Original Article

Neuroprotective Role of Nigella Sativa on Methamphetamine induced Hippocampal Injury in Male Albino Mice

Farhana Rajpar, Samreen Memon, Pushpa Goswami, Fayaz Ali Rajpar

ABSTRACT

OBJECTIVE: To evaluate the neuroprotective effects of Nigella sativa in methamphetamine induced hippocampal injury.

METHODOLOGY: This Experimental study was conducted at Department of Anatomy, Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro, in collaboration with Sindh Agriculture University Tandojam from April 2016 to September 2016 on sixty adult male mice of average 25-40gms. All the animals were housed properly, fed on lab chow and tap water *ad libitum*. The mice were divided into group A (control), B, C and D were experimental groups. Group A was given normal saline orally at the volume of 1 ml/kg. Group B animals were given METH at volume of 10 mL/kg containing 10.0 mg/kg METH. Group C animals were given Kalonji extract at the volume of 3mg/ml of extract, and Group D animals were given METH plus Kalonji extract at the same volume and dose as mentioned earlier. The animals were sacrificed by cervical dislocation after one week of last injection. Brain of each animal was removed, immersed in 10% formaldehyde solution for microscopic studies.

RESULTS: The weight of different groups at baseline was equal in all groups which is reduced at the end of study period. METH treated mice showing hyper cellularity of different layers, in contrast of N.S + METH treated mice showing marked decrease in cellularity of hippocampus.

CONCLUSION: The findings of above study shows neuroprotective effect of Nigella sativa against methamphetamine induced cell injury in hippocampus of mice model.

KEY WORDS: Hippocampus, Histomorphology, Immunohistochemistry, Methamphetamine, Nigella Sativa.

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INTRODUCTION

Methamphetamine (MA) is an illicit drug widely used for its potent psychostimulant effects. It is a drug of choice for abuse due to its low cost, easy availability and having long-lasting psychoactive effects[1,2]. MA notoriously caused more than 71% of global amphetamine-type stimulant (ATS) seizures[3]. It selectively damages the serotonergic and dopaminergic neurons of central nervous system (CNS) and increases the glial fibrillary acidic protein (GFAP) which is an index of gliosis following neuronal injury and drug induced toxicity[4,5]. MA induces neurotoxicity through different mechanisms; proposed mechanisms included apoptosis, DNA damage, inflammatory mediators, damage to blood brain barrier and neuronal over excitatory injury[6,7]. Previous studies also reported ischemic and MA induced injury of brain hippocampus in rat models[8,9]. Nigella sativa (kalonji) is a spicy herb with religious background and medicinal history. Historically, its use dates back to the Kingdom of Assyrians and Ancient Egyptians about 3,000 years ago[10,11]. Hobbenaghi (2014) et al reported that the NS exerted neuroprotective effects in hippocampus neurons in experimental rats with global cerebral ischemia-reperfusion injury. Thymoquinone (TQ) is the most active constituents of N. sativa, attributes to therapeutic actions of N. sativa. TQ is used as chemotherapeutic and chemo preventive agent in various diseases[12,13].

The purpose of this study was to evaluate neuroprotective effects of Nigella sativa in methamphetamine induced hippocampal injury as evidenced by histomorphology and immunohisto-chemistry of tissue sections of hippocampus.

METHODOLOGY

This Experimental study was conducted at Department of Anatomy, Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro, in collaboration with Sindh Agriculture University Tandojam from April 2019 to September 2016. All animal procedures were conducted under animal

protocol approved by Sindh Agriculture University Tando Jam. Sixty adult male mice of average 25-40gm body weight were selected. Sick mice, mice not feeding and female mice were excluded. All animals were housed in stainless steel cages equipped with feed containers and drinkers. They were provided food (lab chow) and tap water *ad libitum*. The light/dark cycle of 12 hours interval is maintained. The mice were divided in to four groups. Control Group A was given normal saline orally at the volume of 1 ml/kg. Experimental Group B animals were given METH at volume of 10 mL/kg [3,9] containing 10.0 mg/kg METH. Experimental Group C animals were given Kalonji extract at the volume of 3mg/ml of extract, and Experimental Group D animals were given METH plus Kalonji extract at the same volume and dose as mentioned earlier. The normal saline, METH and kalonji extract were given in 2 divided doses at the intervals of 8 hours in first three groups. However, the fourth group received the dose of kalonji extract 30 min before the METH.

Body weight and temperature were measured using digital thermometer, rectal temperature was measured before the first injection of METH and 1 hour after each successive drug injection up to 24 hours duration. If the temperature was found raised during the treatment, the animals were cool down by putting them in cages with ice. The animals were sacrificed by cervical dislocation after one week of last injection. Brain of each animal was removed, immersed in 10% formaldehyde solution, and fixed over night at -70^{o}C[12,14].

Preparation of N. sativa extracts

The powdered seed (50 g) was boiled in 500 ml boiling water for 15 min then mixture was filtered and concentrated under reduced pressure at 35°C [12].

Brain Tissue Slicing and Staining was done as Hippocampal sections of 5 µm thick were obtained by microtome, through ethyl alcohol and xylene series and embedded in paraffin blocks, stained with Hematoxylin and Eosin (H&E).

Microphotography was done with all the photographs and measurements were carried out with the help of digital microscope with Digi pro software.

Immunohistochemistry was carried out with commercially available monoclonal antibody against glial fibrillary acidic protein, GFAP-Elisa Kit, USA was used for Immunohistochemistry. [15]

Pre-approved proforma was used to collect and document data during research.

Data was analyzed using SPSS version 22. Quantitative and Qualitative variables were analyzed using analysis of variance, post-Hoc testing and Chi square test respectively. Statistical significance was taken as p≤ 0.005 (Confidence interval 95%).

RESULTS

The present experimental study was conducted to investigate the neuroprotective effect of Nigella sativa on methamphetamine induced hippocampus injury in mice. Brain tissue sections were examined by H & E staining and Immunohistochemistry.

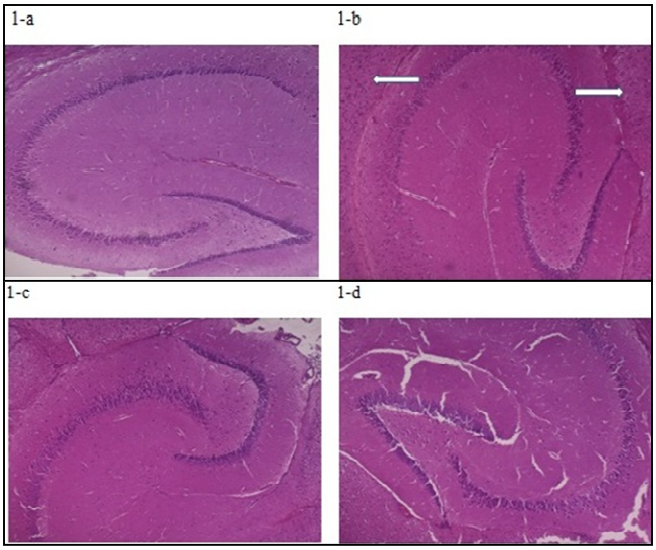
Table I shows the weight of different groups at baseline and at the end of study period. At baseline, the weight of mice was equal in all groups. While at the end of study period, weight was reduced in group B (METH treated mice) (p=0.001). Graph I shows the comparison of weight of different mice groups at baseline with weight at the end of study period.

Histomorphology in photomicrograph I show the changes in different layers of hippocampus in comparison with control group. In (1a) different areas of Hippocampus (1b) of METH treated mice showing hyper-cellularity of different layers, in (1c) section of N.S treated mice showing normal cellularity at hippocampus, in contrast (1d) section of N.S + METH treated mice showing marked decrease in cellularity of hippocampus (H & E x100) .

The panels of Immunohistochemical sections in photomicrograph II were selected to show the changes of different layers of hippocampus in comparison with control group. In (2a) Tissue section of Immunohistochemical staining for Glial fibrillary acidic proteins (GFAP) of hippocampus showing its normal distribution (2b) of METH treated mice showing over expression (GFAP) of different layers of hippocampus, in (2c) section of N.S treated mice showing normal expression of (GFAP) at different layers of hippocampus, in contrast (2d) section of N.S + METH treated mice showing marked changes of hippocampus. (IHC x10)

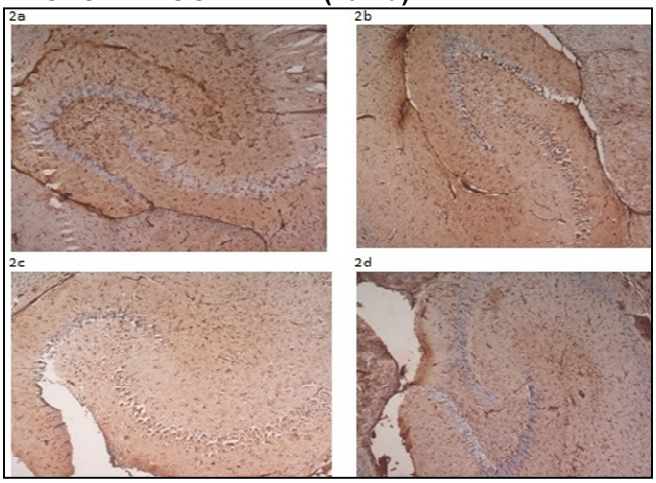
The panels of histomorphology and immunohisto-chemistry of sections in photomicrograph III were selected to show the changes of different layers of hippocampus in comparison with control group. In (3a) M ETH treated mice tissue section showing ventricular derangement/compression. (3b) section of N.S + METH treated mice showing reversal of ventricular damage, in (3c) IHC section METH treated mice showing necrotic lesion at hippocampus (IHC x10) in (3d) Histomorphological section of METH treated mice showing necrotic lesion at hippocampus. (H&E x10)

PHOTOMICROGRAPH I: (1a-1d)



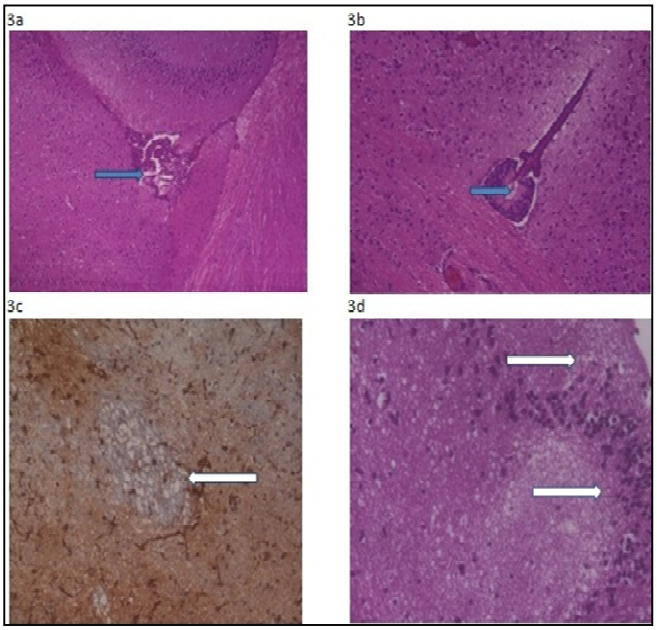
The panels in this photomicrograph were selected to show the changes of different layers of hippocampus in comparison with control group. In (1a) different areas of Hippocampus, the hippocampus (1b) of METH treated mice showing hyper cellularity of different layers, in (1c) section of N.S treated mice showing normal cellularity at hippocampus, In contrast (1d) section of N.S + METH treated mice showing marked decrease in cellularity of hippocampus. (H & E x10).

PHOTOMICROGRAPH II: (2a-2d)



The panels in this Immunohistochemical sections of this picture were selected to show the changes of different layers of hippocampus in comparison with control group. In (2a) Tissue section of Immunohisto-chemical staining for Glial fibrillary acidic proteins (GFAP) of hippocampus showing its normal distribution (2b) of METH treated mice showing over expression (GFAP) of different layers of hippocampus, In (2c) section of N.S treated mice showing normal expression of (GFAP) at different layers of hippocampus, In contrast (2d) section of N.S + METH treated mice showing marked changes of hippocampus. (IHC x10).

PHOTOMICROGRAPH -III

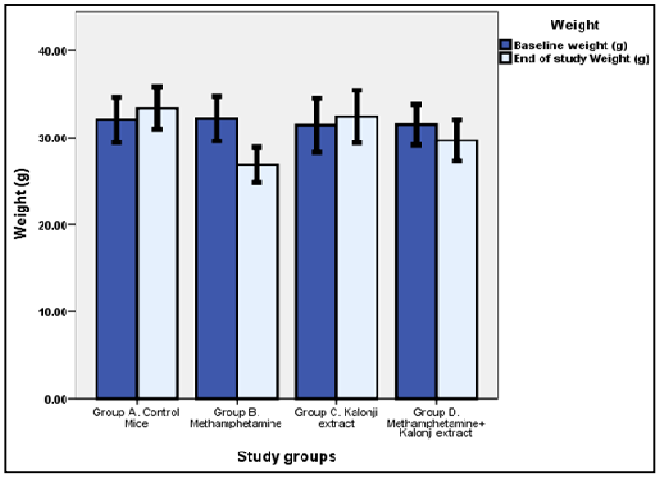


The panels in this section of this figure were selected to show the changes of different layers of hippocampus in comparison with control group. In (3a) M ETH treated mice tissue section showing ventricular derangement/compression. (3b)section of N.S+METH treated mice showing reversal of ventricular damage,In (3c) IHC section METH treated mice showing necrotic lesion at hippocampus. (IHC x10,) In (3c) Histomorphologicalsection METH treated mice showing necrotic lesion at hippocampus. (H&E x10)

TABLE I: WEIGHT (GMS) OF MICE

Groups	Weight at baseline			Weight at the end of study		
Group A. Control Mice	Mean	SD	P value	Mean	SD	P value
Group B. Methamphetamine	32.0	4.6	0.96	33.4	4.4	0.001
Group C. Kalonji extract	32.1	4.5		26.8	3.6	
Group D. Methamphetamine+ Kalonji extract	31.4	5.5		32.4	5.4	
	31.4	4.2		29.6	4.16	

GRAPH I: COMPARISON OF BODY WEIGHT OF MICE AT BASELINE AND END OF STUDY PERIOD



These findings show a neuroprotective effect of Nigella sativa against methamphetamine induced cell injury in hippocampus of mice model.

DISCUSSION

Many people take Methamphetamine(MA) due to its attention-enhancing effects. A literature search showed several studies on methamphetamine induced injury of brain hippocampus in rat models and its neuroprotective effects by Nigella sativa[10,11,16].

The findings of present experimental study, conducted on 60 mice model, Brain tissue sections examined by H & E staining and Immunohistochemistry are consistent with findings of other studies as well[17,18].

A previous study has reported neuroprotective effects of NS on the chronic toluene induced neuronal injury at sub cellular levels. The changes of neuronal injury completely disappeared in the NS treated animals and histopathological examination showed reversal of cell injuries[19].

Highly neuroprotective effect of NS and TQ was observed against toluene induced hippocampus injury by other researchers as well[20,21].

In present study, the methamphetamine treated mice showed neurodegenerative changes. Most damaging effect was shown by Astrocytosis (hypercellular glial tissue) and necrosis of different layers of hippocampus. While NS treated mice reversed the amphetamine induced neuronal injury. Ventricular injury was not found and cellularity was noted normal in NS treated hippocampus (Photomicrograph-3b). These findings are in support of above mentioned studies. Group D- mice received methamphetamine with Nigella sativa and were compared with Group B-methamphetamine treated mice. Significant differences were observed between group B and D. Overall the NS exerted significant neuroprotective effects against methamphetamine.

Another novel part of present study was the immunohistological examination as shown in Photomicrograph 02. Over expression of GFAP was not observed in the controls and NS + methampheta-mine treated mice. On the contrary, a high expression of GFAP was noted in the group B- methamphetamine treated mice hippocampus.

Nigella sativa treated mice showed normal expression of GFAP as shown in Photomicrograph 2a. GFAP showed normal expression in various layers of hippocampus. Methamphetamine + Nigella sativa treated mice showed no abnormality in expression of GFAP as show in Photomicrographs 2c. Normal expression of GFAP was noted in all parts and layers of hippocampus in mice treated by Nigella sativa.

Methamphetamine induced mice showed a body weight reduction as shown in table 1 the finding is supported by previous studies[16,17,22].

Thymoquinone (TQ) showed significant neuroprotective effects in hippocampal neurons in neurodegenerative effects of Alzheimer disease (AD), which is highly consistent with this study as Nigella sativa produced similar effects in the present study against methamphetamine induced injury[22].

Another previous study has reported neuroprotective effects of TQ significantly against hippocampus injury in experimental animals by increasing anti-oxidant enzyme activity[24].

From the above discussion and findings of histopathological and immunohistochemical analysis of hippocampus of mice of present study suggests a neuroprotective effect of Nigella sativa

CONCLUSION

The findings of above study shows neuroprotective effect of Nigella sativa against methamphetamine induced cell injury in hippocampus of mice model. Nigella sativa is commonly available herb can be used in clinical practice and will be beneficial for community at very affordable cost.

Ethical Permission: ERC approval letter of LUMHS No. LUMHS/REC/342 dated: 07-05-2015.

Conflict of interest: There was no any conflict of interest.

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