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Neuroprotective effects of *Indigofera tinctoria* on noise stress affected Wistar albino rat brain

Sakthivel Srinivasan, Wankupar Wankhar, Sheeladevi Rathinasamy, Ravindran Rajan

Department of Physiology, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Taramani campus, Chennai-600 113, India.

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ABSTRACT

The objective of the study is to evaluate the DNA damage, behavior and free radical scavenging enzymes level when exposed to noise. Noise stress was performed using broadband white noise generator after pre-treated oral administration of *Indigofera tinctoria* (300mg/Kg .b.w.). Significance increase in nitric oxide and lipid peroxidation level in stressed rat shows possibility of neurodegeneration and this is justified by genomic DNA damage in brain discrete region. Increase in enzymatic and decrease in non-enzymatic level suggest that oxidative imbalance persist in animal when expose to noise in both brain and adrenal. Anxiety and altered motor coordination was also observed in our studies, this finding could be attributed to the detrimental effects of noise not only at the biochemical level but also the molecular and psychological behavior of the rat. However, oral administration of *I.tinctoria* significantly prevented noise induced oxidative damages. These results conclude that *I.tinctoria* may possess neuroprotective effects and the antioxidant property of the plant may have resulted in its therapeutic efficacy.

INTRODUCTION

Stress is an integral part of human life, living beings when continuously exposed to stressful stimuli has been known to affect several physiological and psychological processes. Noise is a stressful stimulus if exceeded 90 dBA becomes a source of stressor (Ramsey, 1982). Stress can stimulate the Hypothalamo-Pituitary-Adrenal (HPA) and increase the release of Corticotrophin Releasing Hormone (CRH) from the hypothalamic paraventricular nucleus, causing the secretion of adrenocorticotropin (ACTH) from anterior pituitary, which in turn stimulates the secretion of glucocorticoids from the adrenal cortex axis (Kvetnansky *et al*., 2002). Elevation in the corticosterone level accelerates the generation of free radical (McIntosh and Sapolsky, 1996). Generation of free radicals is a fundamental feature of normal cellular functions. However, excessive generation and inadequate removal of free radical results in destructive and irreversible damage to the cells (Lopaczyski and Zeisel, 2001). Over production of free radicals can cause oxidative damage to biomolecules (lipids, proteins, DNA), eventually leading to many chronic diseases such as cardiovascular diseases, chronic inflammation, stroke, septic shock, aging and other degenerative diseases in humans (Yun-Zhong *et al*., 2002). Various stresses have been associated with enhanced free radical generation causing oxidative damage. Oxidative stress arises from the imbalance between pro-oxidants and antioxidants leading to oxidative damage. Neural tissue is especially sensitive to oxidative stress because of the fact that brain cells are the almost vulnerable to free radical damage caused by lipid peroxidation compare with other tissues, owing to their highest percentage of unsaturated fats (Antonio, 2001). Antioxidants are classified as exogenous (natural or synthetic) or endogenous compounds, responsible for scavenging free radicals or inhibiting the binding of metal ions needed for catalysis of ROS generation (Gilgun-Sherki *et al*., 2001). *Indigofera tinctoria* (*I.tinctoria*) (Fabaceae) has been a part of Indian and Chinese medicinal systems since ancient times. The plant has been used for treatment of nervous disorders, liver ailments, epilepsy, cancer, inflammation, bronchitis and as ointment for sores, old ulcers and haemorrhoids (Renukadevi *et al*., 2011). But the effectiveness of aqueous extract of *I.tinctoria* in prevention of noise-stress induced animal models has not yet been studied.

** Corresponding Author*
Ravindran Rajan, Assistant Professor, Department of Physiology, Dr ALM PG IBMS, University of Madras, Taramani Campus, Chennai- 600113, India. Email: drravindranrajan [at] gmail.com

Hence, this present study was undertaken to evaluate the efficacy of *I.tinctoria* extract against the physiological and psychological changes in the rats when exposed to noise.

MATERIALS AND METHOD

Extraction of plant extract

The plant *I. tinctoria* was collected (May to November 2013) from the KSG Enterprises **(**Tindivanam, Tamil Nadu, India) and authenticated by Dr. D. Aravind (Department of Medical Botany, and National Institute of Siddha, Chennai, India). Voucher specimens were deposited at the Herbarium of National institute of Siddha, Reg no: NIS/MB/83/2013. The collected plants were separated from unwanted materials and dried in shade. The leaves were ground to coarse powder with the help of a suitable grinder. The powder was then stored in an airtight container, kept in a cool, dark and dry place until the analysis.

Experimental design

Wistar strain male albino rats weighing 180–220 g were randomly selected. The animals were maintained under standard laboratory condition and fed ad libitum with food (M/S Hindustan Lever Limited, Bombay, India) and water. All the rats were housed under condition of controlled temperature (26 ± 2°C) with 12 h light and 12 h dark exposure. The animals were divided into four groups with six animals in each group. Group I served as the control, Group II animals were subjected to noise-stress for 4 h daily for 15 days (Sub-acute exposure), group III were treated with *Indigofera tinctoria* (IT) for 48 days and experiments were carried out on 49th day and Group IV consisted of noise with IT-treated animals. These animals were pre-treated with IT for 33 days and then exposed to noise stress for 15 days. During the noise stress period, they were also given IT extract by the oral route and all the experiments were done on the 49th day. Ethical clearance was obtained before the commencement of experiments from the ethical committee (IAEC No: 22/02/2013) and the Committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Noise stress induction

Broadband white noise at 100-dBA intensity was produced by a white noise generator, amplified by an amplifier connected to a loud speaker fixed 30 cm above the animal cage. A sound level meter was used to measure the intensity of the noise (Samson *et al*., 2005).

Sample Collection

Isolation of brain region was performed between 8 to 10 a.m. to avoid circadian rhythm induced changes. The animals were sacrificed under deep anesthesia using Pentothal sodium (40mg/kg b.w). The discrete regions of brain (cerebral cortex, cerebellum, striatum, brain stem, hippocampus and hypothalamus) were dissected according the method given by Glowinski and Iverson, (1996). The brain regions was excised, washed in ice cold saline and blotted to dryness. Quickly weighed and the brain sample were homogenized by using Teflon glass homogenizers. 10% homogenate of this tissue was prepared in phosphate buffer (0.1 M, pH 7.0) and centrifuged at 3000 g at 4°c for 15 min to remove cell debris and the clear supernatant was used for further biochemical assays.

Biochemical determinations

Protein was estimated as per the method described by Lowry *et al*. (1951). Lipid peroxidation was determined in the tissue samples as described by Ohkawa *et al*. (1979). Nitric oxide (NO) levels were measured as total nitrite + nitrate levels with the use of the Griess reagent by the method of Moshage, (1995). Protein thiol by Sedlack and Lindsay, (1968) was determined. Superoxide dismutase (SOD) according to Marklund and Marklund, (1974) and catalase (CAT) according to the method of Sinha, (1972). The activity of glutathione peroxidase (GPx) was estimated by the methods of Rotruck *et al*. (1973). Reduced glutathione (GSH) in the tissue samples was estimated by the method of Moron *et al*. (1979). The vitamin-C (ascorbic acid) content in the tissue was determined according to the method of Omaye *et al*. (1979).

DNA fragmentation assay

Separation of DNA molecules from the extracted samples was performed by (Borriello *et al*., 2002).

Behavioral analysis

Motor co-ordination was assessed using the conventional Rota rod test (Dunham *et al*., 1957) and narrow beam (Kolb and Whishaw, 1983). Elevated plus maze was used to evaluate the anxiety status in animals (Pellow and File, 1986).

Statistical analysis

The results are presented with mean±S.D. The data statistically evaluated using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests in SPSS-20.

RESULT AND DISCUSSION

Noise is defined as disturbing, unwanted sound and its damaging effects particularly the productions of free radicals are not limited to the auditory organ (Reha *et al*., 2009). Oxygen free radicals can attack protein, nucleic acids and lipid membranes thereby disrupting normal cellular functions and integrity (Manikandan *et al*., 2005). In this respect, it is worthy to note that a brain antioxidant level is a main target of free radicals toxicity due to its high oxygen consumption.

Free radicals

Free Radicals are unpaired electron molecules and these molecules are highly reactive. Free radicals attack three main cellular components (Lipids, proteins and DNA). Nitric oxide is a

highly reactive signal molecule in the CNS. It is a unique messenger molecule that serves diverse physiological functions throughout the body. The NO levels in various groups are presented as bar diagram with mean ± S.D (Fig 1). Control groups when compared with *I.tinctoria* unaccompanied groups there is no changes observed. In sub-acute noise stress the NO levels are significantly ($P < 0.001$) increase in discrete region of brain and adrenal. Elevated levels of NO have been found in increased nitrosative stress, leading to cell degeneration and necrosis. Predominantly the hypothalamus and adrenal showed continue increased nitric oxide level indicating that these areas are affected the most during sub-acute noise exposure. This justify that noise stress generate nitric oxide level in the brain and adrenal. This is agreement with Lidija *et al*. (2007) who reported that, the increase of NO production in distinct brain regions functionally connected via afferents and efferent suggests that these regions are affected by the injury. However, the plant *I. tinctoria* treated noise exposed group shows significant decrease in nitric oxide level by down-regulating the activity. This because of plants contain several phytochemicals which possess strong antioxidant activities (Senthilkumar and Venkatesalu, 2009). The LPO data from various groups are presented as bar diagram with mean ±S.D (Fig 2). LPO is a free-radical-mediated process. In sub-acute noise stress the LPO level were significantly ($P < 0.001$) increased in rat brain and adrenal region, which also supports the generation of free radicals. This increased ROS leads to excessive membrane damage in brain region and the generation of reactive free radicals overcomes the antioxidant defense (Ashok and Sheeladevi, 2012). The *I. tinctoria* with noise group LPO level were significantly decreased in cortex ($P < 0.05$), cerebellum ($P < 0.01$), and hypothalamus ($P < 0.01$) when compare with control and sub-acute noise exposure LPO level were decreased in cortex ($P < 0.01$). Protein thiol level are presented as bar diagram with mean ±S.D (Fig 3). The activities of protein thiol are significantly ($P < 0.001$) decreased in 15 days noise-stress group compare with control. This may have occurred due to oxidation of one of the major thiol substance. The present study also shows that, sub-acute noise stress lead to a significant elevation in free radical scavenging enzymes SOD, GST, CAT, GPx and decrease in GR levels hen compared with control, in brain and adrenal. SOD, CAT, and GPx are among the key enzymes that defend the contents of cells against presented as bar diagram with mean ±S.D (Table1). In sub-acute noise stress SOD level in hippocampus, hypothalamus and adrenal continually increased, this representing that these areas are affected. Vidyasagar *et al*. 2004 who reported that, increased SOD levels were only partially effective in combating the oxidative damage. Since the increase in SOD activity may have caused by excess superoxide radical production, elevated lipid peroxidation in brain region is justified. In this respect, it is worthy to note that a brain antioxidant level is a main target of ROS toxicity due to its high oxygen consumption. This explains the reason for the increased production of free radicals in noise stress observed in this study. CAT and GPx from various groups are presented as bar diagram with mean ±S.D (Table 2 & 3). The CAT and GPx levels are significantly ($P < 0.001$) increase in 15 days noise exposure in all the regions of the brain and adrenal when compared with control. Increased SOD activity could naturally accumulate the super oxides and H_2O_2 which justify the increase in CAT and GPx for their increased activity after noise exposure. Catalase has been shown to be responsible for the detoxification of significant amounts of H_2O_2. Glutathione peroxidase metabolizes peroxides such as H_2O_2 and protects cell membranes from lipid peroxidation.

GST levels from various groups are presented in Table 4 with mean ±SD. In sub-acute noise stress the GST levels were significantly ($P < 0.001$) increased in both brain and adrenal. In noise stress exposure the adrenal to shows an increase GST activity indicating that these areas are affected. Glutathione reductase (Table 5) are significantly ($P < 0.001$) decreased in both brain discrete regions and adrenal when exposed to noise stress. This is clearly justified that noise cause GST and GR levels in brain. The decrease in GST and GR activity could lead to imbalance in redox status due to decrements in the antioxidant activity ad this could be attributed to the decrease in major substrate such as GSH levels (Fig 4) in brain and adrenal. There is a chance between alterations in GSH with neurodegenerative disorders such as Alzheimer's disease, amyotrophic lateral sclerosis, schizophrenia, and Parkinson's disease are reported (Bharath *et al*., 2002). Loss of glutathione and consequent oxidative damage have been suggested to be early signaling events in apoptotic cell death. The decreased GSH levels is presumably due to enhanced utilization of reduced glutathione by glutathione peroxidase in detoxification of H_2O_2 generated by noise induced oxidative stress. Noise stress induced elevated free radical scavenging enzymes are significantly reduced in pre-treated *I. tinctoria* with noise stressed animals. The plant *I. tinctoria* has been reported that, contain potential of antioxidant and free radical scavenging activity (Nagarajan and Sellamuthu, 2013). This is explain that plant may enhance the free radical scavenge enzyme to scavenging the free radicals. The activity of Vit-C (Fig 5) were significantly ($P < 0.001$) decreased in 15 days noise-stress group. Vitamins C and E have been found to be protective against noise-induced cochlear damage (Seidman, 2000). In our study the sub-acute stress decreased the Vit C level. Vitamin C is a hydrophilic reducing agent which directly reacts with superoxides, hydroxyls and various lipid hydro peroxides more effectively than any other water soluble antioxidant. *I.tinctoria* treated with noise group displays significant increase in Vit-C levels compare with the stress group particularly in cerebellum, striatum, hippocampus and striatum, brainstem when compare with sub-acute noise group. This justify that plant contain natural antioxidant. This in agreement with Nagarajan *et al.* (2013) who reported that, the plant *I.tinctoria* contain potential of antioxidant and free radical scavenging activity. This results justify that, noise stress-induced alteration in the biochemical abnormalities was prevented in *I.tinctoria* supplemented group. Therefore the present study suggests that *I.tinctoria* supplementation may utilise antioxidant effect and can be regarded as a protective drug against stress.

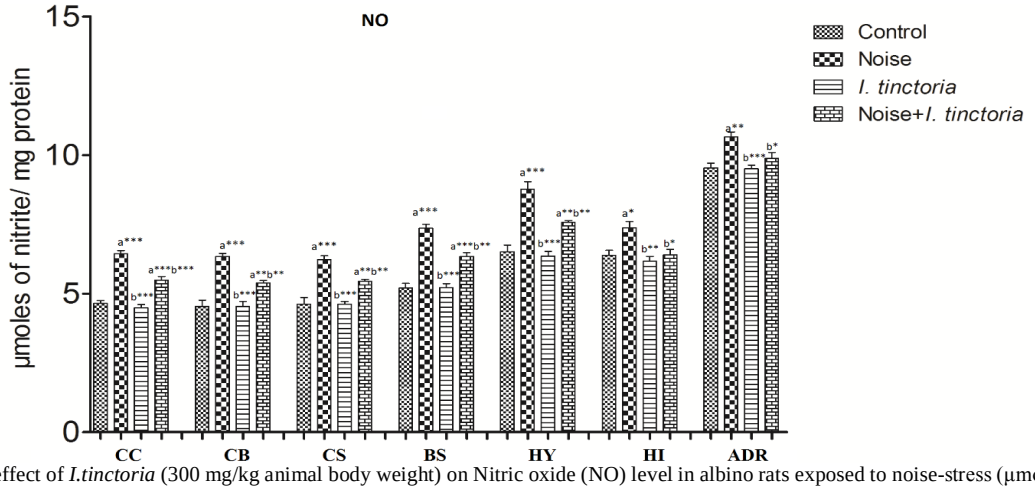

Fig. 1: Protective effect of *I.tinctoria* (300 mg/kg animal body weight) on Nitric oxide (NO) level in albino rats exposed to noise-stress (µmoles of nitrite/ mg protein). Values are expressed as mean±S.D of six animals. Significance at *p < 0.05; Significance at **p < 0.01; and c- Significance at ***p < 0.001. (CC-Cortex, CB-Cerebellum, CS-Striatum, BS- Brain stem, HY- Hypothalamus, HI- Hippocampus and ADR- Adrenal)

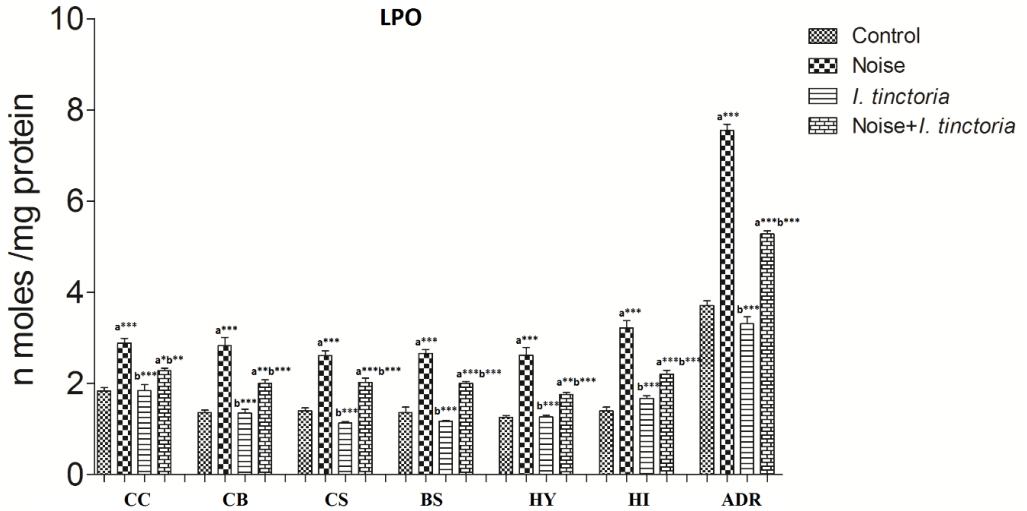

Fig. 2: Protective effect of *I.tinctoria* (300 mg/kg animal body weight) on Lipid Peroxidation (LPO) level in albino rats exposed to noise-stress (n moles /mg protein). Values are expressed as mean±S.D of six animals. Significance at *p < 0.05; Significance at **p < 0.01; and c- Significance at ***p < 0.001. (CC-Cortex, CB-Cerebellum, CS-Striatum, BS- Brain stem, HY- Hypothalamus, HI- Hippocampus and ADR- Adrenal)

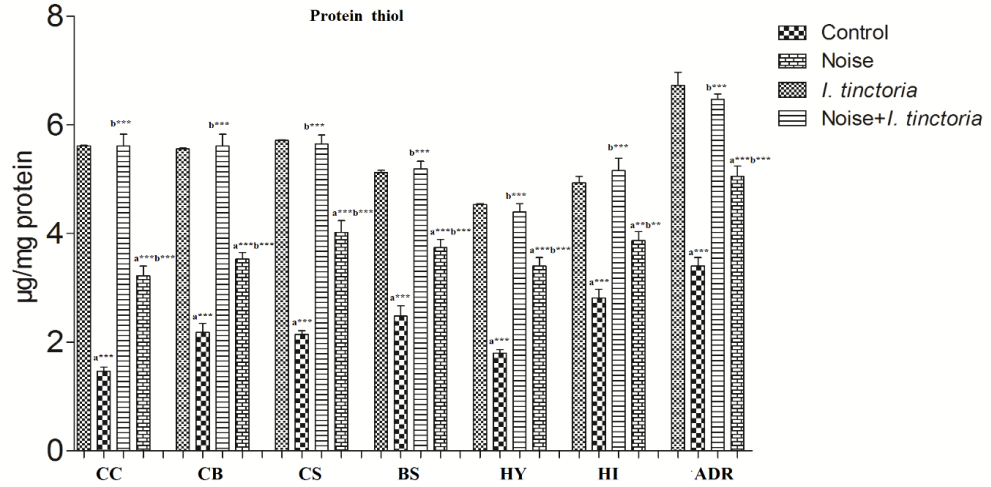

Fig. 3: Protective effect of I.tinctoria (300 mg/kg animal body weight) on Protein thiol level in albino rats exposed to noise-stress (µg/mg protein). Values are expressed as mean±S.D of six animals. Significance at *p < 0.05; Significance at **p < 0.01; and c- Significance at ***p < 0.001. (CC-Cortex, CB-Cerebellum, CS-Striatum, BS- Brain stem, HY- Hypothalamus, HI- Hippocampus and ADR- Adrenal)

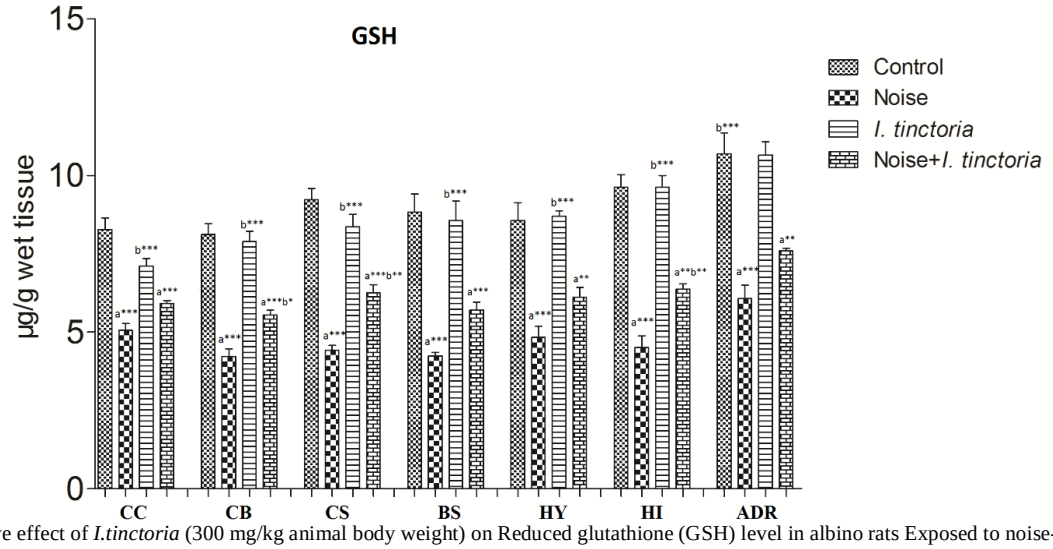

Fig. 4: Protective effect of *I.tinctoria* (300 mg/kg animal body weight) on Reduced glutathione (GSH) level in albino rats Exposed to noise-stress (µg/g wet tissue). Values are expressed as mean±S.D of six animals. Significance at *p < 0.05; Significance at **p < 0.01; and c- Significance at ***p < 0.001. (CC-Cortex, CB-Cerebellum, CS-Striatum, BS- Brain stem, HY- Hypothalamus, HI- Hippocampus and ADR- Adrenal)

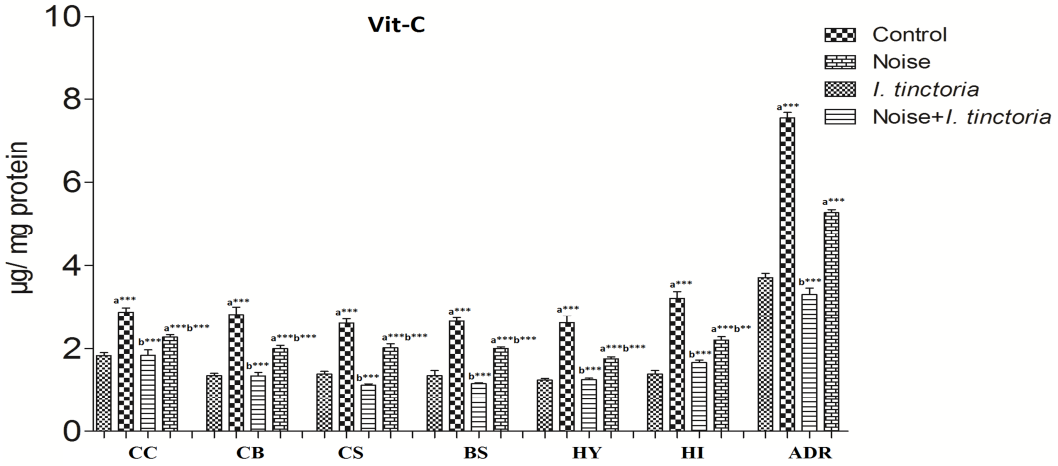

Fig. 5: Protective effect of *I.tinctoria* (300 mg/kg animal body weight) on Vitamin C (Vit-C) level in albino rats exposed to noise-stress (µg/ mg protein). Values are expressed as mean±S.D of six animals. Significance at *p < 0.05; Significance at **p < 0.01; and c- Significance at ***p < 0.001. (CC-Cortex, CB-Cerebellum, CS-Striatum, BS- Brain stem, HY- Hypothalamus, HI- Hippocampus and ADR- Adrenal)

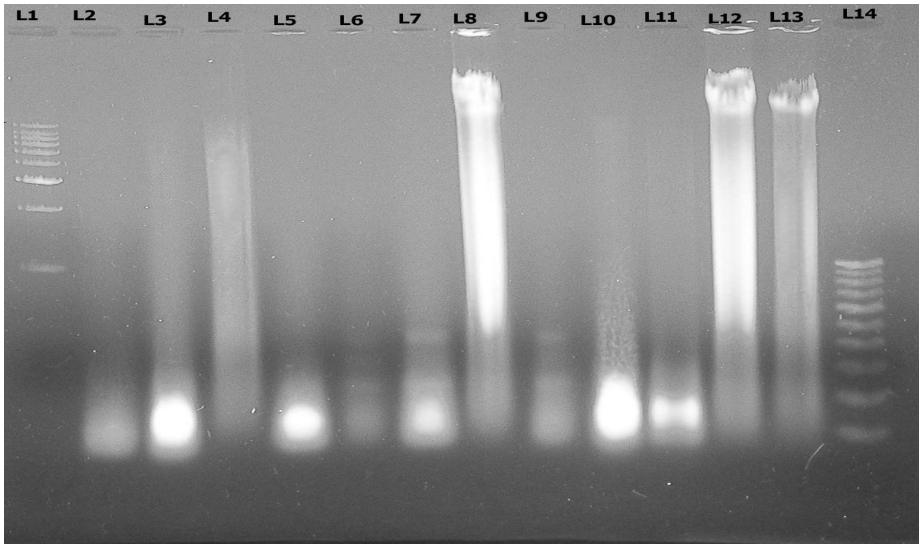

Fig. 6: Protective effect of *I.tinctoria* (300 mg/kg animal body weight) on noise induced DNA fragmentation (L1-1KB Ladder, L2-Control CC, L3-*I.tinctoria* CC, L4- Noise CC, L5-Noise with *I.tinctoria* CC, L6- Control CB, L7-*I.tinctoria* CB, L8- Noise CB, L9- Noise with *I.tinctoria* CB, L10-Control HI, L11-*I.tinctoria* HI, L12- Noise HI, L13-Noise with *I.tinctoria* HI, L14- 100bp Ladder) (CC- Cerebral Cortex, CB-Cerebellum, and HI- Hippocampus)

Table 1: Protective effect of *I.tinctoria* (300 mg/kg animal body weight) on SOD level in albino rats exposed to noise-stress (units/mg protein).

Organ	Group I	Group II	Group III	Group IV
Cortex	0.601±0.019	1.037±0. 090 ^{a***}	0.600±0.037 ^{b***}	0.820±0.09 $^{a*** b***}$
Cerebellum	0.587±0.033	0.805±0.062 ^{a***}	0.580±0.027 ^{b***}	0.679±0.101 $^{a* b*}$
Striatum	0.661±0.052	0.791±0.097 ^{a*}	0.634±0.082 ^{b*}	0.631±0.078 ^{b*}
Brain stem	0.555±0.067	1.365±0.335 ^{a***}	0.562±0.048 ^{b***}	1.097±0.143 $^{a*** b*}$
Hypothalamus	1.871±0.155	4.274±0.86 ^{a***}	1.819±0.082 ^{b***}	2.728±0.431 $^{a* b***}$
Hippocampus	1.294±0.068	3.535±0.664 ^{a***}	1.207±0.078 ^{b***}	2.284±0.258 $^{a** b***}$
Adrenal	1.803±0.289	2.983±0.296 ^{a***}	1.888±0.213 ^{b***}	2.296±0.068 $^{a** b***}$

Data are represented as mean ± S.D. (n=6).a- compared with control; b- compared with noise; Significance at $*p < 0.05$; Significance at $**p < 0.01$; and c-Significance at $***p < 0.001$.

Table 2: Protective effect of *I.tinctoria* (300 mg/kg animal body weight) on catalase level in albino rats exposed to noise-stress (µ moles of H_2O_2 consumed / mg protein).

Organ	Group I	Group II	Group III	Group IV
Cortex	4.810±0.108	9.845±1.142 ^{a***}	4.817±0.307 ^{b***}	6.634±0.846 $^{a** b***}$
Cerebellum	4.876±0.205	10.726±1.041 ^{a***}	4.812±0.233 ^{b***}	6.174±0.618 $^{a** b***}$
Striatum	4.835±0.239	9.869±1.118 ^{a***}	4.647±0.593 ^{b***}	7.234±0.506 ^{a***} ^{a***}
Brainstem	4.979±0.634	2.783±2.987 ^{a***}	4.909±0.488 ^{b***}	8.233±0.857 $^{a* b***}$
Hypothalamus	5.334±0.441	11.743±2.285 ^{a***}	5.457±0.250 ^{b***}	7.689±1.186 $^{a* b***}$
Hippocampus	5.637±0.401	13.366±2.437 ^{a***}	5.436±0.397 ^{b***}	8.201±0.99 $^{a* b***}$
Adrenal	6.557±1.027	16.793±1.857 ^{a***}	6.611±0.750 ^{b***}	11.714±1.850 $^{a*** b***}$

Data are represented as mean ± S.D. (n=6). a- compared with control; b- compared with noise; Significance at $*p < 0.05$; Significance at $**p < 0.01$; and c-Significance at $***p < 0.001$.

Table 3: Protective effect of *I.tinctoria* (300 mg/kg animal body weight) on Glutathione Peroxidase (GPX) level in albino rats exposed to noise-stress (µg of GSH consumed / mg protein).

Organ	Group I	Group II	Group III	Group IV
Cortex	4.928±0.295	9.617±0.893 ^{a***}	4.661±0.264 ^{b***}	6.983±0.394 $^{a*** b***}$
Cerebellum	4.127±0.164	9.207±1.037 ^{a***}	4.172±0.265 ^{b***}	6.742±0.82 $^{a*** b***}$
Striatum	4.825±0.357	9.178±1.160 ^{a***}	4.482±0.731 ^{b***}	6.212±0.537 $^{a* b***}$
Brainstem	4.559±0.605	9.750±1.682 ^{a***}	4.366±0.340 ^{b***}	6.328±0.627 $^{a* b***}$
Hypothalamus	3.977±0.377	7.705±1.438 ^{a***}	3.895±0.318 ^{b***}	5.296±0.659 $^{a* b***}$
Hippocampus	4.219±0.193	9.474±1.669 ^{a***}	4.236±0.420 ^{b***}	6.259±0.909 $^{a** b***}$
Adrenal	9.164±1.409	15.773±1.954 ^{a***}	9.092±0.896 ^{b***}	12.277±0.93 $^{a** b**}$

Data are represented as mean ± S.D. (n=6). a- compared with control; b- compared with noise; Significance at $*p < 0.05$; Significance at $**p < 0.01$; and c-Significance at $***p < 0.001$.

Table. 4: Protective effect of *I.tinctoria* (300 mg/kg animal body weight) on GST level in albino rats exposed to noise-stress (CDNB conjugate formed / min / mg protein).

Organ	Group I	Group II	Group III	Group IV
Cortex	1.433±0.294	2.791±0.153 ^{a***}	1.199±0.170 ^{b***}	2.123±0.127 $^{a*** b***}$
Cerebellum	1.364±0.235	2.658±0.221 ^{a***}	1.338±0.291 ^{b***}	2.221±0.138 $^{a*** b*}$
Striatum	1.289±0.152	2.279±0.273 ^{a***}	1.196±0.151 ^{b***}	1.813±0.182 $^{a** b**}$
Brainstem	1.377±0.220	3.303±0.228 ^{a***}	1.351±0.129 ^{b***}	2.274±0.203 $^{a*** b***}$
Hypothalamus	1.524±0.313	3.464±0.348 ^{a***}	1.669±0.153 ^{b***}	2.578±0.256 $^{a*** b***}$
Hippocampus	1.553±0.255	3.383±0.164 ^{a***}	1.784±0.245 ^{b***}	2.523±0.223 $^{a*** b***}$
Adrenal	2.056±0.571	4.937±0.663 ^{a***}	1.929±0.482 ^{b***}	2.966±0.334 $^{a* b***}$

Data are represented as mean ± S.D. (n=6). a- compared with control; b- compared with noise; Significance at $*p < 0.05$; Significance at $**p < 0.01$; and c-Significance at $***p < 0.001$.

Table 5: Protective effect of *I.tinctoria* (300 mg/kg animal body weight) on GR level in albino rats exposed to noise-stress (nM of NADPH oxidized / min / mg protein).

Organ	Group I	Group II	Group III	Group IV
Cortex	0.077±0.003	0.045±0.006 ^{a***}	0.072±0.003 ^{b***}	0.058±0.004 $^{a*** b**}$
Cerebellum	0.059±0.003	0.037±0.0009 ^{a***}	0.060±0.001 ^{b***}	0.045±0.003 $^{a*** b***}$
Striatum	0.061±0.005	0.043±0.001 ^{a***}	0.061±0.003 ^{b***}	0.049±0.003 ^{a***b*}
Brainstem	0.059±0.005	0.038±0.005 ^{a***}	0.061±0.003 ^{b***}	0.046±0.004 ^{a**b*}
Hypothalamus	0.064±0.003	0.032±0.006 ^{a***}	0.073±0.005 ^{b***}	0.045±0.005 $^{a*** b**}$
Hippocampus	0.065±0.006	0.027±0.005 ^{a***}	0.065±0.004 ^{b***}	0.042±0.005 $^{a*** b**}$
Adrenal	0.085±0.008	0.053±0.002 ^{a***}	0.083±0.001 ^{b***}	0.074±0.002 $^{a** b***}$

Data are represented as mean ± S.D. (n=6). a -compared with control; b- compared with noise; Significance at $*p < 0.05$; Significance at $**p < 0.01$; and Significance at $***p < 0.001$.

Table 6: Protective effect of *I. tinctoria* (300 mg/kg animal body weight) on motor behavior in Albino Rats Exposed to noise-stress.

Parameters	Group I	Group II	Group III	Group IV
Rota Rod (time spent in rota rod)	189.1±19.2	83.5±11.8^{a***}	206.333±20.9^{b***}	157±15.5$^{a* b***}$
Narrow beam(time taken to cross in seconds)	5.166± 1.1	8.33± 1.5^{a**}	4.83± 0.7^{b***}	6± 1.4^{b*}
Narrow beam (number of slips)	1± 0.6	3± 0.8^{a**}	0.833± 0.7^{b**}	2.33± 1.0^{a*}

Data are represented as mean ± S.D. (n=6).a -compared with control; b- compared with noise; Significance at $*p < 0.05$; Significance at $**p < 0.01$; and Significance at $***p < 0.001$.

Table 7: Protective effect of *I.ntigofera* (300 mg/kg animal body weight) on Elevated plus maze (anxiety) in albino rats exposed to noise-stress

Parameters	Group I	Group II	Group III	Group IV
No of open arm entry	8.5± 1.0	2± 1.6 ^{a***}	7.16± 2.6 ^{b***}	3.33± 0.8 ^{a***}
Time spent in open arm entry	274.8± 14.5	107± 19.0 ^{a***}	233.8± 50.0 ^{b***}	153± 32.8^{a***b*}
No of closed arm entry	2.66± 0.8	5.66± 1.0 ^{a***}	3± 1.0 ^{b**}	4.83±0.9 ^{a**}
Time spent in closed arm entry	130.6± 19.8	248.8± 28.5^{a***}	132.6± 26.8^{b***}	192± 26.5^{a**} ^{b**}

Data are represented as mean ± S.D. (n=6). a -compared with control; b- compared with noise; Significance at *p < 0.05; Significance at **p < 0.01; and Significance at ***p < 0.001.

DNA fragmentation

Electrophorosis of DNA isolated from cortex, cerebellum, hippocampus regions and its shows smear pattern (Fig 6). This result indicated that noise had the potency to induce DNA damage in the brain region. This result justify by Luann *et al*. (2002) who reported that, oxidative DNA damage is associated with intense noise exposure in the rat. One of the possibility is that DNA can be an early target of damage in mammals, such that strand breakage occurs before detectable lipid peroxidation or protein damage (Schraufstatter *et al*., 1986). The damage may result from direct ROS activity or may be an indirect result of ROS lipid peroxidation. *I. tinctoria* with noise exposed animal brain regions are not showing any fragmentation and this result further concluded that *I. tinctoria* clearly reduced the DNA damage.

Locomotor and anxiety level

The effect on motor coordination are assessed using a rota-rod apparatus. The results are summarized in Table 6. In the present study noise stress significantly (P < 0.001) decreased the motor coordination. Plant extract showed significant decrease in the motor coordination score and fall of time of the rat from the rotating rod justify that plant restored the motor coordination activity. In the present study, the rats were tested for the balance and motor coordination on the narrow beam (Table 6). The results of narrow beam showed compromised motor function in sub-acute noise exposed rats. The time taken to cross and number of slips the beam are significantly (P < 0.01) increase in sub-acute exposed rats when compare with control group. The noise-stress induced change in the motor coordination are significantly decrease number of slips and time taken to cross enhanced in noise-stress with *I. tinctoria* group. The same result suggested that Satyndra *et al*. (2013) the treatment of intoxicated mice with plant seed extract improves motor behavior, due to reduction in oxidative stress in striatum of the brain.

Elevated plus maze is used to evaluate psychomotor performance and emotional aspects of rats. Anxiety disorders are due to involvement of GABAergic, serotonergic, involvement. Values are the number of entries into open and closed arms in 5min (Mean ± S.D) (Table 7). The adrenergic and dopamanergic system have also been shown to play a role in anxiety (Kishore *et al*., 2012). In sub-acute noise stress, significantly (P < 0.001) decrease in the open arm entry and time spend in open arm. Results shows that on sub-acute exposure of noise cause anxiety in rats. Results shows that plant extracts with noise stressed rats exhibited significant increase in the number of open arm entries. The number of arm entries, but decreases in time spent in closed arm reflects plants revealed anxiolytic activity. Earlier reports on the chemical constituents of plants and their pharmacology suggest that plants containing flavonoids, alkaloids, phenolic acids, essential oil, saponins and tannins possess activity against many locomotor, anxiety disorders (Bhatacharya and Satyan, 1997). Renukadevi *et al*. 2011 who reported that *I. tinctoria* contain flavonoids and phenolics abundantly. The results of behavioral tests showed that impaired motor function and anxiety in noise exposed group. Interestingly, *I. tinctoria* with noise exposed rats shows significant improvement in all the behavior test. This justify *I. tinctoria* is potential to renovate locomotory and anxiety level.

CONCLUSION

The results indicate that oxidative damage occurred in the rat brain and adrenal when exposed to noise-stress. The aqueous extract *I. tinctoria* treatment restored the activities and levels of antioxidant machinery in the rat brain upon noise-stress. Interestingly, *I. tinctoria* treated group prevented the cortical neuronal and adrenal alteration which is observed in noise-stress exposed rat. This effect might be due to the antioxidant action of the *I. tinctoria.*

So the present study proposes that *I. tinctoria* supplementation may utilize antioxidant effect and can be regarded as a neuroprotective drug against stress.

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CONFLICT OF INTERESTS

No conflict of interests to declare.

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