Mangiferin ameliorates intracerebroventricular-quinolinic acid-induced cognitive deficits, oxidative stress, and neuroinflammation in Wistar rats

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Abstract:

INTRODUCTION: Mangiferin (MGF), a xanthone polyphenol, confers neuroprotection via combating oxidative stress and inflammation. The current investigation aimed to assess the neuroprotective potential of MGF on behavioral and neurochemical anomalies evoked by administration of quinolinic acid (QA) through intrastriatal injection in male Wistar rats and to reveal the associated mechanisms.

MATERIALS AND METHODS: QA (300 nm/4 µl saline) was administered intracerebroventricular in the striatum (unilaterally) once. Thereafter, MGF 20 and 40 mg/kg (peroral) was administered to the animals for 21 days.

RESULTS: QA administration caused marked alteration in motor activity (rotatod), footprint analysis, and cognitive function (Morris water maze test, and novel object recognition test). Furthermore, oxido-nitrosative stress (increased nitrite content, lipid peroxidation, with reduction of GSH), cholinergic dysfunction, and mitochondrial complex (I, II, and IV) dysfunction were observed in hippocampus and striatal region of QA-treated rats in comparison to normal control. Pro inflammatory mediators (tumor necrosis factor-alpha TNF-α and interleukin-1β) were noted to increase in the hippocampus and striatum of QA-treated rats. In addition, we observed BDNF depletion in both the hippocampus and striatum of QA-treated animals. MGF treatment significantly ameliorated memory and motor deficits in QA-administered rats. Moreover, MGF treatment (40 mg/kg) restored the GSH level and reduced the MDA, nitrite level, and pro-inflammatory cytokines in striatum and hippocampus. Furthermore, QA-induced cholinergic dysfunction (AChE), BDNF depletion and mitochondrial impairment were found to be ameliorated by MGF treatment.

CONCLUSION: The results suggest that MGF offers the neuroprotective potential that may be a promising pharmacological approach to ameliorate cognitive deficits associated with neurodegeneration.

Keywords: Hippocampus, mangiferin, oxido-nitrosative stress, quinolinic acid, striatum

Introduction

Oxidative stress and inflammation elicited by free radical ions are the common factors responsible for cognitive deficits. A previous report expected to rise in the number of dementia patients to 115 million in 2050. In India, 22% of the elder population is combating with cognitive impairment.[1] The elucidation of pathophysiological mechanisms underlying cognitive impairment leads to the development of novel pharmacological interventions.

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Quinolinic acid (QA) is one of the potent endogenous neurotoxicants found in the mammalian brain. QA is an intermediate neuroactive metabolite of tryptophan which is produced through the kynurenine pathway in a nanomolar concentration. Previous clinical studies reported the enhanced levels of 3-Hydroxykynurenine (precursor of QA) in depressed and cognitive impaired subjects. The elevated level of QA has been implicated in neuronal degeneration. QA activates the NMDA receptor and increases calcium influx in neurons that activates different key enzymes like phospholipases, constitutive nitric oxide synthase, and protein kinases which are involved in oxido-nitrosative stress. QA enhances the free radical ions induced-lipid peroxidation process by forming the complex with Fe (II) which acts as a prooxidant. These excitotoxic events contribute to the neuronal cell death which in turn leads to cognitive impairment. It has been well documented that intrastratal injection of QA in the brain causes neuronal damage via activation of microglial cells. Intrastratal injection of QA induces motor and behavioral alterations in the experimental animals.

Mangiferin (MGF) is a xanthanoid polyphenol extracted from Mango fruit, peel, bark, and leaves in Cyclopia species (honeybush tea). MGF has been noted to possess tremendous potential to combat oxidative stress and inflammation that improve behavioral function including cognition. In addition, it possesses cardiostimulating, hepatoprotective, anticancer, antiviral, immunomodulatory properties. Thus, the current investigation was planned to explore the neuroprotective potential of MGF in intracerebroventricular (ICV) QA-induced behavioral and neurochemical alterations into Wistar rats.

Materials and Methods

Drug and chemicals
MGF and QA were procured from Sigma Aldrich Chemicals, India. Tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and BDNF kits were procured from Elabscience, India. All other chemicals were freshly prepared and were of analytical grade.

Animals
For this investigational study Male young Wistar Rats (200–250 g) were bought from the NIB, Noida, India. The experimental protocol (Approval no. IAEC/ KSOP/E/18/07) was reviewed and accepted by IAEC. Under the standard laboratory conditions, animals were kept at 23°C ± 2°C temperature with 70% relative humidity maintained on 12 h light/dark periodic pattern with free access to food and water. To prevent possible chances of infection due to wet cage, animal beddings were changed daily. The animals were sacrificed on scheduled time-interval as per the study plan to assess the various parameters.

Experimental design
Rats were arbitrarily distributed into 5 groups (n = 10): Control group, Sham-operated, QA (300 nM/4 µl QA in Normal saline), QA + MGF-20 mg/kg (300 nM/4 µl QA in Normal saline with MGF: 20 mg/kg), QA + MGF-40 mg/kg (300 nM/4 µl QA in Normal saline with MGF: 40 mg/kg), Drug control (MGF-40 mg/kg). MGF was dissolved in 0.1% dimethylsulfoxide in 0.9% normal saline and given through oral route. Doses selection of MGF was based on our previously designed investigation on MGF.

Normal saline was administered to one group while another group receives QA (300 nM/4 µl in Normal saline) on day 0. The drug treatment was given for the entire study time. No animal mortality was observed during the study period. At the end of study i.e., 21st day, locomotor activity and footprint analysis were performed to analyze the locomotor activity and gait behavior. Spatial learning memory was assessed on the last 5 days of the study. Novel object recognition test (NORT) and Rotarod were performed on the last 3 days of the study period. Biochemical parameters were analyzed in the striatum and hippocampus of the rats after cervical dislocation.

Surgical procedure (intracerebroventricular administration)
All the surgical were sterilized with 70% ethanol. The rat was anesthetized by injecting the ketamine. The rat hairs were shaved from the top of the shoulder to the space between the eyes. The anesthetized rat was positioned on the stereotaxis apparatus. The head was held with the ear bars and disinfected the shaved area with the help of ethanol-dipped cotton swab followed by an iodine-dipped cotton swab. After the incision of the skin, a hole was drilled in the skull according to the coordinates. Hamilton syringe (10 µl) with a needle was cleaned and sterilized for the administration of QA. After the drug administration, the syringe was kept for 5 min to inhibit the oozing of the drug from the hole. Then incision was closed with suturing. The temperature was maintained throughout the surgery to prevent hypothermia. The rats were daily monitored after the surgery for the 1st week. To avoid irritation from the suture thread, 5-0 nylon suture was removed in 7–10 days following surgery.

Behavioral assessment
Morris water maze testing
This maze represents a more specific test of spatial memory. To perform the Morris water maze (MWM) test, a round black water-filled pool of 180 cm in diameter...
and 40 cm height was taken. The water was filled in the pool up to 30 cm. The temperature of the pool water was maintained at 25°C ± 0.1°C. A 13 cm² sized platform made up of plexiglass square was positioned in center of one quadrant, ten mm below the water level. The test was performed in two trials: (i) training trial and (ii) probe test. All through the visual cue tests and learning trials, the position of the platform has remained the same but it was removed during the probe test. The learning trial was performed for four successive days. The probe trial was conducted without platform on the 5th day. In the training trial, the location of the platform was changed every time, but the visual pattern of cue remains unchanged. In each trial, rat was allowed to swim till they reached the platform. Once the animal reached the platform, the rat was permitted to halt on this platform for 30 s. The time span utilized by the animal to arrive at platform was noted. During this trial, individual animal was permitted to swim in the MWM for two min. The time consumed in the target quadrant (%) was recorded.

Rotarod apparatus
Rotarod test was carried out to determine the grip strength and motor coordination activity of the animal. The training was given to each rat before the test on the final day. 25 rpm was set on the rotatod. The fall-off time was recorded with 180 s cut-off time. Three different trials were carried out with each rat at a 5 min interval.

Novel object recognition test
NORT was used for assessment of recognition memory. Three different phases were carried out to perform NORT i.e., (a) habituation, (b) familiarization, and (c) test phase. The test was executed on a black open box of 50 cm × 50cm × 36 cm. In the first phase, the rat was placed for 5 min in the open box to habituate to the empty open arena. Thereafter, the familiarization phase was conducted in which two objects (a rectangular plastic ball and a wooden rectangle) placed at the box. Freedom was given to the animal for 10 min in the box for area exploration. In the next phase, the wooden rectangular-shaped object was replaced with a pyramid-shaped object. Individual rat was permitted for 180 s. To evade olfactory cues, the objects as well open field box was frequently cleaned with alcohol after each phase of the test. The time taken for exploring the novel and familiar object was recorded. Recognition index was calculated by taking the ratio of time spent by the animal to explore the novel object over the complete time consumed by the animal to explore novel and familiar objects during the 3 min test session. Results were calculated and expressed as percentage recognition index.

Locomotor activity
A laser beam photoactometer was used to measure the locomotor behavior of each rat to determine horizontal activity. The rat was placed between the beam emitter and detector for one min inside the activity box to get acclimatized so that it could give the normal reading. The activity counts by interrupting the photobeam for 5 min was noted to evaluate the basal activity score.

Foot print analysis
The gait abnormalities in rats were assessed by footprint analysis. Summarily, the footprints of the rats were obtained by dipping their feet in four different colored food dyes and were allowed to run on an inclining gangway with dimensions 80 cm × 10 cm × 8 cm. The runway floor was covered with a white sheet. In order to get a clear footprint, the animals were supposed to walk up the runway into a dim section. Afterward, the rats were cleaned with warm water to remove the dye. The footprints were scanned and the ‘stride length’ was calculated by using a scale. Stride length was calculated by measuring the distance between sequential placements of the same rat’s paw.

Biochemical procedure
Preparation of tissue homogenate
On the last day i.e., 21st day, the rats from each group were killed. The hippocampus and striatum region of the brain was cautiously and speedily isolated on the ice-cold Petri dish. 0.1M PBS of pH 7.4 was used to make 10% tissue homogenate. Homogenate was then centrifuged for 15 min at a low temperature. Finally, the supernatant was separated from homogenate and was stored at −20°C for further biochemical analysis.

Estimation of lipid peroxidation
50 µl of hippocampus/striatum homogenate was mixed thoroughly with 50 µl of SDS (8.1%). Into this mixture, an equal amount (350 µl) of acetic acid (20%) and thiobarbituric acid (0.8%) solution was added and 1.5 ml volume was make up with the water. Afterward, the prepared solution in the test tube was heated at 95°C for 1 h. The solution was kept at 25°C for cooling followed by centrifugation of the tubes for 10 min at 10000 rpm to collect final supernatant. The MDA level was measured at λmax 532 nm. MDA concentration was estimated and represented as µMol of MDA/mg of total protein.

Estimation of GSH level
In brief, the supernatant and 10% (w/v) TCA were taken in a 1:1 ratio, then centrifugation at 1000 g for 10 min (4°C). In the collected supernatant 1 ml of disodium phosphate (0.3M) and 250 µl of DTNB (0.001M) were added. The absorbance (Abs) was recorded through spectrophotometer at λmax 412 nm.

Estimation of AChE level
The AChE was estimated as described previously. Briefly, the mixture was made that contains Acetylcholine...
Iodide (1 mM), DTNB (2 mM), Monophosphatium phosphate (100 mM, pH-7) and homogenate of hippocampus/stripin in 500 µL. This solution was then incubated at 37°C for 10 min. 500 µL of serine hemisulphate (0.5 mM) was incorporated to terminate this reaction. Finally, the Abs of resulted yellow solution measured at λmax 412 nm. \[20\]

**Estimation of nitric oxide**

An equal amount of striatal/hippocampal supernatant and the Griess reagent were poured in an Eppendorf tube. At room temperature, the mixture was kept for 10 min in a dark chamber. Finally, the Abs was detected at 540 nm and outcomes were expressed as micromoles per mg of total protein. \[7\]

**Assessment of mitochondrial complexes**

**Isolation and preparation of mitochondria**

The hippocampus and striatum parts were separated out from the brain and mitochondria were recovered as described previously (Rosenthal et al.). The isolation medium containing sucrose (77 mM), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (5 mM), bovine serum albumin (1 mg/ml), mannitol (225 mM), and 1 mM ethylene glycol tetra acetie acid (1 mM) was used to homogenize the hippocampus and striatum sample. The homogenized mixture was centrifuged at 2000 g at 4°C for 3 min. Pellets collection was done after discarding the supernatant and again suspended and centrifuged. Afterward, the synaptosomal layer was resuspended in digitonin (0.02%) and followed by centrifuging for 10 min at 12,000 g for further evaluation. \[7,18\]

**NADH dehydrogenase (Complex I) activity**

This method includes catalytic oxidation of NADH into NAD+ and subsequently cytochrome C reduction was takes place. 3 ml of glycylglycine (0.2 mM), NADH (6 mM), sodium bicarbonate (0.02 mM) solution, and 1 mM of cytochrome C was mixed and adjusted to pH 8.5. The sample was added in the mixture and the Abs was detected at 550 nm for the subsequent 180 s. The activity was calculated and represented as nMol of oxidized NADH/min/mg of total protein. \[17\]

**Succinate dehydrogenase (Complex II) activity**

In this method, a mixture containing butanedioic acid (0.6 M), phosphate buffer (0.2 M), 1% BSA, and potassium ferricyanide (0.03M) of pH 7.8 was taken. The reaction was started after adding the sample into the mixture. The Abs was detected at 420 nm for the subsequent 180 s. The activity was calculated and represented as nMol of succinate dehydrogenase/min/mg of total protein. \[19\]

**Cytochrome c oxidase (Complex IV) assay**

Complex IV activity in the sample was assessed as described previously. \[20\] The reaction mixture was taken that contains reduced form of cytochrome C (0.03 mM) in PBS. Thereafter, mitochondrial sample was added to initiate the reaction. The Abs was detected at 550 nm for subsequent 3 min. The activity was calculated and represented as nMol of oxidized cytochrome c/min/mg of total protein.

**Interleukin-1β, tumor necrosis factor-alpha, and BDNF determination**

Hippocampus and striatum were quickly isolated from the rat brain on ice-cold petri-dish. Protease inhibitor cocktail was added in the sample during the homogenization process. IL-1β, TNF-α, and BDNF determination was carried out as per the protocol provided in the kit. IL-1β and TNF-α levels represented as pg/mg of total protein. The BDNF level estimated as ng/mg of total protein.

**Statistical analysis**

All the data values represented as means ± standard error median. Comparison between different experimental group were performed through one-way analysis of variance, followed by Tukey’s post-hoc test (using Graph pad prism software). P < 0.05 was taken into consideration for statistically significance.

**Results**

**Effect of mangiferin on intracerebroventricular-quinolinic acid-induced alterations in behavioral parameters**

The MWM study was performed from the 17th day of experimental study till the last day. The whole trial was performed in two phases: Acquisition phase and probe trial. In the acquisition phase, significance (P < 0.001, P < 0.01, and P < 0.01) changes in the escape latency time was observed on 2nd, 3rd and 4th day, respectively in the QA-treated group as compared to the control group. Whereas, escaped latency time on 3rd and 4th day showed significant reduction (P < 0.05 and P < 0.01) in MGF (40 mg/kg) treated group [Figure 1a]. In the probe trial, ICV-QA-treated rats spent fewer time in the target quadrant (P < 0.001) as compared to the control group. MGF (20- mg/kg) showed nonsignificant result on the time spent in the target quadrant when compared with the QA-treated group. On another hand, MGF (40 mg/kg)-treated ICV-QA rats spent statistically significant (P < 0.01) longer duration in that target quadrant in comparison with ICV-QA rats [Figure 1b]. Thus, MGF (40 mg/kg) alleviated cognition dysfunction caused due to chronic administration of QA.

Furthermore, the QA-treated group exhibited motor in-coodination as evidenced by rotarod test results. We found that the fall-off time of ICV-QA rats was
significantly declined ($P < 0.001$) as compared to the control group [Figure 2a]. MGF (40 mg/kg) treated ICV-QA rats showed significantly ($P < 0.01$) longer fall-off spell as compared to ICV-QA rats. No significant effect was observed in MGF (20 mg/kg) group as compared to the ICV-QA-treated group.

NORT results exhibited that ICV-QA rats were unable to distinguish between the familiar and novel objects thereby showed ($P < 0.01$) lower recognition index in comparison with the control group. MGF (20 mg/kg) treated group exhibited no significant effect on the recognition index in comparison to the ICV-QA-treated group. However, MGF (40 mg/kg) treated ICV-QA rats exhibited significant ($P < 0.05$) increase in recognition index during comparison with ICV-QA-treated group [Figure 2b]. ICV-QA administration, as well as MGF treatment, did not affect the locomotor activity in comparison to normal control rats [Figure 2c].

ICV-QA administration significantly altered the stride length during walking in footprint analysis [Figure 3a]. The stride length on day-7, 14, and 21 significantly ($P < 0.05$, $P < 0.01$, and $P < 0.001$) declined in QA administered rats in comparison to normal control rats. MGF (40 mg/kg) treated animal exhibited significant ($P < 0.05$) increase in stride length as compared to ICV-QA-treated animals on day-21 [Figure 3b]. However, MGF (20 mg/kg) did not produce significant result on stride length in comparison to ICV-QA rats. Thus, our study results indicated that MGF (40 mg/kg) is capable to avert the ICV-QA induced neurotoxicity.

**Effect of mangiferin on intracerebroventricular-quinolinic acid-induced differences in MDA level**

QA-administered group showed marked ($P < 0.001$) rise in the MDA level both in striatum and hippocampus in comparison to the normal control rats. On another hand, MGF (40 mg/kg) exhibited significant ($P < 0.001$) reduction in the MDA level both in the hippocampus and striatum. Nevertheless, MGF (20 mg/kg) treated rats showed significant ($P < 0.05$) MDA reduction in the hippocampus but no changes were observed in the striatum region in comparison to QA-administered rats [Table 1].

**Effect of mangiferin on intracerebroventricular-quinolinic acid-induced differences in GSH level**

ICV-QA group demonstrated significant ($P < 0.01$) decline in the GSH level both in the hippocampal and striatal region during comparison with control group. Administration of MGF (40 mg/kg) significantly ($P < 0.05$) augmented the GSH level in the hippocampus and striatum in comparison to the ICV-QA group. However, no significant effect was observed in MGF (20 mg/kg) group. Similarly, the drug control group failed to show any significant alteration in GSH level in comparison to the control group [Table 1].
**Effect of mangiferin treatment on intracerebroventricular-quinolinic acid-induced variation in nitric oxide**

We found that ICV-QA administration markedly ($P < 0.001$) increased the NO levels in the hippocampus and striatal region in comparison to control rats. MGF (40 mg/kg) treated ICV-QA rats exhibited significantly ($P < 0.05$) decrease of NO level in the hippocampus and striatum as compared to ICV-QA rats [Table 1]. MGF (20 mg/kg) and MGF control group did not exhibit any significant result on NO level in both the brain regions.

**Effect of mangiferin treatment on intracerebroventricular-quinolinic acid-induced alteration in AChE activity**

ICV-QA administration resulted in the marked ($P < 0.001$) augmentation of the AChE activity in the striatal as well as hippocampal region in comparison to normal control group. After 21 days of MGF treatment (40 mg/kg), AChE activity was markedly ($P < 0.01$ and $P < 0.05$) declined in hippocampus and striatum in comparison to ICV-QA rats [Figure 4a]. MGF (20 mg/kg) failed to exert significant action on AChE activity.

**Effect of mangiferin treatment on intracerebroventricular-quinolinic acid-induced alteration in the mitochondrial complex (-I, -II and -IV)**

In the electron transport chain cycle, mitochondrial complex (MC) are involved. Complex I, complex II and complex IV exhibited marked ($P < 0.001$) reduction in the hippocampus as well as striatum of QA-treated groups as compared to control groups [Table 2]. However, MGF (40 mg/kg) treated ICV-QA group displayed significant ($P < 0.01$ and $P < 0.05$) augmentation in the complex I in the striatum and hippocampus. However, the level of complex II was markedly ($P < 0.01$) increased only in the hippocampal region after administration of MGF (40 mg/kg) in ICV-QA rats. Complex IV was increased significantly ($P < 0.05$ and $P < 0.001$) in the hippocampus as well as striatum, respectively in MGF (40 mg/kg) treated ICV-QA rats as compared to ICV-QA treated rats. However, no significant changes in MC activity was noted between the drug control group and normal control rats.

**Effect of mangiferin treatment on intracerebroventricular-quinolinic acid-induced augmentation of interleukin-1β and tumor necrosis factor-alpha level**

Both IL-1β and TNF-α were found to be elevated significantly ($P < 0.001$) in striatum and hippocampus of ICV-QA administered rats in comparison with control group [Figure 4b and c]. However, a higher dose of MGF (40 mg/kg) was associated with significant ($P < 0.001$) reduction in the QA-induced IL-1β level in striatum and hippocampus. Moreover, marked reduction in TNF-α level was noted in the striatum ($P < 0.01$) and hippocampus ($P < 0.001$) of MGF (40 mg/kg)-treated ICV-QA rats.

**Table 1: Effect of mangiferin on quinolinic acid-induced oxido-nitrosative stress in hippocampus and striatum**

| Oxido-nitrosative stress parameters | Brain region   | Control      | Sham         | Quinolinic acid (QA) mg/kg | QA + Mangiferi n=20 mg/kg | QA + Mangiferi n=40 mg/kg | Mangiferi n=40 mg/kg |
|------------------------------------|----------------|--------------|--------------|-----------------------------|-----------------------------|-----------------------------|------------------------|
| MDA (micromoles/mg of protein)     | Hippocampus    | 32.84±1.89   | 39.18±2.84   | 104.11±6.10a                | 83.20±4.23c                 | 44.09±3.92e                 | 30.00±2.20             |
|                                   | Striatum       | 18.91±2.00   | 17.38±3.11   | 62.29±4.45a                 | 49.09±5.10                  | 25.23±3.20e                 | 20.32±1.01             |
| GSH (micromoles/mg of protein)     | Hippocampus    | 91.00±8.37   | 86.23±3.12   | 49.10±7.17b                 | 61.98±7.22                  | 81.83±3.55c                 | 85.82±8.19             |
|                                   | Striatum       | 80.22±6.71   | 81.89±5.29   | 52.29±3.20b                 | 64.20±3.02                  | 71.01±5.20c                 | 74.98±6.66             |
| Nitric Oxide (micromoles/mg of protein) | Hippocampus | 24.72±2.84   | 28.11±3.12   | 45.77±2.11a                 | 38.13±1.28                  | 38.99±1.19d                 | 29.11±1.10             |
|                                   | Striatum       | 12.44±1.19   | 14.81±0.82   | 32.91±2.01b                 | 28.81±4.44                  | 21.84±2.15c                 | 14.94±1.18             |

Data are represented as mean±SEM (n=6). *P<0.001 and †P<0.01 compared to the control group. ‡P<0.05, §P<0.01, and ‖P<0.001 compared with the QA group.
As shown in Figure 4d, the ICV injection of QA significantly (P < 0.001) depleted the level of BDNF in striatal and hippocampal region in comparison with the control group. MGF (40 mg/kg) significantly (P < 0.05) augmented the level of BDNF only in the hippocampus of ICV-QA administered rats. MGF (40 mg/kg) controlled group also demonstrated augmentation of the BDNF level in the hippocampal and striatal region. However, the results were found statistically nonsignificant.

**Discussion**

The current study aims to explore the neuroprotective effect of MGF in QA-administered rats and the possible underlying mechanism. Our findings revealed that MGF significantly alleviated the memory deficits of the QA administered animals. The prevalence of cognitive deficits is very high in neurodegenerative disorders. Moreover, it is associated with a complex and multifactor etiology. It is well known from preclinical as well as from clinical studies that cognitive deficit has strong correlation with higher levels of oxido-nitrosative stress and neuroinflammation. The current study results also support the association of oxidative stress with cognitive function. In addition, we found neuroprotective effects of MGF on QA-induced behavioral and neurochemical deficits in rats. Treatment with MGF alleviated the...
High level of endogenous neurotoxin i.e., QA in the brain associated with behavioral alterations. Intrastriatal administration of QA to experimental animals leads to abrupt and over-stimulation of excitatory NMDA receptors which, in turn causes memory and motor impairments via apoptotic neuronal cell death in different brain regions. The ICV-QA model of behavioral deficits is a well-established experimental model that elicits ROS generation, neuroinflammation, mitochondrial dysfunctioning, and associated apoptotic cell death in the brain. Numerous studies suggested that the ICV-QA administration elicits a vicious cycle of oxido-nitrosative stress and mitochondrial impairment in the brain. In our study, we observed that complex I, II, and IV functions were augmented the mitochondrial damage. Moreover, QA-associate learning as well as memory impairment that reflects the improvement in the hippocampal function. The neuroprotective efficacy of MGF against cognitive function is in accordance with our previously reported investigations.

Mitochondria are a producer and targets of ROS that can elicit oxidative injury via disruption of oxidative phosphorylation. QA acts as mitochondria toxin which causes mitochondria dysfunction via NMDA receptor-mediated excessive calcium entry in the mitochondria. Previous reports suggested that QA-induced the mitochondrial dysfunction that leads to several damaging effects such as mutation in mitochondrial DNA, augmentation of oxidative as well as nitrosative stress, and an increase in the permeability of mitochondrial membrane. Mitochondrial respiratory chain complexes (I-IV) dysfunctions reflect mitochondria anomalies in the cells that lead to mitochondria energy deficits. Among these complexes, complex II and IV are important because their dysfunction leads to the excessive release of free radical ions which further augments the mitochondrial damage. In our study, we observed that complex I, II, and IV functions were reduced in the hippocampus as well as in striatum of QA-injected rats. These results were corroborated with the previous reports. In the present investigation, MGF treatment significantly alleviates the mitochondrial membrane potential loss and swelling via the free radicals scavenging property of MGF. Thus, it can be inferred that MGF protects the mitochondrial function through its potent anti-oxidant activity.
Several experimental and clinical studies have suggested the crucial role for neuroinflammation in neurodegenerative disorders. The activation of inflammatory mediators like IL-1β and TNF-α during inflammation elicits the vicious mechanism that eventually leads to cell death. Oxido-nitrosative stress increases the levels of pro-inflammatory mediators by the involvement of transcription regulator i.e., nuclear factor-kappa B (NF-κB). Thus, neuroinflammation can be prevented either by oxido-nitrosative stress inhibition or repression of NF-κB. Earlier studies also indicated the NF-κB inhibition property of MGF in different animal models. In our study, QA elicits the neuroinflammation and caused augmentation of IL-1β and TNF-α in both hippocampus and striatum. However, MGF a potent anti-oxidant significantly inhibited the up-regulation of pro-inflammatory cytokines in striatum as well as in hippocampus. Thereby, inhibits the further array of mechanism that can progressed toward neuronal cell injury. The observed anti-inflammatory activity of MGF is well corroborated with our previous published reports. BDNF, a crucial neurotrophic factor has been stated to be dysregulated in oxidative stress and neuroinflammation. However, the effect of intrastralatal administration of QA on BDNF has been found contradictory. In our study, we found BDNF depletion in striatum and hippocampus of ICV-QA rats that is corroborated with a recent study. MGF treatment significantly reestablished the BDNF level in the hippocampal region only.

**Conclusion**

The current outcome of the investigation revealed the neuronal protection of MGF against QA-induced behavioral and neurochemical anomalies. The resulted neuroprotective potential attributed to the mitigation of oxidative as well as nitrosative stress (MDA, GSH as well as nitric oxide), neuroinflammation (IL-1β and TNF-α), protection of MC activities and BDNF content. Thus, MGF may provide an intriguing therapeutic approach for the management of behavioral anomalies. However, additional mechanistic studies are warranted to elucidate a more detailed mechanism of MGF.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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