WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 6, 824-841. Research Article ISSN 2277– 7105

# EVALUATION OF ANTIOXIDANT ACTIVITY AND CHEMOPROTECTIVE EFFECT OF DIVINE NONI AGAINST DOXORUBICIN-INDUCED MEMORY IMPAIRMENT IN MICE

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Article Received on
22 Jan. 2018,

Revised on 11 Feb. 2018,
Accepted on 03 March 2018

DOI: 10.20959/wjpr20186-11447

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## ABSTRACT

*Morinda citrifolia* is a medicinal plant commonly known as noni. Traditionally the whole plant is used for various illness including neurological disorders. The aim of the present study was to evaluate antioxidant potential and protective effect of noni juice and divine noni gold concentrate on doxorubicin-induced memory impairment in mice. Antioxidant activity were determined by DPPH free radical scavenging assay. Protective effect of fresh noni juice and divine noni gold concentrate were assessed against doxorubicin induced memory impairment in mice using Morris water maze task. Memory impairment was induced by administrating a single i.p. dose 20 mg/kg b.w. of doxorubicin. Noni juice and divine noni gold concentrate at the dose 0.35 ml/b.w. were administered once daily for 14 days. Estimated endogenous antioxidants, malondialdehyde, acetylcholinestrase activities and histopathology were estimated. In DPPH study $IC_{50}$ value of ascorbic acid, divine noni gold concentrate and noni juice was found to be 3.19 µg/ml, 150.9 µg/ml and 155.7 µg/ml respectively. Doxorubicin caused memory impairment along with increased acetylcholinestrase, malondialdehyde and decreased antioxidants activities which were reversed by noni juice and divine noni gold concentrate adjuvant therapy. The existing result

is confirmed by histopathological analysis of hippocampus. Results suggest that noni juice and divine noni gold concentrate are good source of antioxidants possesses protective effect on doxorubicin induced memory impairment.

**KEYWORDS:** Antioxidant, chemotherapy, memory impairment, radical scavenger, Alzheimer.

## INTRODUCTION

Medicinal plants have been extensively using for centuries to treat numerous disorders. During the last epochs, there has been interest in identifying compounds from plants that can provide beneficial effects on human health. Among these compounds, the antioxidants or free radicals scavengers have received special attention for their pharmacological activity.[1,2]

Phenolic compounds are usually present in herbal products reported to their multiple biological activities.[3] These compounds are most common and active antioxidant available in fruits and vegetables exhibited scavenging free radicals as well as inhibition of oxidases and other enzymes.[4,5,6] The plant antioxidants plays a crucial role in the treatment of cognitive impairment.[7] Occurrence of oxidative stress triggers to develop various pathophysiological conditions i.e. cancer, inflammation, aging and cognitive function.[8,9] This has prompted importance in plant antioxidant i.e. phenolic rich dietary sources and their protective effects on human health.[10] Therefore, over the past few decades, herbal products from folk medicines have become increasingly popular globally owing to their long standing use, efficacy and reduced toxicity.[11] Herbal preparations are recently depicted as complementary and alternative medicine for neurodegenerative disorders.[12]

*Morinda citrifolia* belonging to the family Rubeaceae, is known as noni broadly used as food in tropical regions from Indonesia to Hawaiian Island. Various phytoconstituents are present in leaves, roots, flowers, and fruits like anthraquinones, iridoids, polysaccharides, glycosides, flavonoids, lignins, coumarins have demonstrated a broad range of biological activities in *in-vitro, in-vivo* and clinical studies.[13] Recently reported that commercial noni juice and its constituents are good sources of polyphenols and antioxidants.[14] Noni juice (NJ) being using as an alternative medicine for different kinds of illnesses including mental depression.[15] Various researchers reported that NJ inhibit neuronal damage induced by focal ischemia[16], prevent memory impairment and oxidative stress induced by the amyloid-β peptide[17], prevent scopolamine induced memory impairment is due to its antioxidant property and

inhibition of AChE activity.[18] Divine noni gold concentrate (DNG) is a polyherbal formulation containing NJ and extracts of *Garcinia cambogia* fruit and liquorice. DNG is available in the market, peoples are using as nutraceuticals and for the treatment of various kind of disorders.

Chemotherapy is the most effective and reliable cancer treatment strategies emerges with a high rate of failure and toxicity. Generation of reactive oxygen species (ROS) and initiates lipid peroxidation are common in chemotherapy that decrease antioxidant defence mechanism in normal cells leads to induce various toxicities including neurodegenerative disorders. Chemotherapy-induce memory impairment is an austere challenge being suffering by cancer survivors globally. Cancer patients has been reported attention deficits, memory loss and confused thoughts processes during and after chemotherapy.[19] Breast cancer patients reported cognitive dysfunction who underwent doxorubicin (DOX)-based chemotherapy.[20,21] The mechanism of DOX-induce toxicity in brain tissue is not clear, demonstrated that DOX does not cross blood-brain barrier (BBB) but DOX-induce TNF-α which causes mitochondrial dysfunction in the brain that could be accountable for the memory impairment.[22]

Another fact is that memory impairment is correlate to the dysfunction of cholinergic system in brain.[23] Normal cholinergic activity is very essential for hippocampal neurogenesis and memory improvement.[24] Acetylcholinesterase (AChE) is a specific enzyme present in brain, determination of its activity is crucial to detect the neurotoxic effects of DOX. Several studies reported that free radical production is related to the alteration in the activity of AChE in brain induce memory impairment.[25,26] Therefore, exogenous antioxidants are require to inhibit the production of free radicals to avert memory impairment.

Hence in the present study the antioxidant potential noni is selected. In spite of the various use of noni there have been no studies of it's on chemotherapy-induced memory impairment. Therefore, the aim of the present study is to investigate the protective effect of NJ and DNG on DOX-induced memory impairment in mice.

## MATERIALS AND METHODS

### Drug and Chemicals

Commercial doxorubicin hydrochloride injection IP (NAPRODOX 50 mg) Naprod Life Sciences Pvt. Ltd. was procured from JSS Hospital, Mysuru, India. DPPH• (2,2-diphenyl-1-

picrylhydrazyl) (Sigma-Aldrich Chemical Co®) was used to determine antioxidant activity. All other reagents used were analytical grades.

**Noni samples**

The ripe noni fruits and DNG concentrate were procured from Noni Biotech Pvt. Ltd. Tamil Nadu, India. Batch No. 88510. Fresh noni juice were prepared from fully ripped noni fruits by hand squeezing method.

**Animals**

Swiss albino mice weighing 25-30 g were procured from central animal facility, JSS Medical College, Mysuru, India were used in this study. The animals were kept in polyacrylic cage (22.5 cm × 37.5 cm) and maintained under standard housing conditions (room temperature 24–27° C and humidity 60–65%) with a 12h light and dark cycle. Food and water were provided *ad libitum* but food was not permitted from 1h prior to till completion of behavioral study. Study protocol described were reviewed and approved from the Institutional Animal Ethical Committee (IAEC) of JSS Academy of Higher Education & Research, Mysuru, India, and the obtained IAEC No. for this study was 161/2016.

**Determination of antioxidant activity - DPPH scavenging assay**

DPPH solution were prepared according to the method of Blois, (2000) with minor modification.[27] Individually NJ and DNG 50 µg/ml was pipetted into DPPH solution concentration 50 µg/ml (1:1) to pledge the reaction. After 30 minutes incubation, the absorbance was read at the wavelength 516 nm using UV-spectrophotometer. Methanol was used as a blank and DPPH solution 50 g/ml as standard. Analysis was done in triplicate for standard and each samples. Results were expressed as $IC_{50}$, based on percentage of radical inhibition in relation to the control.

**Treatment schedule**

The healthy mice were divided into six clusters, each cluster contained ten mice and subjected to various daily treatment regimens:

Group-I (Normal-untreated): Mice were fed standard diet and water. Group-II: received DOX 20 mg/kg b.w. i.p on day 1.[28] Group-III: received NJ 0.35 ml/b.w. p.o once daily for 14 successive days.[29] Group-IV: received DNG 0.35 ml/b.w. p.o. once daily for 14 successive days.[29] Group-V: received DOX 20 mg/kg b.w. i.p on day 1 and after half an hour received

NJ 0.35 ml/b.w. p.o once daily for 14 days. Group-VI: received DOX 20 mg/kg b.w. i.p on day 1 and after half an hour received DNG 0.35 ml/b.w. p.o once daily for 14 days.

**Behavioral study**

**Evaluation of memory impairment activity by escape latency time and time spent target quadrant methods in Morris water maze task**

The Morris water maze (MWM) task was performed according to the method of Joseph, (2008).[30] Animals were given pre-treatment training for 14 days of all groups. After two weeks of pre-treatment training, from the day 15$^{th}$ animals were given treatment according to the treatment schedule. Memory impairment activity was assessed once in a week. Mice were released into the water and allowed for 60-90 sec to find the platform in MWM. Then the animal was placed in the dry cage for 60 sec to dry their body after that the next trial was performed. Animals were returned to the home cage after 3 trials/day. In general, animals received three trials/ day with five minutes inter-trial for seven days till the performance became stable. The platform in the water maze was kept at the same position throughout the test to assess the effect on spatial reference memory for escape latency time (ELT) and noted the time consumed to find the platform. The time spent target quadrant (TSTQ) experiment were performed after 3h of ELT experiment. The platform were removed in this experiment and same way animals were placed in the water maze and noted the time consumed in the platform area.

**Assessment of antioxidant molecules, MDA and AChE activity**

After ELT and TSTQ examinations six mice were sacrificed using halothane anesthesia from each group and brains were isolated. The brains of all mice cut into two parts, a part was fixed in 10% formalin for histopathological study and remaining parts were cleaned in ice-cold saline and 10% brain homogenate was prepared with ice cold phosphate buffer and used for the estimation of glutathione (GSH)[31], superoxide dismutase (SOD)[32], catalase (CAT)[33], glutamate-s-transferase (GST)[34], malondialdehyde (MDA)[35], and AChE[36] and absorbance was taken in UV-spectrospectroscopy.

**Statistical analysis**

The result were expressed as mean±SEM. The means were compared by one way and two way analysis of variance (ANOVA) respectively. A significant level of $p<0.05$ was used.

## RESULTS

### Antioxidant activity by DPPH free radical scavenging assay

$IC_{50}$ value were calculated followed by the calibration curve equations. $IC_{50}$ value of the ascorbic acid, DNG and NJ was found to be 3.19 µg/ml **(**Fig. 1a), 150.9 µg/ml **(**Fig. 1b), 155.7 µg/ml **(**Fig. 1c) respectively**.**

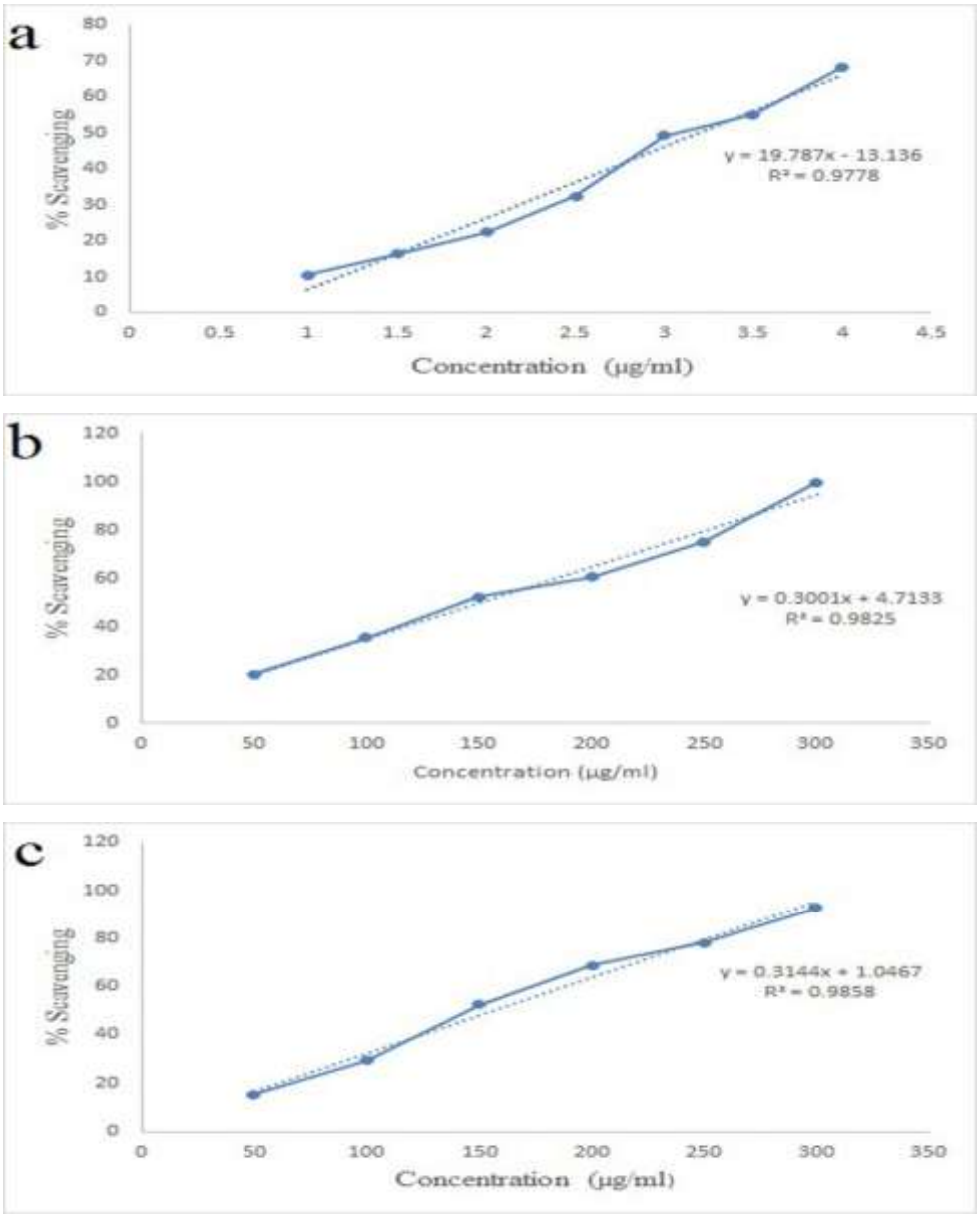


**Fig. 1: Free radical scavenging activity of (a) Ascorbic acid, (b) DNG, (c) NJ.**

### Behavioral study

### Analysis of escape latency time

DOX injection significantly ($p<0.05$) prolonged the ELT on 7$^{th}$ day trial of the accusation period and gradually increased observed in 14$^{th}$ day trial (Group II) in comparison to

untreated (Group I). In combination treatment observed NJ protected and minimised memory impairment by significantly decreasing ELT in DOX challenged mice on 7th and 14th day trials (Group V). Whereas DNG exhibited better protection in DOX challenged mice in both 7th and 14th day trials (Group VI). Whereas individual treatment of NJ and DNG significantly attenuated ELT compared to DOX alone treated animals (Group III and IV) (Fig. 2).

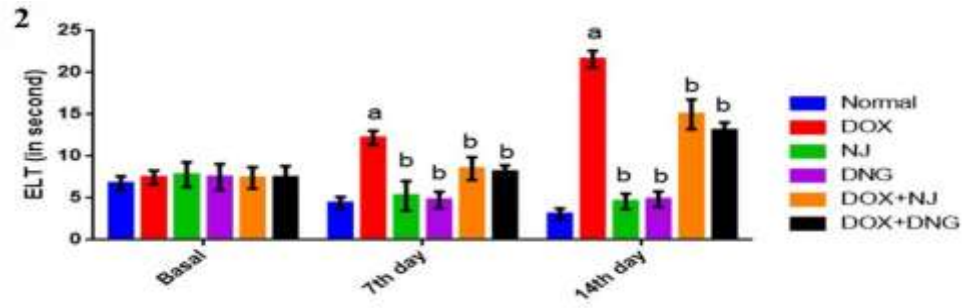


**Fig. 2: All values were expressed as mean±SEM (n=6). The data were analyzed by two-way ANOVA followed by post hoc test. [a]p<0.05 is compared to normal, [b]p<0.05 is compared to DOX.**

**Analysis of time spent target quadrant**

DOX induced memory impairment is confirmed by TSTQ experiment. In the present study, TSTQ significantly (p<0.05) abridged in DOX-treated animals in both 7th and 14th day trials (Group II) in comparison to untreated (Group I) which was significantly (p<0.05) increased observed in combination treatment in both 7th and 14th day trials (Group V and VI). NJ and DNG (Group III and IV) individually significantly (p<0.05) increased TSTQ compared to DOX alone treated animals (Group II). TSTQ method revealed the confirmation of protective effect of NJ and DNG against DOX induced memory impairment (Fig. 3).

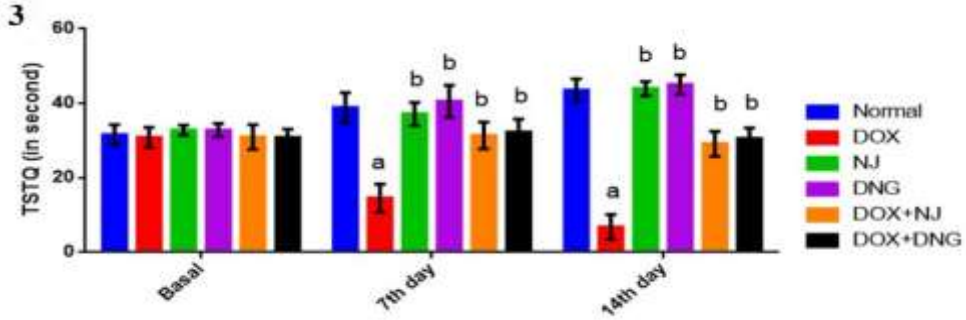


**Fig. 3: All values were expressed as mean±SEM (n=6). The data were analyzed by two-way ANOVA followed by post hoc test. [a]p<0.05 is compared to normal, [b]p<0.05 is compared to DOX.**

**Determination of AChE and MDA activity on hippocampus**

DOX injection significantly ($p<0.05$) augmented the activity of AChE and MDA in hippocampal tissue (Group II) compared with untreated (Group I) which were significantly reverted by NJ and DNG in DOX challenged animals (Group V and VI) compared to DOX alone treated animals (Group II). Whereas, there is no significant difference observed in individual treatment of NJ and DNG (Group III and IV) in comparison to untreated animals (Fig. 4a and 4b).

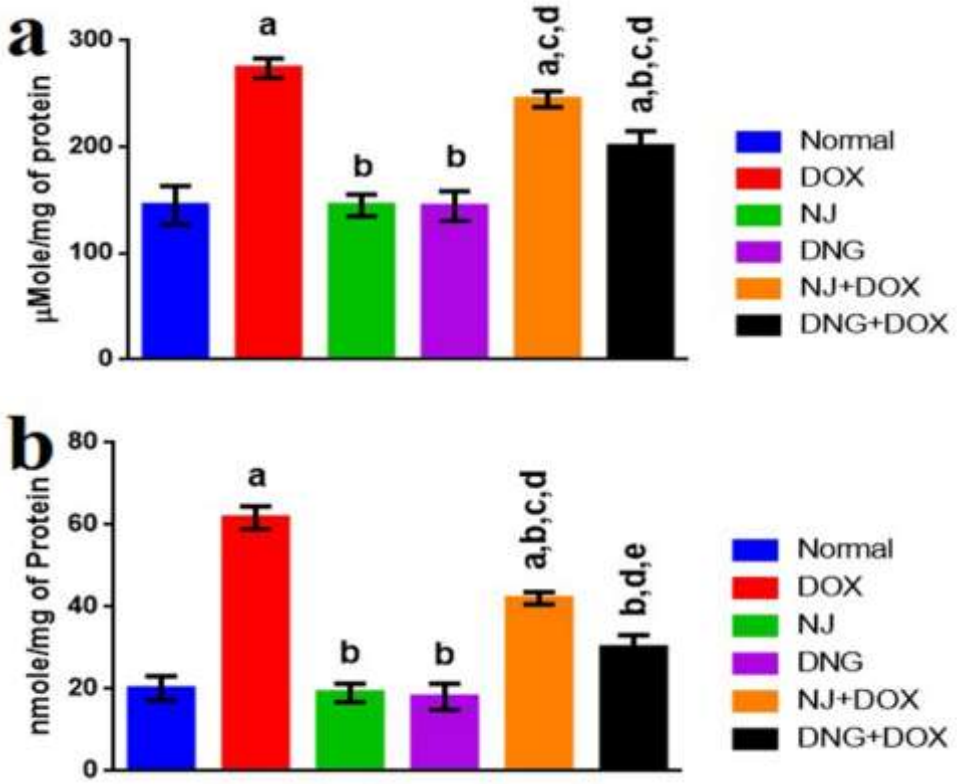


**Fig. 4a (AChE) and 4b (MDA). All the values are mean±SEM of six mice compared with DOX, statistics considered $p<0.05$. [a]$p<0.05$ compared to normal, [b]$p<0.05$ compared to DOX, [C]$p<0.05$ compare to NJ, [d]$p<0.05$ compare to DNG, [e]$p<0.05$ compare to NJ+DOX**

**Determination of the antioxidants activities in brain tissue**

DOX injection significantly ($p<0.05$) depleted the antioxidants activities in hippocampal tissue including GSH, SOD, CAT and GST (Group II). These changes were significantly ($p<0.05$) reversed by adjuvant therapy of NJ and DNG respectively with DOX in combination therapy (Group V and VI). Likewise, individual treatment of NJ and DNG significantly ($p<0.05$) improved GSH, SOD, CAT and GST levels compared to DOX treated animals, on the other hand NJ and DNG significantly ($p<0.05$) improved SOD level whereas

DNG alone significantly ($p<0.05$) increased GSH level (Group III and IV) compared to untreated animals (Group I) (Fig. 5).

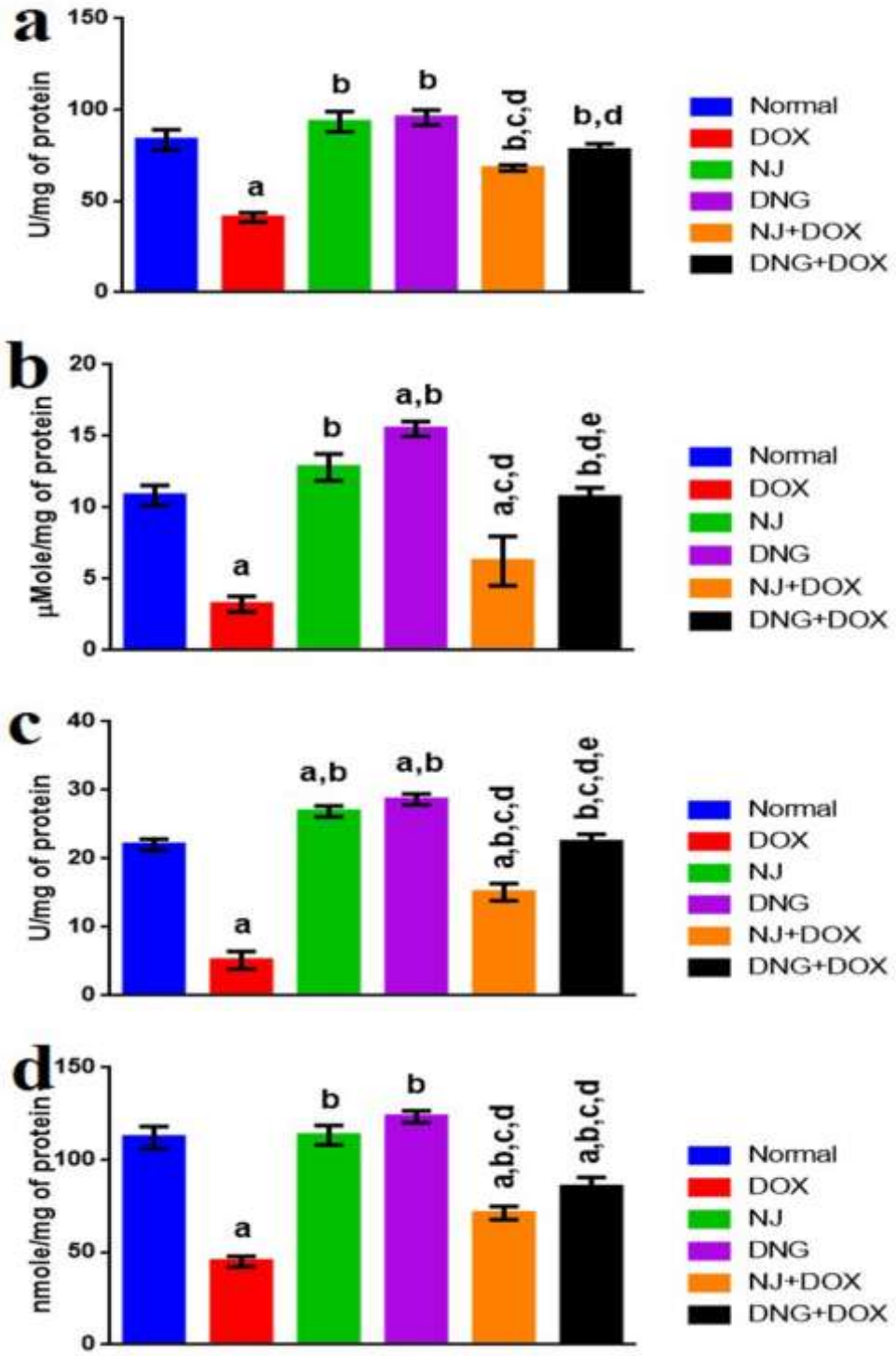


**Fig. 5: (a) CAT, (b) GSH, (c) SOD, (d) GST. All the values were expressed as mean±SEM, (n=6), analysed by one-way ANOVA followed by Tukey's test. [a]p<0.05 compared to normal, [b]p<0.05 compared to DOX, [C]p<0.05 compared to NJ, [d]p<0.05 compared to DNG, [e]p<0.05 compare to NJ+DOX.**

**Histopathological study**

This study was investigated the role of dorsal hippocampal sub-regions CA1 and CA3 in memory for the order of sequentially associated nonspatial elements. In the present study, we have scrutinised CA1 and CA3 regions of mice hippocampus exhibited healthy and normal cellularity in untreated mice brain (Fig.6a, 6b). There is no change occurred in CA1 and CA3 regions in NJ and DNG individual treated animals (Fig.6c, 6d and 6e, 6f) in comparison to untreated animals. DOX exposed animals exhibited cellular damage and degeneration of cells in CA1 and CA3 regions (Fig.6g, 6h) which were protected by adjuvant therapy of NJ and DNG respectively in DOX challenged animals (Fig. 6i, 6j and 6k, 6l).

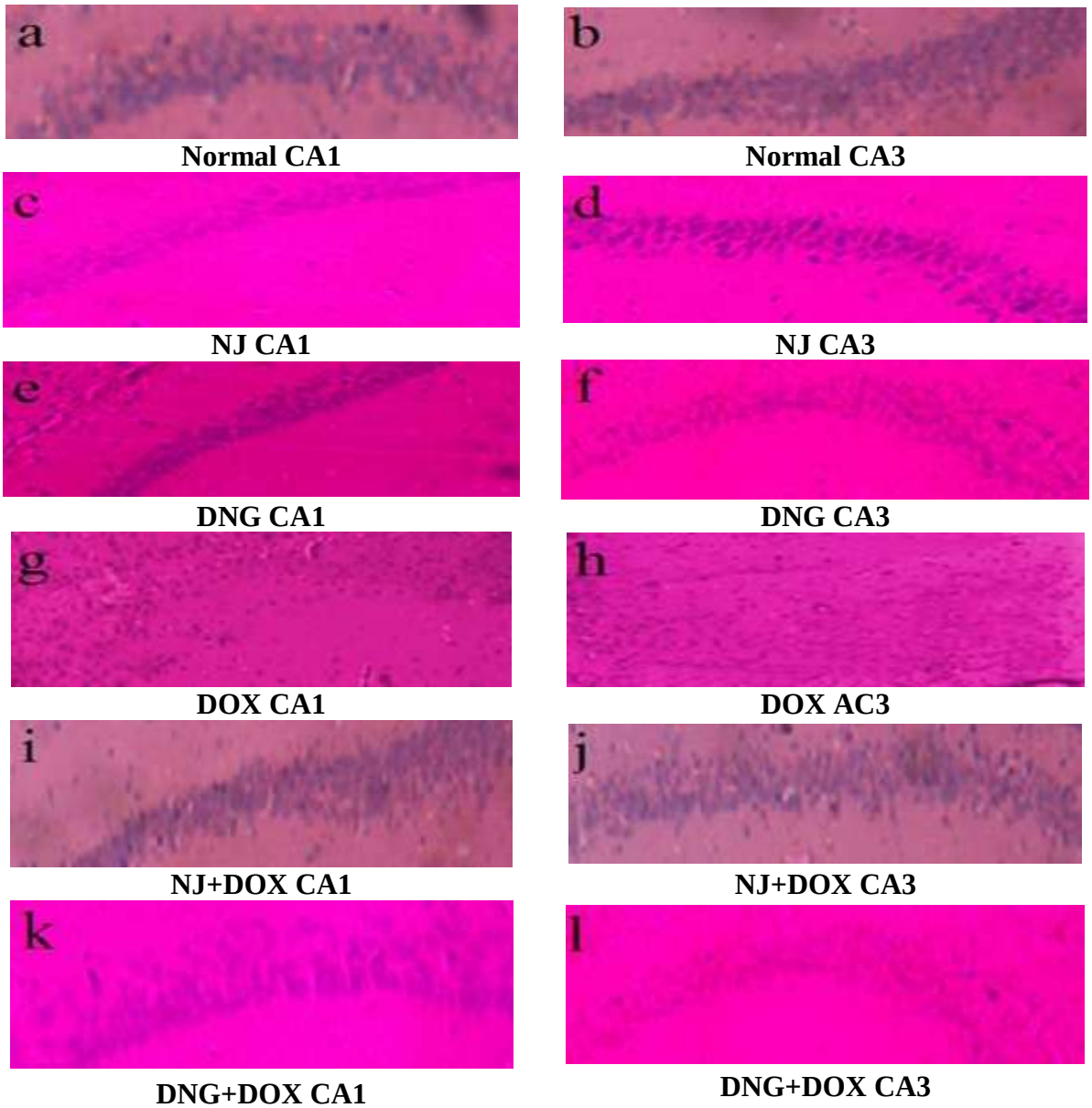


**Fig. 6: Morphological changes visualized under light fluorescence microscope staining haematoxylin and eosin in the brain hippocampus of mice.**

## DISCUSSION

Phenolics are important compounds, many of them naturally occur in a broad range of food and plants. Considered flavonoids are the predominant and best studied group among polyphenols. A variety of plant polyphenols is either being actively developed or already currently using as dietary supplements. Although these compounds play an unknown role in nutrition having antioxidant activity might be potentially beneficial in preventing disease.[37] In the present study, NJ and DNG were exhibited good source of antioxidants having free radical scavenging activity confirmed by performing DPPH free radical scavenging assay, where $IC_{50}$ values exhibited by ascorbic acid 3.19 µg/ml (Fig. 1a), DNG 150.9 µg/ml (Fig. 1b) and NJ 155.7 µg/ml (Fig. 1c). This result is correlated to the study of Dixon *et al.*, reported that phenolic and flavonoid compounds of NJ act as free radicals scavengers and avert numerous diseases.[38]

Based on the free radical scavenging activity of NJ and DNG implemented for further *in-vivo* study to evaluate protective effect against DOX induced memory impairment in mice.

Memory impairment activity was evaluated in MWM task. Since oxidative stress contributes to pathogenesis and histological changes in patients with neurodegenerative disorders.[39] The excessive production of ROS triggers neurotoxicity through thiol and lipid-dependent mechanisms in the cell membrane.[40] This information validated our study in specific use of DOX injection. In the present behavioral study revealed that number of errors and time taken to reach the target area were markedly high in DOX-treated animals, indicating the detrimental effect on spatial reference learning and memory with irritating behavior in DOX alone treated animals when compared to untreated animals (Fig. 2 and 3). Recent findings suggested that this irritating characteristic behavior may be associated with the abnormal function of hippocampus which may be caused due to the generation of ROS in DOX treatment leading to apoptosis cells death in CA1 and CA3 regions.[41] Reported that increased protein oxidation and lipid peroxidation in brain isolated from DOX-treated mice could be involved in the symptoms of chemo-brain observed in patients treated with DOX.[42] In the present study, we got similar result where DOX treated animals exhibited elevate MDA level as well as decline antioxidants activities in brain tissue associated with the degenerated cells in CA1 and CA3 regions of hippocampus observed in histopathological study (Fig. 6g and 6h). Increased superoxide anions may elevate the level of circulating TNF-α that can directly pass through the BBB, which affect in both the cerebral cortex and

hippocampus leads to induce memory deficits.[43] With this view we suggest that the abnormal performance of animals in ELT and TSTQ experiments due to oxidative stress which directly indicates that DOX-induced memory impairment which were protected by NJ and DNG observed in combination therapy (Group V and VI). On the other hand individual treatment of NJ and DNG significantly improved ELT and TSTQ. This result is associated with the outcome of antioxidants activity of NJ and DNG significantly ($p<0.05$) augmented the levels of SOD, GST, CAT and GSH in DOX challenged mice (Group V and VI) compared to DOX alone treated animals (Group II). These observations supports the hypothesis of the mechanism of memory impairment effect of DOX related to depletion of antioxidant defense system. The protective effect of NJ and DNG in the current study is an agreement with Shakti *et al.*, were reported that noni fruit extract can protect memory impairment by preventing oxidative stress induce by scopolamine.[44]

AChE plays a key role in the metabolism of acetylcholine and hence inhibition of AChE has appeared as one of the promising strategy for the treatment of cognitive deficits. The exact mechanism responsible for beneficial effects of noni against DOX induced memory impairment is not known but it may be due to improvement in central cholinergic function of NJ. The most important strategy is to increase cholinergic function by inhibiting AChE activity. In the current study we observed rapid increase AChE activity in DOX exposed animals while NJ and DNG minimised memory impairment by decreasing AChE level in combination therapy respectively (Group V and VI) (Fig. 4a). This result is correlated with Chung *et al.*, Lee *et al.*, Rollinger *et al.*, Orhan *et al.*, and Hernandez *et al.* who demonstrated the presence of quercetin, rutin and scopoletin in noni fruit inhibit AChE activity.[45,46,47,48,49]

The accumulation of MDA, an end product of lipid peroxidation, reflects the extent of oxidative stress and indirectly cellular antioxidant capacity.[50,51] Earlier reports of preclinical study have been demonstrated that chemotherapeutic agents induce central oxidative stress in healthy rodents.[52] As reported earlier, we also have found in the present study significant increase MDA level in mice brain following DOX treatment[42] which is significantly reversed by NJ and DNG observed in combination therapy (Group V and VI) (Fig. 4b). This result clearly indicate that NJ and DNG can prevent the occurrence of memory impairment by inhibiting the generation of ROS in brain. This effect may be due to antioxidant compounds like quercetin and rutin present in noni fruit.[53,54]

In overall study DNG showed more protective effect compared to NJ may be due to high antioxidant potential may be because of containing the extracts of *Garcinia cambogia* fruit. *Garcinia cambogia* is an herbal remedy which is established as potent antioxidant.[55]

## CONCLUSION

In the present study we evaluated antioxidant activity of NJ and DNG which supported to protect memory impairment effect of DOX. Therefore our results suggests that NJ and DNG might offer a useful therapeutic choice in prevention of memory impairment induced by DOX. NJ is having unpleasant odour and bitter taste compare to DNG, moreover DNG exhibited more protective effect than NJ hence we considered DNG to be used as adjuvant therapy for clinical use with chemotherapy.

## ACKNOWLEDGEMENT

Authors sincerely thankful to Dr. T.M. Pramod Kumar, Principal, JSS college of Pharmacy, Mysuru for his kind support and encouragement.

**“Funding”**

The authors sincerely grateful to VGST (Vision Group on Science and Technology), grant no. VGST/K-FIST (LI) (14-15)/15-16. Department of information Technology, Biotechnology and Science and Technology, Govt. of Karnataka for funding this project under the K-FIST (Karnataka Fund for Improvement of Science and Technology).

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