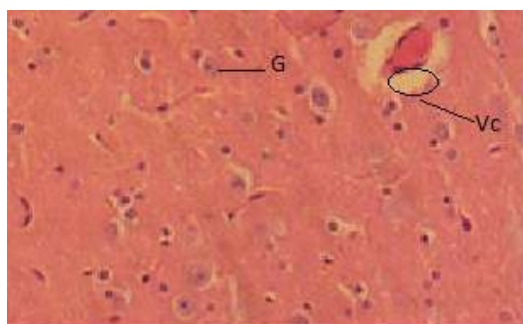
Group IV



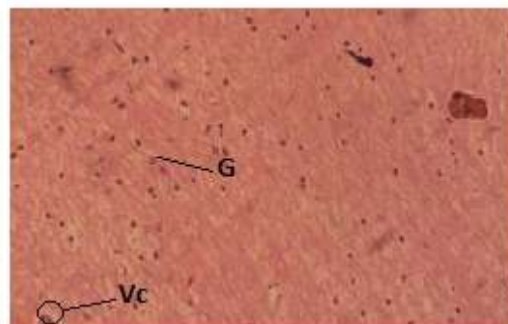
Group V



Group VI



Group VII



Group VIII

Fig 8. Histopathological changes of cerebral cortex of rats in different groups.

Group I: Control group showing usual histopathological structure of cerebral cortex. Group II: Reserpine treated group revealed the existence of ghost cells (G), vacuolated cytoplasm (Vc) indicating damage to cerebral cortex. Group III: Morin hydrate (50 mg/kg, p.o.), Group IV: Morin hydrate (100 mg/kg, p.o.), Group V: Gemigliptin (50 mg/kg, p.o.), Group VI: Gemigliptin (100 mg/kg, p.o.), Group VII: Morin hydrate (50 mg/kg, p.o.) + Gemigliptin (50 mg/kg, p.o.), Group VIII: Vit. E (10 mg/kg, p.o.). These groups also showed the presence of ghost cells (G) and vacuolated cytoplasm (Vc), however the number of ghost cells and vacuolated cytoplasm is lesser compared to reserpine group (Group II).

Rotenone induced neurodegeneration

Effect of morin hydrate and Gemigliptin on motor activity: Group II showed significant ($p < 0.001$) decrease in latency to fall off from rotarod as compared to Group I. Group IV ($p < 0.01$) and Group V, VI, VII ($p < 0.001$) showed significant increase in latency to fall off from rotarod as compared to Group II. Group III showed non-significant increase in latency to fall off from rotarod as compared to Group II. Group VIII also showed significant ($P < 0.001$) significant increase in latency to fall off from rotarod as compared to Group II.

Rota-Rod Test

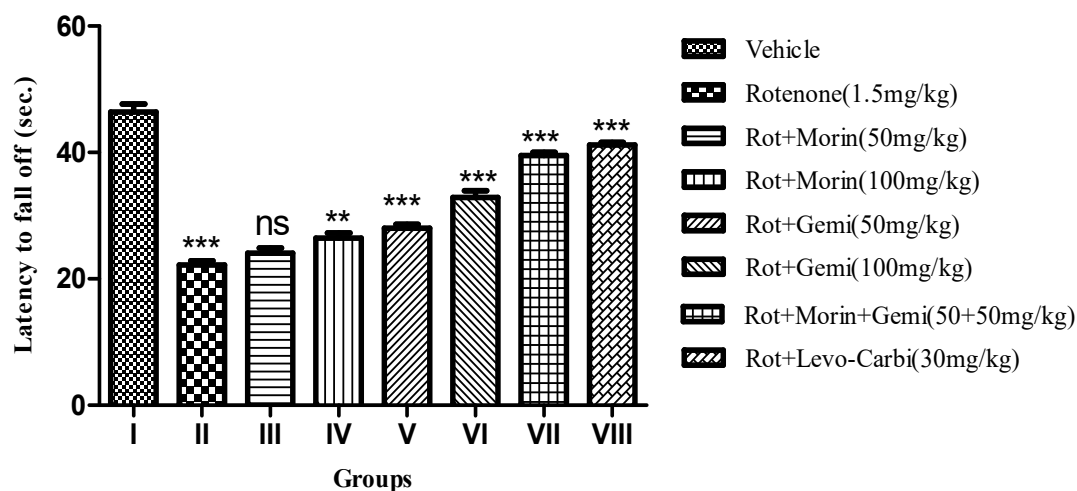


Fig.9 Effect of morin hydrate and Gemigliptin on motor activity.

Values expressed as mean \pm S.E.M. Group II is compared with group I. Groups III, IV, V, VI, VII and VIII compared with group II. One-Way ANOVA followed by Dunnett's test. ns – Non significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Effect of morin hydrate and Gemigliptin on number of pokes in hole board test: Group II

showed significant ($p < 0.001$) decrease in nose pokes as compared to Group I. Group III ($p < 0.05$), Group V ($p < 0.01$) and Group IV, VI, VII ($p < 0.001$) showed significant increase in nose pokes as compared to Group II. Group VIII also showed significant ($p < 0.001$) increase in nose pokes as compared to Group II.

Hole Board Test

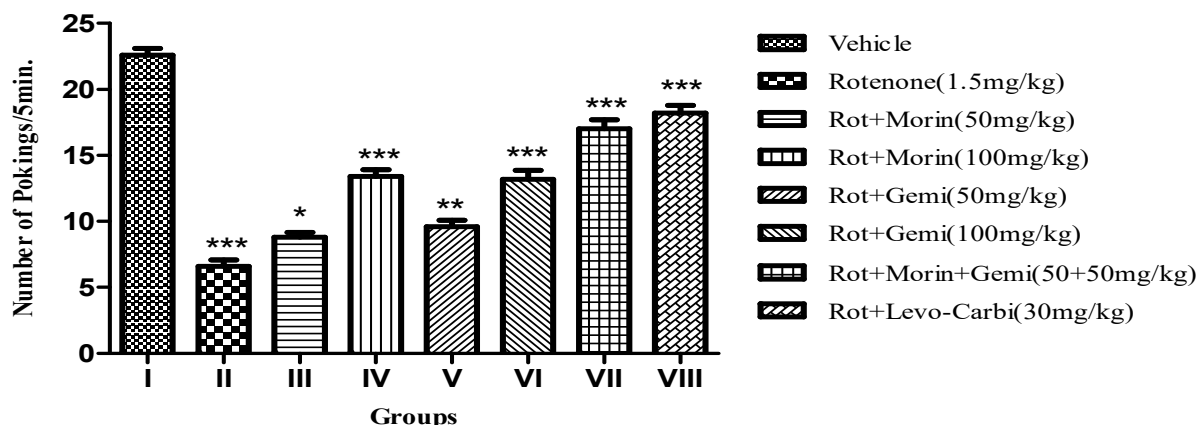


Fig. 10 Effect of morin hydrate and Gemigliptin on number of pokes in hole board test.

Values expressed as mean \pm S.E.M. Group II is compared with group I. Groups III, IV, V, VI, VII and VIII are compared with group II. One-Way ANOVA followed by Dunnett's test. ns – Non significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Biochemical parameters:

Effect of morin hydrate and Gemigliptin on catalase (CAT) level in rat brain: Group II showed

significant ($p < 0.001$) decrease in CAT level as compared to Group I. Group IV, VI, VII showed significant ($p < 0.001$) increase in CAT level as compared to Group II whereas Group III and Group V showed non-significant increase in CAT level as compared to Group II. Group VIII also showed significant ($p < 0.001$) increase in CAT level compared Group II.

CAT Activity

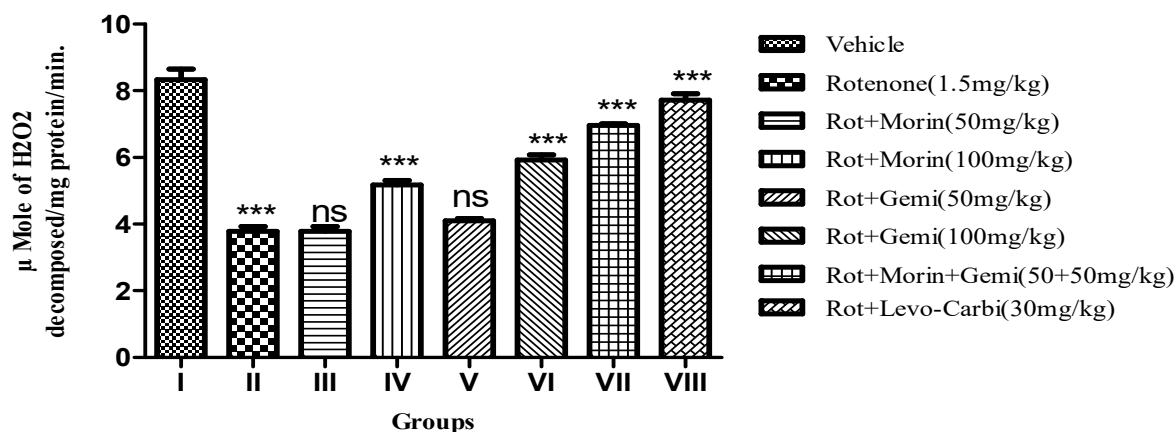


Fig. 11 Effect of morin hydrate and Gemigliptin on catalase (CAT) level in rat brain.

Values expressed as mean \pm S.E.M. Group II were compared with group I. Groups III, IV, V, VI, VII and VIII compared with group II. One-Way ANOVA followed by Dunnett's test. ns – Non significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Effect of morin hydrate and Gemigliptin on reduced glutathione (GSH) level in rat brain:

Group II showed significant ($p < 0.001$) decrease in GSH level as compared to Group I. Group IV, VI, VII ($p < 0.001$) and Group V ($p < 0.01$) showed significant increase in GSH level compared to Group II. Group III showed non-significant increase in GSH level as compared to Group II. Group VIII also showed

significant ($p < 0.001$) increase in GSH level as compared to Group II.

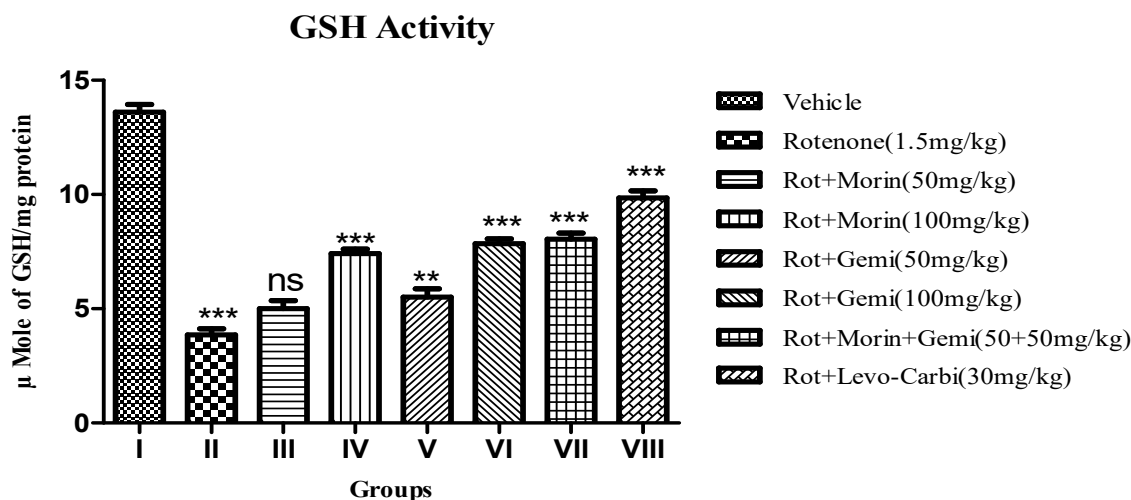


Fig. 12 Effect of morin hydrate and Gemigliptin on reduced glutathione (GSH) level in rat brain.

Values expressed as mean \pm S.E.M. Group II were compared with group I. Groups III, IV, V, VI, VII and VIII were compared with group II. One-Way ANOVA followed by Dunnett's test. ns – Non significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Effect of morin hydrate and Gemigliptin on superoxide dismutase (SOD) level in rat brain:

Group II showed significant ($p < 0.001$) decrease in SOD level as compared to Group I. Group III, V ($p < 0.05$) and Group IV, VI, VII ($p < 0.001$) showed significant increase in SOD level as compared to Group II. Group VIII also showed significant ($p < 0.001$) increase in SOD level as compared to Group II.

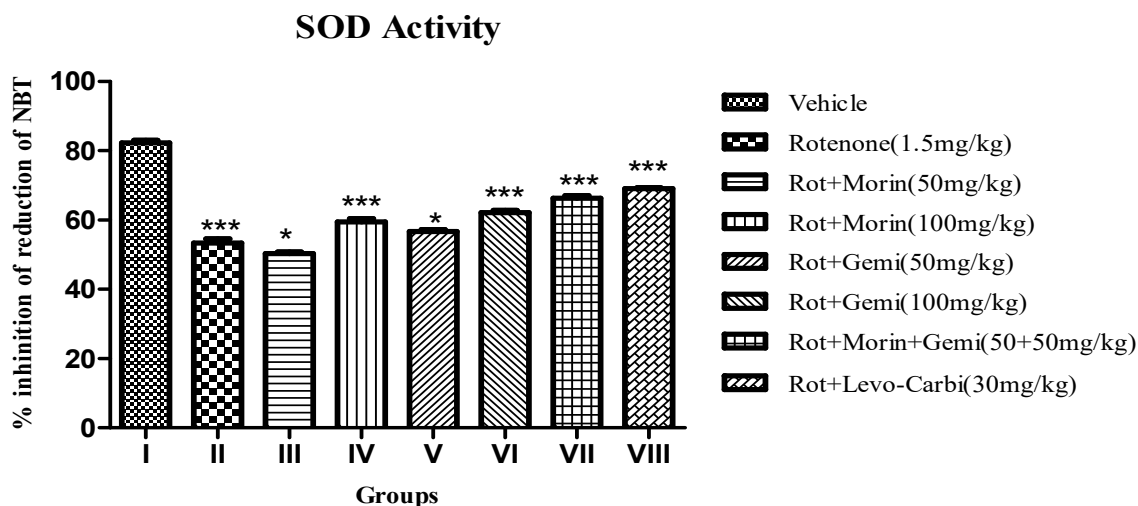


Fig. 13 Effect of morin hydrate and Gemigliptin on superoxide dismutase level in rat brain.

Values expressed as mean \pm S.E.M. Group II compared with group I. Groups III, IV, V, VI, VII and

VIII compared with group II. One-Way ANOVA followed by Dunnett's test.

ns – Non significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Effect of Morin hydrate and Gemigliptin on lipid peroxidation (LPO) level in rat brain: Group II showed significant ($p < 0.001$) increases in LPO level as compared to Group I. Group V ($p < 0.01$), Group IV,

VI, VII ($p < 0.001$) showed significant decrease in LPO level as compared to Group II. Group III showed non-significant decrease in LPO level as compared to Group II. Group VIII ($p < 0.001$) showed significant decrease in LPO level as compared to Group II.

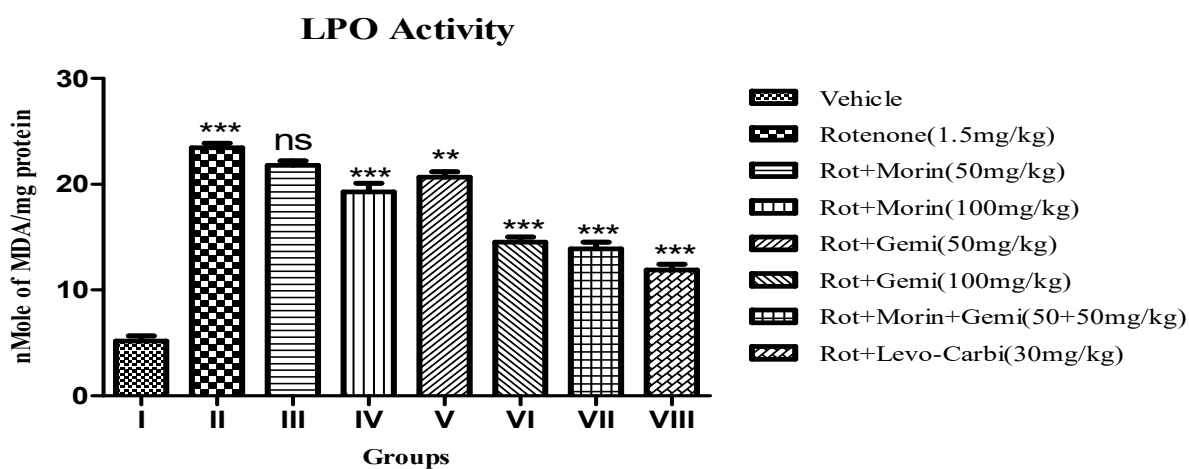
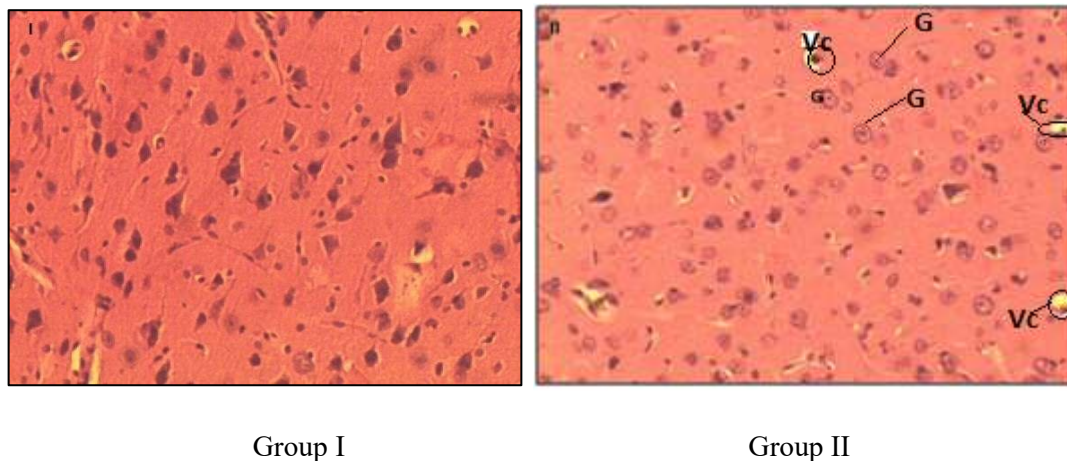


Fig.14 Effect of morin hydrate and Gemigliptin on lipid peroxidation (LPO) level in rat brain.

Values expressed as mean \pm S.E.M. Group II compared with group I. Groups III, IV, V, VI, VII and VIII compared with group II. One-Way ANOVA

followed by Dunnett's test. ns – Non significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Histopathological analysis



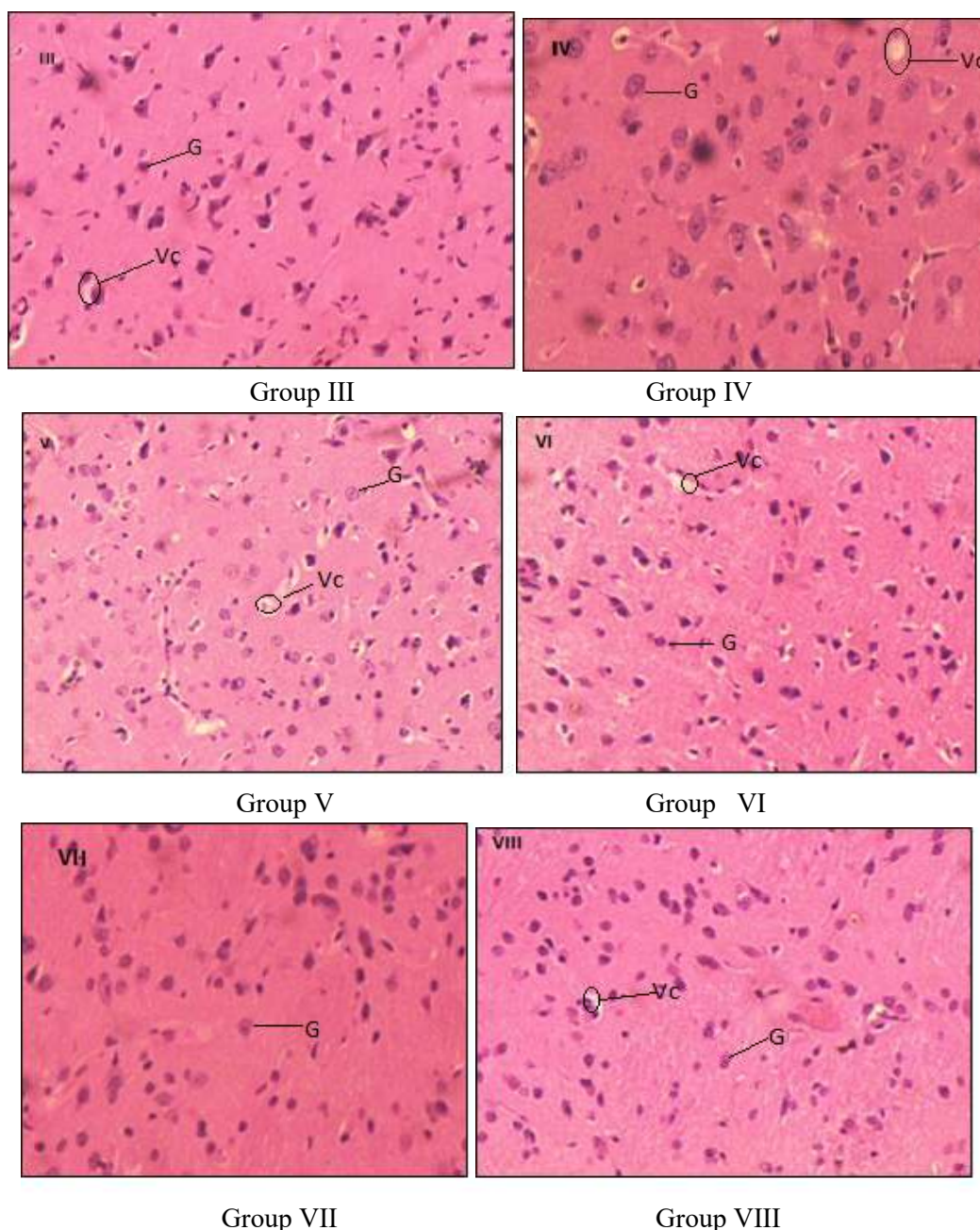


Fig.15 Histopathological changes of cerebral cortex of rats in different groups.

Group I: Control group, histopathological structure of cerebral cortex showing normal architecture. Group II: Rotenone treated group revealed the presence of ghost cells (G), vacuolated cytoplasm (Vc). Group III: Morin hydrate (50 mg/kg, p.o.), Group IV: Morin hydrate (100 mg/kg, p.o.), Group V: Gemigliptin (50 mg/kg, p.o.), Group VI: Gemigliptin (100mg/kg, p.o.), Group VII: Morin hydrate (50 mg/kg, p.o.) + Gemigliptin (50 mg/kg, p.o.), Group VIII: Vit.E (10 mg/kg, p.o.) These mentioned groups showed the existence of ghost cells (G) and vacuolated cytoplasm (Vc), however the number

of ghost cells and vacuolated cytoplasm is lesser compared to rotenone group (Group II).

DISCUSSION

Tardive dyskinesia (TD), caused by long-term administration of neuroleptic drugs, has become an evident clinical problem during schizophrenia treatment due to its duration from months to years after ceasing to take the drug. In some patients, the symptoms are irreversible. Reserpine (RES) is an indole alkaloid and had been used for decades as an antipsychotic and antihypertensive reagent to relieve psychotic symptoms and control blood pressure, respectively. Previous

reports have linked reserpine with the development of TD and behavioural symptoms of parkinson disease (PD) by depleting catecholamines. [22] In the first animal model of this study, animals were treated with reserpine (1 mg/kg, s.c.) on every other day in 5 days. Development of orofacial dyskinesia was characterized by increased vacuous chewing movements, tongue protrusions and orofacial bursts. The total locomotor activity was significantly decreased in reserpine treated groups as compared to vehicle group. During the treatment schedule, water and food intake of animals were reduced. The combination treatment of morin hydrate (50 mg/kg p.o.) and Gemigliptin (50 mg/kg p.o.) showed significant effect as compared to other groups in locomotor activity of animals. However, morin hydrate (50mg/kg) and Gemigliptin (50mg/kg) showed non-significant increase in locomotor activity of animals. Treatment with morin hydrate (100 mg/kg) and Gemigliptin (100 mg/kg) showed significant increase in locomotor activity in animals. Diarrhoea was observed in animals treated with Gemigliptin as its side effect.

VCMs include some pharmacological and neurochemical features with parkinsonian resting tremor, suggesting that evaluation of these movements may be used to investigate the efficacy of anti-parkinsonism agents. [23] Tongue protrusion frequency and vacuous chewing movements are considered as evaluation parameters in orofacial dyskinesia. Reserpine (1mg/kg, s.c.) treated group showed significant increase in tongue protrusion frequency and vacuous chewing movement. Morin hydrate (50 and 100 mg/kg p.o.) showed decrease in tongue protrusion frequency and VCMs as compared to reserpine treated group. Gemigliptin (50 and 100mg/kg, p.o.) also showed the same effect as morin hydrate. Combination of morin hydrate and Gemigliptin showed significant decrease in VCMs and tongue protrusion frequency. The behavioural alterations have been related to dysfunctions in some areas concerned with PD pathophysiology, such as striatum and globus pallidus, and it has also been linked to changes in the dopamine levels in the nigrostriatal system. [24]

Biochemical parameters also get affected due to the administration of reserpine. Antioxidant enzymes such as CAT, GSH, SOD levels get decreased after the administration of reserpine and hence proved the generation of reactive oxygen species resulted in elevated lipid peroxidation. In biochemical studies significant results were observed with morin hydrate

(100mg/kg), Gemigliptin (100 mg/kg) and their combination. The decreased levels of defense enzymes CAT, SOD, and GSH due to reserpine (1 mg/kg) administration were significantly normalized by morin hydrate, Gemigliptin and their combination. Morin hydrate and Gemigliptin and their combination showed significant decrease in lipid peroxidation as compared with reserpine treated group. Vitamin E (10 mg/kg) was used as standard drug to discuss results of treated group. In histopathological finding, reserpine treated group showed the presence of ghost cells (G) and vacuolated cytoplasm (Vc) compared to vehicle treated group. Whereas, morin hydrate and Gemigliptin treated group showed fewer number of ghost cells and vacuolated cytoplasm as compared with reserpine treated group which indicated the protective effect of morin hydrate and Gemigliptin. Therefore, from the results it was observed that morin hydrate and Gemigliptin showed neuroprotective effect against reserpine-induced orofacial dyskinesia in rats.

Rotenone can easily cross the BBB because of its high lipophilic nature and inhibits the activity of complex-1 in the mitochondrial respiratory chain, thereby raise oxidative insult, inflammation and decreased neurotransmitters level. It resembles many of key pathological features of human PD. [25] Thus, in this study rotenone (1.5 mg/kg) was given to animals for 10 days which produces neurological symptoms. Rotenone administration significantly decreased the motor coordination, which was established by reduced hypokinetic movements in hole board test, and decreased grip strength activity in rotarod. Behavioural parameter such as rotarod-test showed decreased latency to fall off in rotenone treated group as compared with vehicle treated group. Rotarod test was used to assess muscle strength and balance. Morin hydrate (50 mg/kg) showed non-significant increase in latency to fall off while 100 mg/kg showed significant increase in latency to fall from the rotarod. Gemigliptin (50 and 100 mg/kg) showed significant increase in latency to fall off. Combination of morin hydrate and Gemigliptin also showed increase in latency to fall off from rota rod in significant manner. In hole board test, number of nose pokes of animals were evaluated for 5 min. In rotenone treated group, number of nose poking were significantly decreased as compared with vehicle treated group. Morin hydrate and Gemigliptin at doses 100mg/kg showed significant increase number of nose pokes as compared to

rotenone treated group. Levodopa-Carbidopa (30mg/kg) was used as standard drug which showed significant results in behavioural parameters as compared to rotenone treated group. Oxidative harm was also increased significantly by the rotenone administration as compared to control animals. It was observed by the increased lipid peroxidation and decreased levels of SOD, CAT and GSH in animals. This play key role in the brain by eliminating free radicals formed during the metabolism.

Morin hydrate and Gemigliptin showed significant results in biochemical parameters. Morin hydrate (100 mg/kg) and Gemigliptin (100 mg/kg) showed significant increase in CAT level. Combination of morin hydrate and Gemigliptin also showed significant increase in CAT level in rat brain. While evaluating effects on SOD and GSH, morin hydrate (100mg/kg) and Gemigliptin (100 mg/kg) and their combination showed significant increase in levels of SOD and GSH. Lipid peroxidation level was increased in rotenone administered group. Lipid peroxidation increases due to the increased reactive oxygen species. Morin hydrate (100 mg/kg) and Gemigliptin (100 mg/kg) showed significant decrease in lipid peroxidation. As well as combination of both the drugs were also showed effective results in reducing lipid peroxidation. In histopathological analysis, rotenone treated group showed the presence of ghost cells (G) and vacuolated cytoplasm (Vc) compared to vehicle treated group. Whereas, Morin hydrate and Gemigliptin treated group showed fewer number of ghost cells and vacuolated cytoplasm as compared with rotenone treated group. Gemigliptin act as DPP-IV inhibitor which showed neuroprotection by increasing circulating GLP-1 levels. Stimulation of GLP-1 receptor subsequently turns on downstream AMPK/Sirt 1 pathway which protects mitochondrial function and suppresses intracellular ROS accumulation. However, dipeptidyl peptidase-4 (DPP-IV), a serine peptidase, inactivates GLP-1 leading to a very short half-life. [9] Morin has been reported to inhibit ROS formation, caspase-3 activation and apoptosis. Gemigliptin has been reported to reduce endoplasmic reticulum stress and apoptosis signalling molecules. From all these results and data obtained, it has been observed that morin hydrate and Gemigliptin showed protective effects against reserpine-induced orofacial dyskinesia and rotenone-induced neurodegeneration.

CONCLUSION

From the results of the present study, it has been concluded that morin hydrate and Gemigliptin showed protective effect and thus could be the potential therapeutic agents for the treatment of parkinson's disease. Morin hydrate showed protective effect by reducing the oxidative stress and improved the motor function and biochemical parameters in treated animals. Gemigliptin acts through GLP-1 receptors by inhibiting DPP-IV enzyme and showed neuroprotective effect by improving motor function and biochemical parameters. Combination of these drugs also showed neuroprotective effect by reducing oxidative stress and acting on GLP-1 receptors and thus could be the new treatment strategy for parkinson's disease.

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