

Neuroprotective Activity of *Crataeva nurvala* Buch-Ham Stem Bark against Scopolamine-Induced Cognitive Impairment via Antioxidative Activities in Rats

Atanu Bhattacharjee^{*1}, Shastry Chakrakodi Shashidhara² and Santanu Saha¹

¹Department of Pharmacognosy, NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangalore – 574 018, Karnataka, India

²Department of Pharmacology, NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangalore – 574 018, Karnataka, India

ABSTRACT

Background: *Crataeva nurvala* Buch-Ham is a well explored traditional Indian medicinal plant, routinely used as folkloric medicine to treat various ailments in particular urolithiasis and neurodegenerative disorders associated with cognitive dysfunction.

Objectives: The present study was undertaken with the objective to investigate the antioxidant and anti-dementia activities of *Crataeva nurvala* stem bark extract.

Methods: The antioxidant and anti-dementia activities of *Crataeva nurvala* extract was carried out using superoxide dismutase (SOD), catalase (CAT) specific activities, total content of reduced glutathione (GSH), malondialdehyde (MDA) level (lipid peroxidation) and Hebb's-William maze assays respectively in Wistar rats subjected to scopolamine (1 mg/kg i.p.) induced dementia.

Results: Sub-acute exposures (daily, for 7 successive days) of *Crataeva nurvala* extract (100, 200 and 400 mg/kg) significantly increased antioxidant enzyme activities (SOD and CAT), total content of reduced GSH and reduced lipid peroxidation (MDA level) in rat brain homogenates in dose dependent manner, suggesting the antioxidant potential. Moreover, in Hebb's-William maze test, *Crataeva nurvala* extract showed significant improvement in transfer latency, suggesting anti-dementia activity.

Conclusion: Taken together, our results suggest combination of antioxidant and anti-dementia activities may be associated with the neuroprotective effect of *Crataeva nurvala* extract against scopolamine-induced oxidative stress in rat brain.

Keywords- *Crataeva nurvala*, Neuroprotection, Antioxidant, Anti-dementia.

INTRODUCTION

Alzheimer disease (AD) is an irreversible age-related form of dementia that slowly erodes the brain. It slowly robs the person memory and cognitive skills and causes changes in personality and behavior¹. The progression of the erosion is accompanied with neurodegeneration and characterized by abnormal amyloid β protein plaques, neurofibrillary tangles of tau protein and loss of cholinergic cells, and thereby decline in cholinergic neurotransmission^{2,3}.

Scopolamine, a muscarinic antagonist, had been widely used to study cognitive deficits in experimental animals⁴. Recently, it was reported that memory impairment induced by scopolamine in rats was associated with increased oxidative stress in brain⁵. The damaging effect of the oxidative stress was most vulnerable in AD⁶. Moreover, increased MDA level, one of the most harmful effects of oxidative stress had been considered as important biomarker for *in-vivo* lipid peroxidation^{7,8}, previous studies indicated oxidative damage as an important early-stage factor in neurodegenerative disorders associated with cognitive dysfunctions⁹. Hence, it may be possible to delay the progression of neurodegeneration in AD with antioxidants.

Several synthetic drugs are used clinically to improve cognitive function and bolstering body's defense against free radicals and thereby provide protection against AD. But the resulting side-effects associated with these synthetic drugs have made their utility limited¹⁰. Therefore, it is worthwhile to explore the efficacy of traditional medicines to treat various neuropharmacological disorders as they are considered to be safe and economical¹¹.

Crataeva nurvala (*C. nurvala*) Buch-Ham (Family: Cappariaceae) commonly known as Varuna, a well explored traditional Indian medicinal plant of Western ghats routinely used as folkloric

medicine against urolithiasis and neurological disorders^{12,13}. It is a medium sized branched deciduous plant distributed throughout the river banks of southern India and other tropical, sub-tropical countries of the world, wild or cultivated¹⁴. Hence, the present study was designed to evaluate the neuroprotective potentiality of *C. nurvala* through *in-vivo* neuropharmacological assay and biochemical estimations of several antioxidant parameters in Wistar rat model.

MATERIALS AND METHODS

Extraction and phytochemical analysis

The stem bark of *C. nurvala* was collected from the stream sides of Western ghat, India (voucher specimen no. NGSMIPS/Hb-04/2011), extracted by cold maceration with ethanol and concentrated through rotary flash evaporator¹⁵. The yield was found to be 17 % w/w. A portion of concentrated ethanolic extract was subjected to different qualitative phytochemical screening to identify the presence of various phytoconstituents as described by Harborne¹⁶.

Drugs and chemicals

Scopolamine hydrobromide, piracetam, sodium carboxy methyl cellulose (Sodium-CMC), Acetylthiocholine iodide, 5, 5'-Dithiobis (2-nitrobenzoic acid) (DTNB) were collected from Sigma-Aldrich, Bangalore, India. All the toxic, standard and test drugs (suspended in 0.6% w/v of sodium-CMC solution) were administered in the morning session i.e. 9 AM- 10 AM on each day.

Acute toxicity study

The acute toxicity of ethanolic extract of *C. nurvala* stem bark was evaluated in female Wistar rats as per OECD guidelines 425 (Up and Down Procedure). A fixed dosage study was adopted for acute toxicity study where the

limit dose is 2000 mg/kg body weight of test animal. Clinical signs of toxicity, body weight changes, cage side parameters and mortality rate were observed every hour for the first 6 hours and every day for 7 days¹⁷.

Experimental animals

Healthy young Wistar rats of either sex aged between 8-12 week old and weighing between 150-200 g obtained from the institutional animal house were used for *in-vivo* study. Prior to experiment animals were housed in a room under standard environmental condition for 7 days (25⁰C; 50-70% relative humidity; 12/12 h light/dark cycle) and fed with standard rat pellet and water *ad libitum*¹⁸. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) under the IAEC no.: KSHEMA/AEC/34/2011.

Experimental design

Sixty healthy Wistar rats of either sex were selected randomly (n=60) and divided into ten groups of six animals each to evaluate their responses on exteroceptive behavior models. The groupings of animals were summarized below.

- Group I (Normal control): Vehicle (0.6% w/v sodium CMC) p.o.
Group II (Negative control): Scopolamine (1 mg/kg i.p.)
Group III (Positive control): Piracetam (50 mg/kg) p.o.
Group IV: *C. nurvala* ethanolic extract (100 mg/kg) p.o.
Group V: *C. nurvala* ethanolic extract (200 mg/kg) p.o.
Group VI: *C. nurvala* ethanolic extract (400 mg/kg) p.o.
Group VII: Piracetam (50 mg/kg) p.o. + Scopolamine (1 mg/kg i.p.)
Group VIII: *C. nurvala* ethanolic extract (100 mg/kg) p.o. + Scopolamine (1 mg/kg i.p.)
Group IX: *C. nurvala* ethanolic extract (200 mg/kg) p.o. + Scopolamine (1 mg/kg i.p.)

Group X: *C. nurvala* ethanolic extract (400 mg/kg) p.o. + Scopolamine (1 mg/kg i.p.)

The dosing for all groups were done for a period of 7 days; after 45 minutes of administration of the last dose on 7th day, amnesia was induced by administration of scopolamine (1 mg/kg i.p.) to Group II, VII, VIII, IX and X respectively. The negative control group (Group II) received just one dose of scopolamine (1 mg/kg i.p.) on 7th day and 45 minutes after the administration of scopolamine, trials were taken on Hebb's-William maze and the retention was observed after 24 hours of the first exposure i.e. on day 8¹⁹.

Exteroceptive behavioral model

Hebb's-William maze

The Hebb's-William Maze is an incentive based exteroceptive behavioral model useful for measuring spatial and working memory of rats^[20]. A typical Hebb's-William maze (75x75x 25 cm) is a rectangular wooden box divided into three components viz. animal chamber (start box), which is attached to the middle chamber (exploratory area) and a reward chamber at the other end of the maze in which the reward (food) is kept.

The experiment was conducted under zero watt red coloured bulb to minimize the nocturnal cycle disturbances. During study, the animals were exposed to food and water *ad libitum* only for 1 h after the maze exposure to ensure motivation towards reward chamber.

Each rat was placed in animal chamber and the door of start box was closed immediately after the animal moved into the exploratory area to prevent its back entry. Time taken (in seconds) by the animals to reach reward chamber from start box (initial transfer latency, ITL) was noted for each animal. Each animal was allowed to explore the maze for additional 20 seconds. After experiment, the animals were returned

to their home cages and transfer latency was recorded again after 24 hours of the first exposure (retention transfer latency, RTL). A fall in transfer latency on subsequent maze exposures was taken as an index of successful retention²¹.

Estimation of antioxidant status

Collection of brain sample

Immediately after the experiment animals were sacrificed by cervical decapitation under light anesthesia and whole brain was carefully removed from the skull. The fresh whole brain was weighed, kept on ice bath, rinsed with ice-cold isotonic saline and homogenized (approximately 20 mg of tissue/ml of phosphate buffer (pH 8.0; 0.1M) in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for the estimation of antioxidant status²².

Estimation of lipid peroxidation

Lipid peroxidation is an autocatalytic process, which is common consequence of cell death. Malondialdehyde (MDA), one of the end products of lipid peroxidation is formed during oxidative degeneration. Hence, to measure MDA, thiobarbituric acid (TBA) test was performed because of its higher sensitivity and simplicity in operation. During the process, MDA react with TBA and form a red colour complex which was extracted with n-butanol-pyridine mixture and estimated by UV-Visible spectrophotometer at absorption maxima 532 nm. Lipid peroxide levels were expressed in terms of $\mu\text{Mol MDA/mg protein}$ ²⁴.

Estimation of super oxide dismutase (SOD)

Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2)

and elemental oxygen (O_2) and as such provides an important defense against the toxicity of the superoxide radical²⁵. In the assay, conversion of xanthine to uric acid and hydrogen peroxide is mediated via superoxide ions (O_2^-), generated by xanthine oxidase (XOD). Superoxide ions (O_2^-) convert NBT to NBT-diformazan, a blue colour complex with absorption maxima at 560 nm. SOD reduces the superoxide ion (O_2^-) concentration and thereby lowers the rate of NBT-diformazan formation. The extent of reduction in NBT-diformazan formation is a measure of SOD activity present in the sample. The absorbance was recorded for 3 minutes at 1 minute interval and % inhibition of NBT-diformazan formation by SOD was converted to the relative concentration. SOD levels were expressed in terms of unit/mg wet tissue²⁶.

Estimation of catalase (CAT)

Catalase (CAT) is an antioxidant enzyme ubiquitously present in mammalian and non-mammalian aerobic cells containing a cytochrome system. In UV range H_2O_2 shows continuous increase in absorption with decreasing wavelength. Catalase catalyses the rapid decomposition of H_2O_2 to water and oxygen. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit is a measure of catalase activity²⁷.

Estimation of reduced glutathione (GSH)

Reduced glutathione (GSH) is a tripeptide (γ -glutamylcysteinylglycine) that contains a free thiol group. GSH is a major tissue antioxidant that provides reducing equivalents for the glutathione peroxidase (GPx) catalyzed reduction of lipid hydroperoxides to their corresponding alcohols and hydrogen peroxide to water²⁸.

DTNB (5, 5'-Dithiobis (2-nitrobenzoic acid)), known as Ellman's Reagent, was developed for the detection of

thiol compounds. DTNB reacts with free thiol group of glutathione (GSH) and generates 2-nitro-5-thiobenzoic acid and glutathione disulfide (GSSG). Since 2-nitro-5-thiobenzoic acid is a yellow colored compound, GSH concentration in the sample can be determined by UV spectrophotometer at 412 nm. GSH is generated from GSSG by glutathione reductase, and reacts with DTNB again to produce 2-nitro-5-thiobenzoic acid. Therefore, this recycling reaction improves the sensitivity of total glutathione detection²⁹.

Statistical significance

The results were expressed as mean \pm SEM (standard error of mean). The difference between the control and treated means were analyzed using one way analysis of variance (ANOVA) followed by Tukey test. P-values < 0.05 were taken to be statistically significant.

RESULTS

Phytochemical analysis

Preliminary phytochemical analysis showed presence of alkaloids, phenolic compounds and tannins, phytosterols and triterpene, flavonoids, saponins, and coumarins in ethanolic extract of *C. nurvala* stem bark.

Acute toxicity study

No mortality was observed following oral administration of *C. nurvala* ethanolic extract even with the highest dose (2000 mg/kg). Moreover, no significant changes in body weight and behavior were observed. Hence, *C. nurvala* could be safe up to the dose of 2000 mg/kg body weight of the animal. Hence doses of 100, 200 and 400 mg/kg were selected for *in-vivo* behavioral assay.

Hebb's-William maze test

Results suggest pre-treatment with ethanolic extract of *C. nurvala* (100, 200 and 400 mg/kg body weight) for seven successive days did not exhibited much difference in ITL compared to normal control group but in presence of amnesia, higher dose of *C. nurvala* ethanolic extract (400 mg/kg body weight) afforded a significant (^{###} $p < 0.001$) decrease in TL (score: 220.66 ± 6.05) compared to other lower doses groups (score: 286.33 ± 5.10 and 259.33 ± 5.87 respectively) and closely approximated to standard drug Piracetam (score: 204.66 ± 5.48). However, all the three doses of extract showed improvement spatial learning and memory activity in dose dependant manner. Moreover, in Group VIII, IX and X, significant decrease in RTL on Day 8 compared to Day 7 were observed which elaborate the drugs responses to overcome the learning and memory deficits produced by scopolamine (Table 1 and Figure 1).

Measurement of lipid peroxidation

Results suggest pre-treatment with ethanolic extract of *C. nurvala* (100, 200 and 400 mg/kg body weight) for seven successive days did not exhibited much difference in MDA level compared to normal control group but in presence of amnesia, higher dose of *C. nurvala* ethanolic extract (400 mg/kg body weight) afforded a significant (^{###} $p < 0.001$) decrease in MDA level (score: 0.77 ± 0.022) compared to other lower doses groups (score: 1.025 ± 0.080 and 0.82 ± 0.033 respectively) and closely approximated to standard drug Piracetam (score: 0.606 ± 0.031). However, all the three doses of extract showed decline in MDA level in dose dependant manner (Table 2 and Figure 2).

Estimation of superoxide dismutase (SOD)

In presence of amnesia, higher dose of *C. nurvala* ethanolic extract (400 mg/kg



body weight) afforded a significant ($^{###}p < 0.001$) increase in SOD level (score: 1.10 ± 0.073) compared to other lower doses groups (score: 0.75 ± 0.042 and 0.94 ± 0.077 respectively) and closely approximated to standard drug Piracetam (score: 1.35 ± 0.045). However, all the three doses of extract showed increase in SOD level in dose dependant manner (Table 2 and Figure 3).

Estimation of catalase (CAT)

In presence of amnesia, higher dose of *C. nurvala* ethanolic extract (400 mg/kg body weight) afforded a significant ($^{###}p < 0.001$) increase in catalase level (score: 4.25 ± 0.16) compared to other lower doses groups (score: 3.55 ± 0.246 and 3.951 ± 0.248 respectively) and closely approximated to standard drug Piracetam (score: 4.50 ± 0.220). However, all the three doses of extract showed increase in catalase level in dose dependant manner (Table 2 and Figure 4).

Estimation of reduced glutathione (GSH)

In presence of amnesia, higher dose of *C. nurvala* ethanolic extract (400 mg/kg body weight) afforded a significant ($^{###}p < 0.001$) increase in GSH level (score: 0.541 ± 0.013) compared to other lower doses groups (score: 0.443 ± 0.024 and 0.520 ± 0.021 respectively) and closely approximated to standard drug Piracetam (score: 0.580 ± 0.028). However, all the three doses of extract showed increase in GSH level in dose dependant manner (Table 2 and Figure 5).

DISCUSSION

Many clinical studies have reported oxidative stress as one of the early causative factors involved in the pathogenesis of AD³⁰. Recent studies postulated that memory impairment in scopolamine-induced amnesic model is associated with

increased oxidative stress within the brain^{31,32}. Oxidative stress is the disturbance of balance between production of reactive oxygen species (ROS) and antioxidant defense systems. Therefore, it may be possible to delay the progression of AD with antioxidants³³. The current hypothesis about the mechanisms by which neurons come into necrotic or apoptotic processes has led to believe that the therapeutic use of antioxidants may be beneficial in aging and neurodegenerative disorders like AD³⁴.

Increased MDA level, one of the most harmful effects of ROS has been considered as important biomarker for *in-vivo* lipid peroxidation³⁵. The results of antioxidant study showed higher MDA level and lower levels of SOD, CAT, and GSH in scopolamine induced group compared to normal control group. Pretreatment with *C. nurvala* extract, significantly improve the levels of SOD, CAT and GSH whereas MDA level was decreased significantly. This demonstrated the antioxidant potentiality of *C. nurvala* extract. The findings from Hebb's - William maze test suggest *C. nurvala* ethanolic extract showed protective effect in transfer latency against scopolamine induced amnesia in dose dependent manner. Moreover, the decrease in transfer latency during retention period suggested the drug response to overcome learning and memory deficit produced by scopolamine which provides an adequate scientific promise to validate the anti-dementia potency of *C. nurvala* extract.

CONCLUSION

Therefore, in the present study we observed that *C. nurvala* extract (i) showed improvement in antioxidant enzyme status (ii) inhibited lipid peroxidation in brain homogenate and (iii) showed improvement in memory of Wistar rats when tested on Hebb's- William maze test. Thus, a combination of antioxidant and anti-



dementia effects exhibited by *C. nurvala* extract may be responsible for its neuroprotective potentiality.

Conflict of interest

We declare that we don't have any conflict of interest.

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Table 1. Effect of *C. nurvala* extract on transfer latency (in seconds) on Hebb's-William maze

Groups	Treatment	ITL on Day 7 (sec)	RTL on Day 8 (sec)
Group I: (Normal control)	Vehicle (0.6% w/v sodium CMC)	189.50 ± 1.40	174.56 ± 2.39
Group II: (Negative control)	Scopolamine (1mg/kg i.p)	317.83 ± 3.42 ***	302.32 ± 1.45 ***
Group III: (Positive control)	Piracetam (50 mg/kg) orally	184.28 ± 2.29 ###	174.28 ± 2.29 ###
Group IV:	<i>C. nurvala</i> ethanolic extract (100 mg/kg) orally	191.36 ± 2.40 ###	178.33 ± 2.35 ###
Group V:	<i>C. nurvala</i> ethanolic extract (200 mg/kg) orally	192.41 ± 1.16 ###	187.54 ± 5.12 ###
Group VI:	<i>C. nurvala</i> ethanolic extract (400 mg/kg) orally	187.2 ± 1.35 ###	182.19 ± 3.29 ###
Group VII	Piracetam (50 mg/kg) orally + Scopolamine (1mg/kg i.p.)	204.66 ± 5.48 ###	190.42 ± 4.65 ###
Group VIII	<i>C. nurvala</i> ethanolic extract (100 mg/kg) orally + Scopolamine (1mg/kg i.p.)	286.33 ± 5.10 ****	256.14 ± 3.23 ****
Group IX	<i>C. nurvala</i> ethanolic extract (200 mg/kg) orally + Scopolamine (1mg/kg i.p.)	259.33 ± 5.87 ****	240.62 ± 2.56 ****
Group X	<i>C. nurvala</i> ethanolic extract (400 mg/kg) orally + Scopolamine (1mg/kg i.p.)	220.66 ± 6.05 ****	198.32 ± 2.89 ****

Data were analyzed through one way ANOVA followed by Tukey's test. Values were expressed as (mean ± S.E.M.), n = 6 in each group. *** p<0.001, ** p<0.01 and * p< 0.05 when compared to normal control; ### p< 0.00, ## p< 0.01 and # p< 0.05 when compared to negative control.



Table 2. Effect of *C. nurvala* extract on lipid peroxidation and antioxidant enzymes

Groups	Treatment	MDA level μ Mol MDA/mg protein	SOD (unit/mg wet tissue)	Catalase (unit/mg wet tissue)	mMol GSH/mg wet tissue
Group I (Normal control)	Vehicle (0.6% w/v sodium CMC)	0.358 ± 0.026	1.70 ± 0.131	5.563 ± 0.172	0.655 ± 0.004
Group II (Negative control)	Scopolamine (1mg/kg i.p)	1.605 ± 0.206 ***	0.34 ± 0.046 ***	2.416 ± 0.252 ***	0.373 ± 0.031 ***
Group III (Positive control)	Piracetam (50 mg/kg) orally	0.287 ± 0.038 ###	1.71 ± 0.193 ###	5.438 ± 0.452 ###	0.623 ± 0.009 ###
Group IV	<i>C. nurvala</i> ethanolic extract (100 mg/kg) orally	0.358 ± 0.228 ###	1.68 ± 0.160 ###	5.316 ± 0.239 ###	0.631 ± 0.022 ###
Group V	<i>C. nurvala</i> ethanolic extract (200 mg/kg) orally	0.328 ± 0.044 ###	1.81 ± 0.186 ###	5.333 ± 0.215 ###	0.608 ± 0.017 ###
Group VI	<i>C. nurvala</i> ethanolic extract (400 mg/kg) orally	0.315 ± 0.031 ###	1.86 ± 0.160 ###	5.480 ± 0.251 ###	0.618 ± 0.024 ###
Group VII	Piracetam (50 mg/kg) orally + Scopolamine (1mg/kg i.p)	0.606 ± 0.031 ###	1.35 ± 0.045 ###	4.50 ± 0.220 ###	0.580 ± 0.028 ###
Group VIII	<i>C. nurvala</i> ethanolic extract (100 mg/kg) orally + Scopolamine (1mg/kg i.p)	1.025 ± 0.080 ***#	0.75 ± 0.042 ***#	3.55 ± 0.246 ***#	0.443 ± 0.024 ***#
Group IX	<i>C. nurvala</i> ethanolic extract (200 mg/kg) orally + Scopolamine (1mg/kg i.p)	0.82 ± 0.033 ***#	0.94 ± 0.077 ***#	3.951 ± 0.248 ***#	0.520 ± 0.021 ***#
Group X	<i>C. nurvala</i> ethanolic extract (400 mg/kg) orally + Scopolamine (1mg/kg i.p)	0.77 ± 0.022 ***#	1.10 ± 0.073 ***#	4.25 ± 0.16 ***#	0.541 ± 0.013 ***#

Data was analyzed by one-way ANOVA followed by Tukey's post-hoc test. Values were expressed as mean ± S.E.M., n = 6 in each group. *** p < 0.001, ** p < 0.01 and * p < 0.05 when compared to control; ### p < 0.001, ## p < 0.01 and # p < 0.05 when compared to negative control.



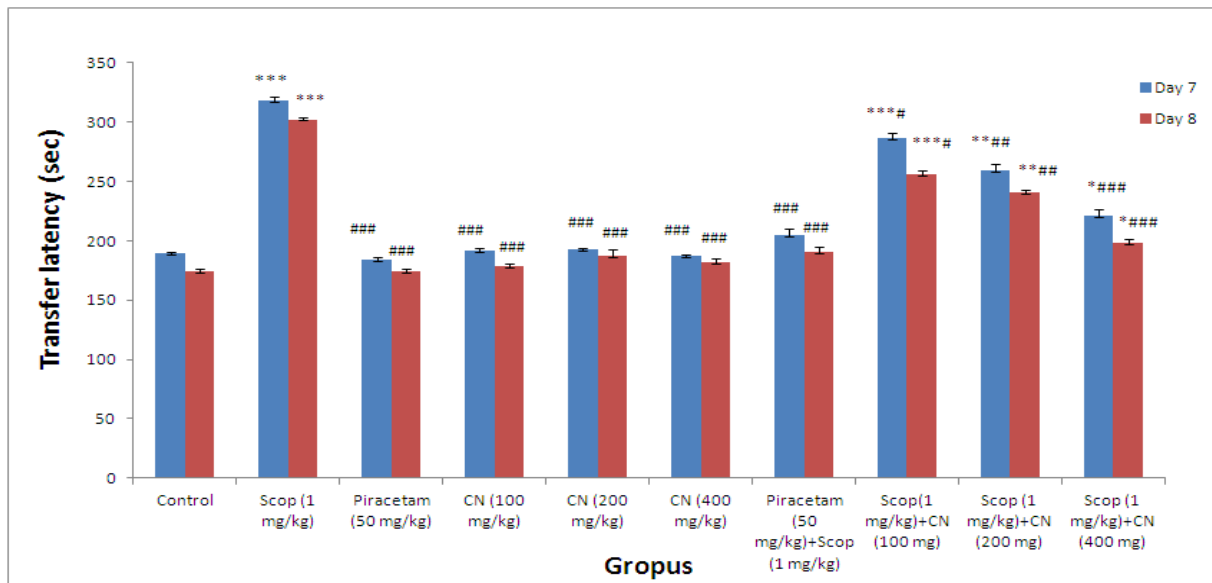


Figure 1. Effect of *C. nurvala* extract on transfer latency (in seconds) on Hebb's-William maze

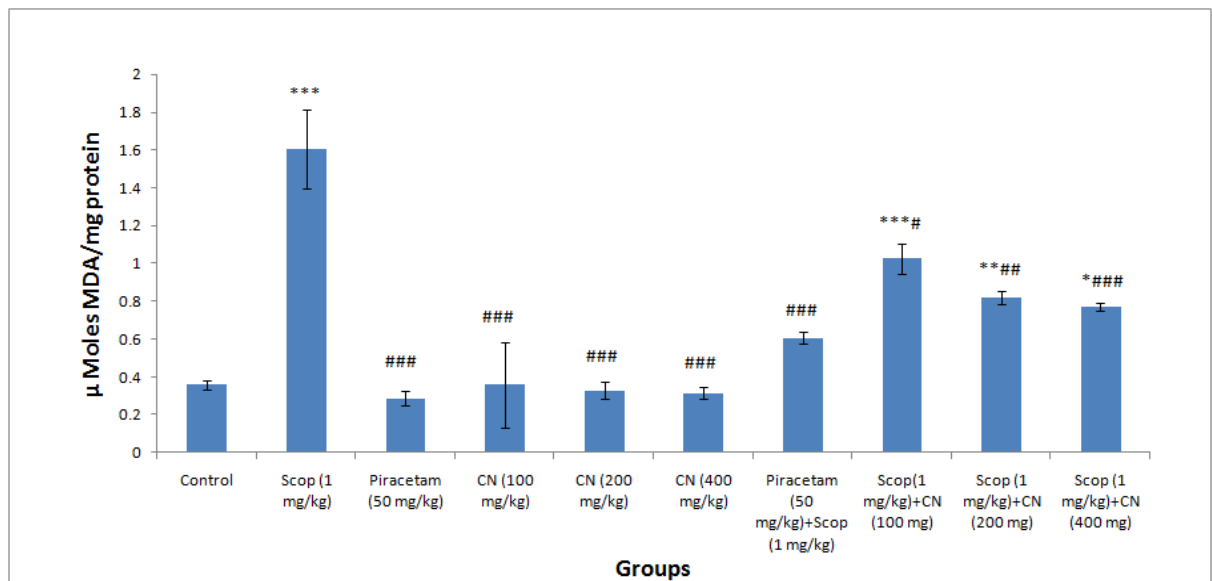


Figure 2. Effect of *C. nurvala* extract on lipid peroxidation



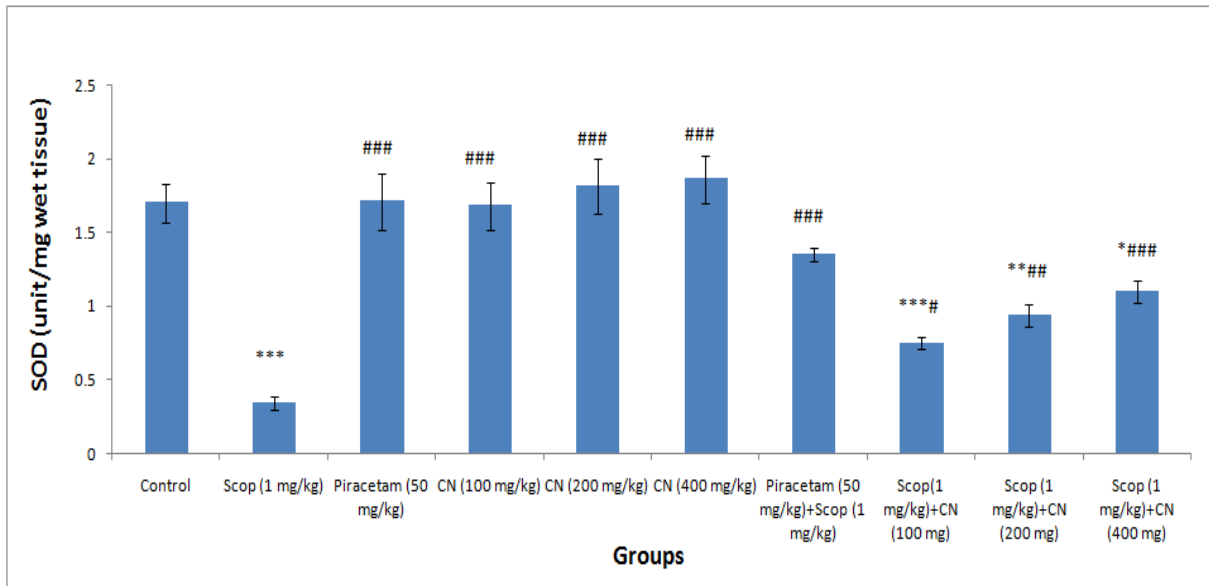


Figure 3. Effect of *C. nurvala* extract on SOD enzyme

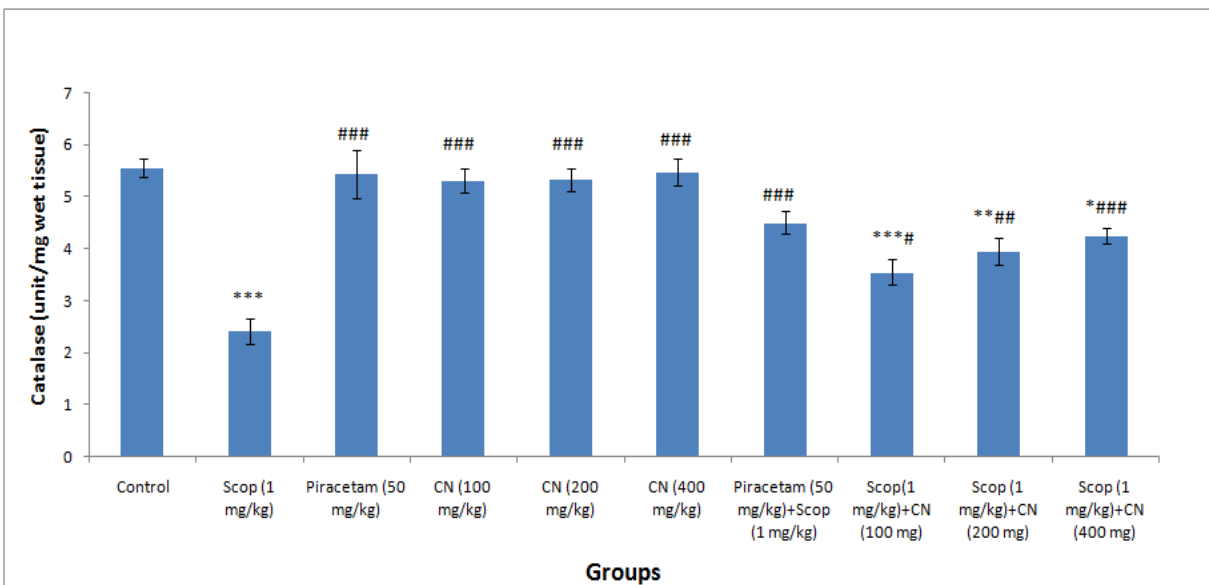


Figure 4. Effect of *C. nurvala* extract on catalase enzyme



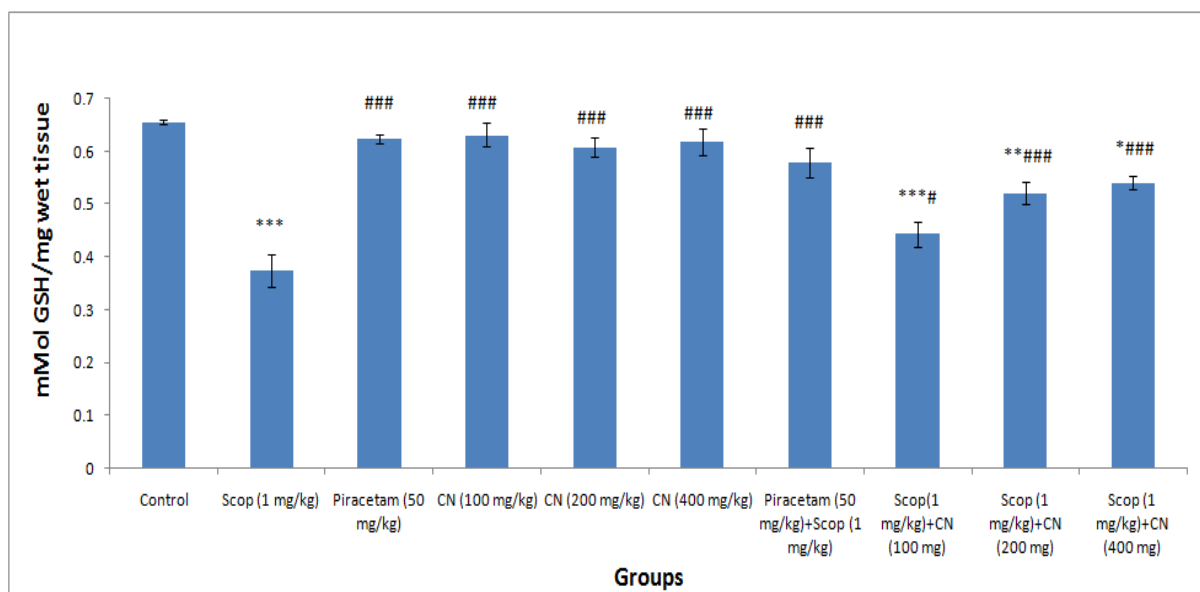


Figure 5. Effect of *C. nurvala* extract on GSH enzyme

