INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD ISSN: 2455-0620 Volume - 6, Issue - 11, Nov – 2020
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value: 86.87 Impact Factor: 6.719
Received Date: 13/11/2020 Acceptance Date: 28/11/2020 Publication Date: 30/11/2020

Amelioration of Endosulfan Induced Neurotoxicity in the Cerebrum of Swiss Mice by Resveratrol, Lipoic Acid and Vitamin E

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Abstract: *Endosulfan, an organochlorine pesticide had been in global use as a part of pest management strategy. In the present study, a comprehensive effort has been made to elucidate the protective effects of certain antioxidants viz., trans- resveratrol, alpha- lipoic acid and vitamin E against endosulfan induced neurotoxicity in different regions of the brain of Swiss albino mice on the basis of altered histochemical localization of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities. It was observed that exposure of endosulfan at a dose of 2.45 mg/kg body weight/day for 15 days altered the distribution pattern of these neurotransmitter enzymes distinctively delineating its toxic effects on the mice brain. The antioxidant treated groups were administered resveratrol 5 mg/kg body weight/day, alpha-lipoic acid 20 mg/kg body weight/day and vitamin E 50 mg/kg body weight/day, for 15 days. In other antioxidant plus endosulfan treated groups all the doses of antioxidants were administered along with endosulfan one hour prior to endosulfan administration for 15 days. It was observed that endosulfan induced AChE, BChE inhibition in different nuclei and fiber tracts of various brain regions involved in learning, intelligence, thought processing and perception, interpretation of sensory impulses, motor function, planning, organizational abilities, tactile responses, memory, emotion, language comprehension, perception of pain, muscle stretch, co ordination, eye movements and regulation of other normal body functions etc. Hence, the study clearly delineates toxic effects of endosulfan on the brain of mice which were ameliorated on administration of antioxidants.*

Key words: *Endosulfan, Neurotoxicity, Antioxidants, AChE, BChE.*

1. INTRODUCTION:

To arrange the rising demand of food necessities for a growing human population, intense quantity of pesticides is being used, to enhance crop production across the planet, as a part of pest management policy. Massive application of pesticides has resulted in severe health consequences to man and his macro and micro environments. Endosulfan, the pesticide which is being considered in the current study as an inducer of neurological disorders, belongs to the group of organochlorine pesticides. Worldwide production of endosulfan was once estimated to be 10,000 metric tonnes in 1984 (1) but is now out of patent now after its use for more than five decades.

Brain is inclined of undergoing oxidative stress as it has relatively high concentration of peroxidizable fatty acids that act like substrate to the lipid peroxide formation, high oxygen consumption, and high level of oxidizable dopamine molecules and poor cellular defense system (2,3). Therefore, the central nervous system served as the major target of endosulfan toxicity in humans and animals. Endosulfan has been reported to be a strong neurotoxin in animals as well as in mammals, including human beings (4, **5,** 6). It has been reported to get accumulated in the brain as it has the ability to cross the blood brain barrier (BBB), to cause its functional mutilation (7). Conditions such as cerebral palsy, epilepsy and increased risk of Parkinson's disease have also been linked to endosulfan exposure (8,9).

Antioxidants are a diverse group of compounds that work by squelching free radicals. As antioxidants are known to protect against cellular damage induced by free radicals and other oxidative processes, three neuroprotective antioxidants namely resveratrol, alpha-lipoic acid and vitamin E have been used as research materials to counteract endosulfan induced toxicity. Resveratrol, quantified in grapes, mulberries, peanuts has been attributed to possess neuroprotective properties against ischemia, seizure, Alzheimer`s and Parkinson's disease due to its anti-inflammatory, antioxidant, estrogenic and hypolipemic properties (10,11). Alpha-lipoic acid (ALA- also known as thioctic acid) , found in a variety of foods, notably kidney, heart and liver meats as well as spinach, yeast, broccoli and potatoes has been reported to be beneficial in Alzheimer's disease, memory dysfunction and CNS disorders (12). Vitamin E is a potent lipid soluble antioxidant, found in green leafy vegetables, lean meats, poultry, fishes, beans, eggs, nuts, vegetable oils, whole grains and fortified cereals. Supplementation of Vitamin E has been found to be helpful in protection against cognitive impairment, Parkinson's and Alzheimer's disease (13).

As the pesticides are well known nerve poisons, they potentially affect the balance of CNS by bringing alterations in the neurotransmitter enzymes. Hence, the main focus of the present study has been to assess and evaluate the differential qualitative distribution of neurotransmitter enzymes in cerebrum region of the brain under the influence of detrimental exogenous agent endosulfan and also to monitor whether certain beneficial agents such as resveratrol,

alpha-lipoic acid and vitamin E had the propensity to protect brain from exogenous influences. The biomarkers considered are acetylcholinesterase and butyrylcholinesterase, these has been assessed on the basis of histochemical localization profile.

2. MATERIALS AND METHODS:

Test Chemical and Antioxidants

The neurotoxicity was induced by using technical grade endosulfan of 99% purity (CAS No 115-29-7) obtained from Shree Pesticides Pvt. Ltd., Udaipur (Rajasthan, India). The antioxidants vitamin E and alpha- lipoic acid were purchased from Hi Media, India. Stilbene trans- resveratrol was procured from Cayman, USA.
Acetyl thiocholine iodide, butyryl thiocholine iodide were obtained from Hi Media, India.

Animal models - Healthy, male, adult Swiss albino mice, *7*- 8 weeks old and weighing 28±7 gms were maintained in plastic cages, bedded with sterilized rice husk in a well ventilated room at 35± 2°C temperatures with relative humidity of 50-55% and 12±1 hour's dark and light phase. They were fed with a standard diet and were given *ad libitum* access to water.

EXPERIMENTAL DESIGN

The mice were divided into ten groups, minimum of 6 mice per group, and their dose protocol was as follows:
a) Group I: Control group mice which were administered olive oil as a vehicle per day for 15 days
b) Group II: Experimental group mice which were administered endosulfan (2.45 mg/kg body weight/day for 15 days)
c) Group III: Experimental group mice which were administered resveratrol (5 mg/kg body weight/day for 15 days)
d) Group IV: Experimental group mice which were administered endosulfan (2.45 mg/kg body weight/day for 15 days) + resveratrol (5 kg/kg body weight /day for 15 days)
e) Group V: Experimental group mice which were administered alpha- lipoic acid (20 mg/kg body weight day for 15 days)
f) Group VI: Experimental group mice which were administered endosulfan (2.45 mg/kg body weight/day for 15 days) + Alpha- lipoic acid (20 mg/kg body weight/day for 15 days)
g) Group VII: Experimental group mice which were administered Vitamin E (50 mg/kg body weight/day for 15 days)
h) Group VIII: Experimental group mice which were administered Endosulfan (2.45 mg/kg body weight/day for 15 days) + Vitamin E (50 mg/kg body weight for 15 days)
i) Group IX: Experimental group mice which were administered Resveratrol (5 mg/kg body weight/day for 15 days) + Alpha- lipoic acid (20 mg/kg body weight/day) + Vitamin E (50 mg/kg body weight/day for 15 days)
j) Group X: Experimental group mice which were administered Endosulfan + Resveratrol (5 mg/kg body weight/day for 15 days) + Alpha- lipoic acid (20 mg/kg weight/day for 15 days) + Vitamin E (50 mg/kg body weight/day for 15 days).

All the doses of antioxidants and endosulfan were prepared by dissolving them in olive oil and were administered orally by means of tuberculin syringe according to their body weight.

At completion of dose administration, the animals were killed by cervical dislocation. Cranium was dissected opened to expose the brain which was excised immediately, blotted free of blood and weighed on Sartorius balance. Subsequently, tissue was further processed for histoenzymological studies.

HISTOENZYMOLOGICAL STUDIES

Brain was fixed in chilled calcium formol (4^0C) and kept in a refrigerator, for 18-20 h and was further processed for localization of various enzymes.

1.) Histochemical method for AChE and BChE

Direct coloring method described by Karnovsky and Roots (14) was adapted for the demonstration of AChE.

3. OBSERVATIONS AND RESULTS:

3.1. Distribution of AChE in the different nuclei and fiber tracts of cerebrum of mice

The distribution of AChE activity in the following nuclei and fiber tracts of cerebrum was observed.

Cerebral cortex

In control group, lack of AChE activity was observed in the cerebral cortex (CC) and negative reaction was found in area cinguli of the cortex (CCA). Mild activity was seen in truncus corpus callosum (TCC) and cingulum (Cg). External capsule (EC) showed mild to minor AChE activity (Plate 1.1 and Plate 1.2, Fig.1; Table 1). In endosulfan treated group, AChE activity was markedly reduced in CCA and TCC as compared to control group (Plate 1.1 and Plate 1.2 , Fig.2; Table 1).

Basal ganglia

The basal ganglion is a unified mass of grey matter nuclei, located in the forebrain, midbrain and diencephalon. It is involved in the control of movement by receiving inputs from the motor areas of the cortex.

In control group, strong AChE activity was observed in nucleus caudate putamen (NCP) due to which internal capsule could not be seen (Plate 1.1 and Plate 1,2, Fig.1; Table 1). In endosulfan treated group, decline in AChE activity was observed in NCP (Plate 1.1 and Plate 1.2, Fig.2; Table 1).

Area Septalis

Lack of AChE activity was observed in the nucleus dorsalis septi (DS) and nucleus lateralis septi (LS) in the control group. Moderate to mild AChE activity was observed in nucleus medialis septi (MS) and nucleus diagonalis band of broca (NFDB). Mild AChE activity was seen in nucleus accumbens septi (NAS) and commisure anterior (CA) (Plate 1.1, Fig.1; Table 1). Comissure anterior pars anterior (CAP) revealed mild to very less activity. Mild AChE activity was observed in nucleus fimbrialis septi (NFS) and nucleus lateralis septi (NLS) where as minor activity was observed in nucleus triangularis septi (NTS) (Plate 1.2, Fig.1; Table 1).

However, in endosulfan administered group decline in AChE activity was observed in the whole area septalis region as compared to control (Plate 1.2, Fig.2; Table 1).

The enzyme reaction in all the regions was found to be elevated in antioxidants groups as compared to control (Plate 1.1 and Plate 1.2, Fig.3, 5, 7, and 9; Table 1) where as it was found that pre treatment with antioxidants was able to check inhibition of AChE enzyme activity by endosulfan (Plate 1.1 and Plate 1.2, Fig.4, 6, 8 and 10; Table 1).

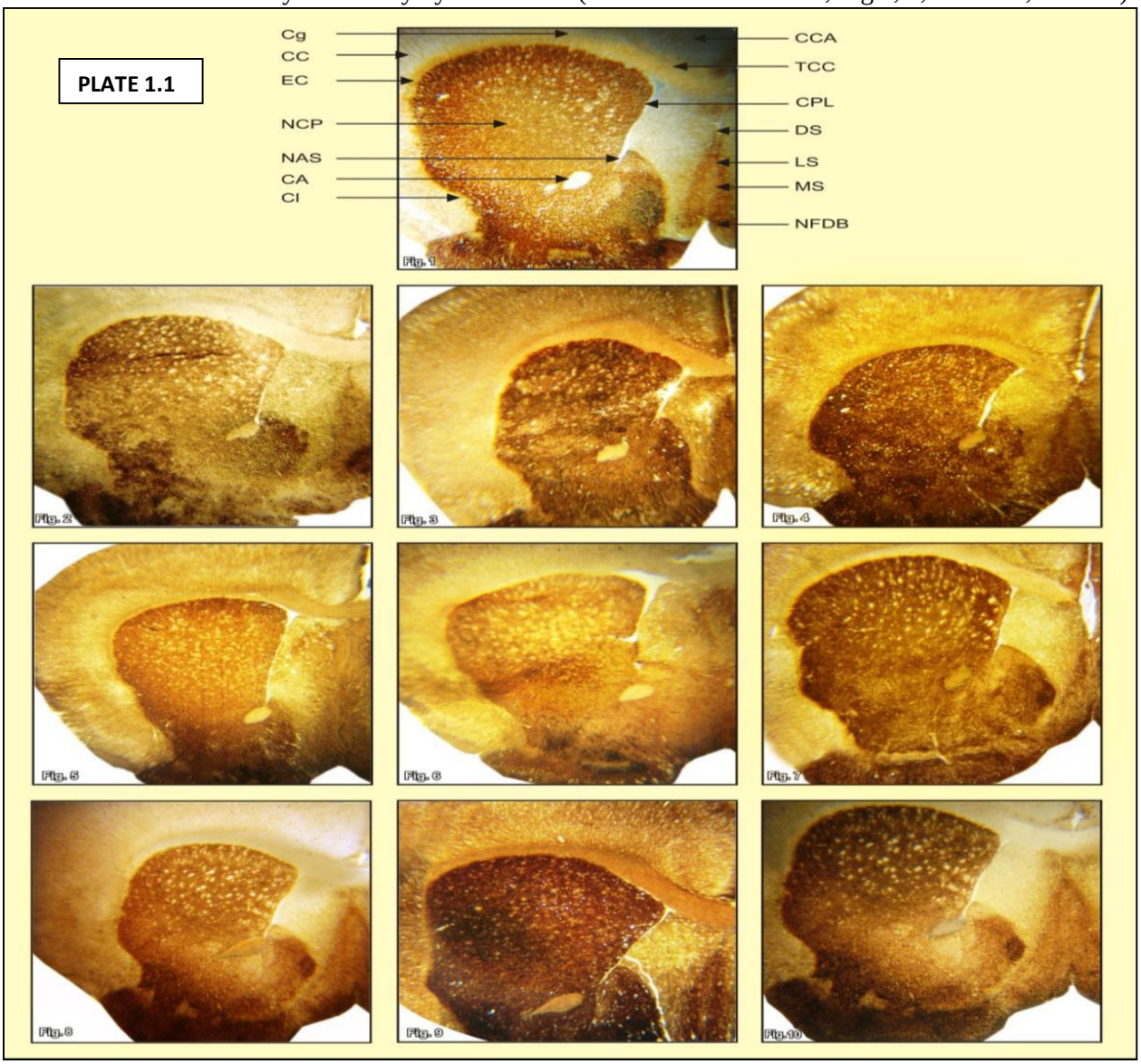

PLATE 1.1 : HISTOCHEMICAL DISTRIBUTION OF AChE ACTIVITY IN DIFFERENT NUCLEI AND FIBER TRACTS OF CEREBRUM (25 X)

Fig: 1. Control group showing strong AChE activity in NCP (nucleus caudate putamen). MS (nucleus medialis septii) and NFDB (nucleus fasciculus diagonalis band of broca) revealed moderate activity. Mild activity was observed in

TCC (truncus corpus callosum), Cg (cingulum), CA (commisure anterior), EC (capsula externa) and NAS (nucleus accumbens septi). Cerebral cortex (CC) revealed mild to negligible activity.

Fig: 2. Endosulfan treated experimental group showing markedly reduced AChE activity in NCP, Cg, CA, MS, TCC, NFDB and EC.

Fig: 3, 5, 7, and 9. Resveratrol, alpha- lipoic acid and vitamin E solitary and combined treated experimental groups showing strong AChE activity in NCP. Moderate activity was observed in TCC and NAS.

Fig: 4, 6, 8 and 10. In resveratrol plus endosulfan, alpha- lipoic acid plus endosulfan, vitamin E plus endosulfan and combined antioxidants plus endosulfan treated experimental groups**,** amelioration in AChE activity was observed in NCP, Cg, CA, MS, TCC, NFDB and EC.

PLATE 1.2 - HISTOCHEMICAL DISTRIBUTION OF AChE ACTIVITY IN DIFFERENT NUCLEI AND FIBER TRACTS OF CEREBRUM (25 X)

Fig: 1. Control group showing strong AChE activity in NCP (nucleus caudate putamen). Mild activity was observed in TCC (truncus corpus callosum), EC (capsula externa), NFS (nucleus fasciculus septi) and NAS (nucleus accumbens septi). Negligible activity was observed in mfb (median forebrain bundle).

Fig: 2. Endosulfan treated experimental group showing markedly reduced AChE activity in EC, NCP, TCC, NAS, NFS and mfb.

Fig: 3, 5, 7, and 9. Resveratrol, alpha- lipoic acid and vitamin E solitary and combined treated experimental groups showing strong AChE activity in NCP ,EC, NFS and NLS. Mild to moderate activity was observed in NCP and CAP (commisure anterior pars anterior).

Fig: 4, 6, 8 and 10. Resveratrol plus endosulfan, alpha- lipoic acid plus endosulfan, vitamin E plus endosulfan and combined antioxidants plus endosulfan treated experimental groups showing amelioration in AChE activity in NCP, TCC, NAS, NFS and mfb.

3.2. Distribution of BChE in the different nuclei and fibre tracts of cerebrum of mice

The distribution of BChE activity in the following nuclei and fibre tracts of cerebrum was observed.

Cerebral Cortex

In control group, mild to negligible BChE activity was observed in the cerebral cortex (CC) and negative reaction was found in area cinguli of the cortex (CCA). Strong activity was seen in truncus corpus callosum (TCC) and cingulum (Cg). External capsule (EC) showed moderate BChE activity (Plate 2.1 and Plate 2.2, Fig.1; Table 2). In endosulfan treated group, BChE activity was markedly reduced in CCA and TCC as compared to control group (Plate 2.1 and Plate 2.2, Fig.2; Table 2).

Basal Ganglia

The basal ganglion is an unified mass of grey matter nuclei, located in the forebrain, midbrain and diencephalon. It is involved in the control of movement by receiving inputs from the motor areas of the cortex.

In control group, strong BChE activity was observed in nucleus caudate putamen (NCP) due to which internal capsule could not be seen (Plate 2.1 and Plate 2.2, Fig.1; Table 2). In endosulfan treated group, decline in BChE activity was observed in NCP (Plate 2.1 and Plate 2.2, Fig.2; Table 2).

Mild and moderate BChE activity was observed in the nucleus dorsalis septi (DS) and nucleus lateralis septi (LS) repectively in the control group. Strong BChE activity was observed in nucleus medialis septi (MS) and moderate activity was seen in nucleus diagonalis band of broca (NFDB). Mild BChE activity was seen in nucleus accumbens septi (NAS) and strong reaction was observed in commissure anterior (CA) (Plate 2.1, Fig.1; Table 2). Commissure anterior pars anterior (CAP) revealed very strong activity. Strong BChE activity was observed in nucleus fimbrialis septi (NFS) and nucleus lateralis septi (NLS) where as moderate activity was observed in nucleus triangularis septi (NTS) (Plate 2.2, Fig.1; Table 2).

However, in endosulfan administered group decline in BChE activity was observed in the whole area septalis region as compared to control (Plate 2.2, Fig.2; Table 2).

The enzyme reaction in all the regions was found to be elevated in antioxidants groups as compared to control (Plate 2.1 and Plate 2.2, Fig.3, 5, 7, and 9; Table 1) where as it was found that pre treatment with antioxidants was able to check inhibition of BChE enzyme activity by endosulfan (Plate 2.1 and Plate 2.2, Fig.4, 6, 8 and 10; Table 2).

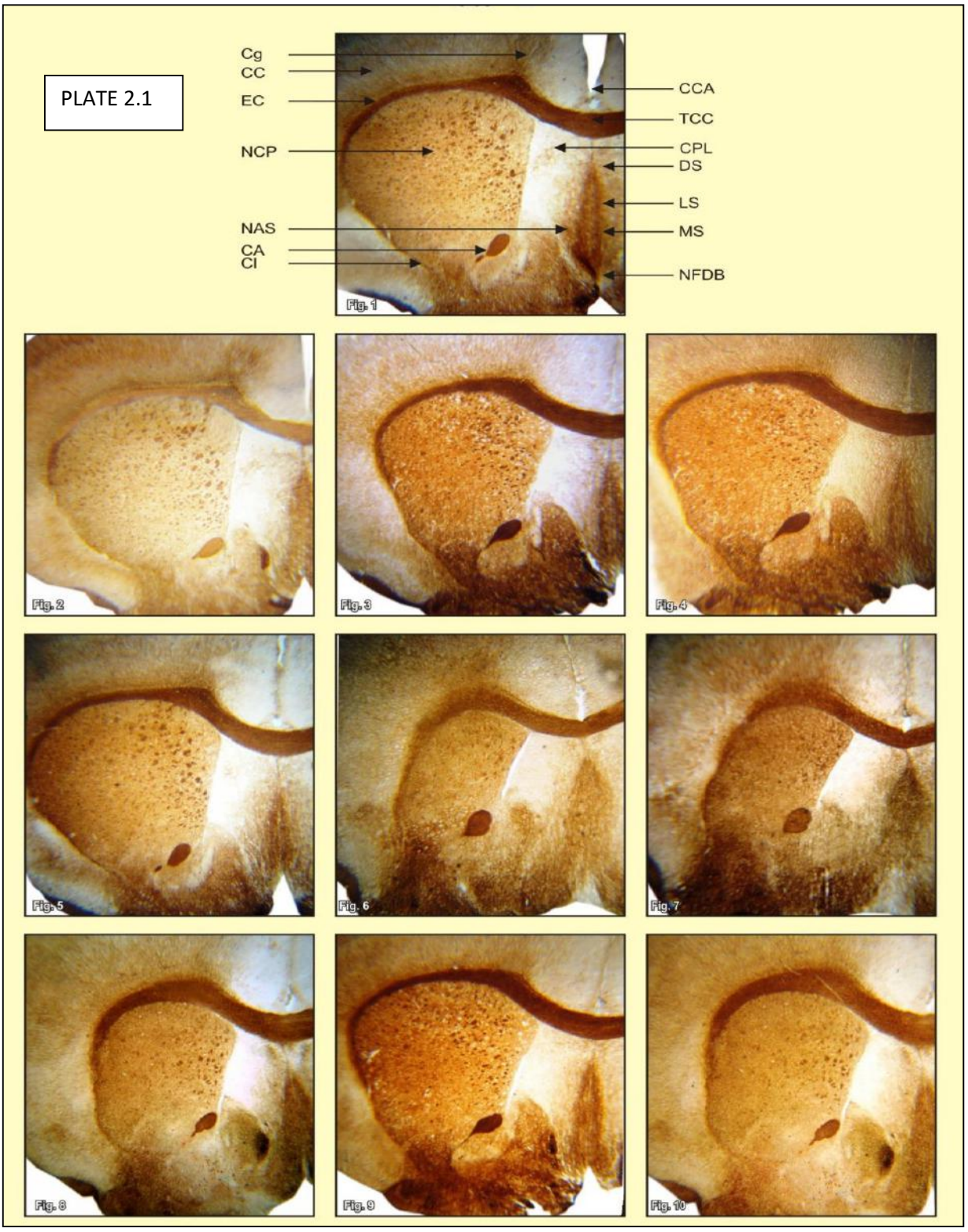

PLATE 2.1: HISTOCHEMICAL DISTRIBUTION OF BChE ACTIVITY IN DIFFERENT NUCLEI AND FIBER TRACTS OF CEREBRUM (25 X)

Fig: 1. Control group showing strong BChE activity in TCC (truncus corpus callosum), Cg (cingulum), MS (nucleus medialis septii) NCP (nucleus caudate putamen) and CA (commisure anterior) and NFDB (nucleus fasciculus diagonalis band of broca). EC (capsula externa) revealed moderate activity. Mild activity was observed in NAS (nucleus accumbens septi). Cerebral cortex (CC) revealed mild to negligible activity.
Fig: 2. Endosulfan treated experimental group showing markedly reduced BChE activity in NCP, Cg, CA, MS, TCC, NFDB and EC.

Fig: 3, 5, 7, and 9. Resveratrol, alpha- lipoic acid and vitamin E solitary and combined treated experimental groups showing very strong BChE activity in NCP. Moderate activity was observed in TCC and NAS.
Fig: 4, 6, 8 and 10. In resveratrol plus endosulfan, alpha- lipoic acid plus endosulfan, vitamin E plus endosulfan and combined antioxidants plus endosulfan treated experimental groups**,** amelioration in BChE activity was observed in NCP, Cg, CA, MS, TCC, NFDB and EC.

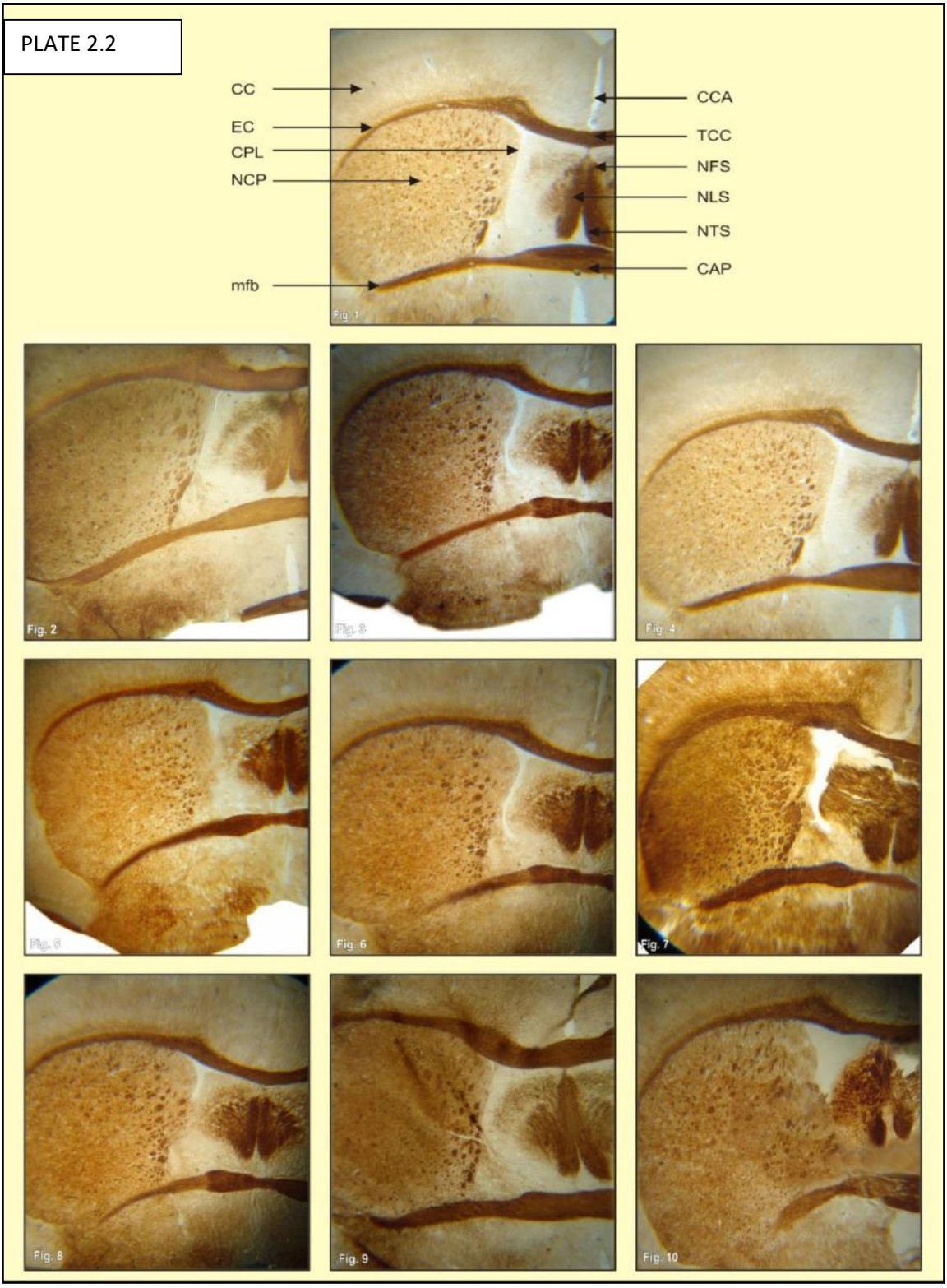

PLATE 2.2: HISTOCHEMICAL DISTRIBUTION OF BChE ACTIVITY IN DIFFERENT NUCLEI AND FIBER TRACTS OF CEREBRUM (25 X)

Fig: 1. Control group showing strong BChE activity in NCP (nucleus caudate putamen), TCC (truncus corpus callosum) and NFS (nucleus fasciculus septi). Moderate activity was observed in EC (capsula externa), Mild activity was observed in NAS (nucleus accumbens septi). Very strong activity was observed in mfb (median forebrain bundle).
Fig: 2. Endosulfan treated experimental group showing markedly reduced BChE activity in EC, NCP, TCC, NAS, NFS and mfb.
Fig: 3, 5, 7, and 9. Resveratrol, alpha- lipoic acid and vitamin E solitary and combined treated experimental groups showing very strong to strong BChE activity in NCP,EC, NFS, CAP and NLS
Fig: 4, 6, 8 and 10. Resveratrol plus endosulfan, alpha- lipoic acid plus endosulfan, vitamin E plus endosulfan and combined antioxidants plus endosulfan treated experimental groups showing amelioration in BChE activity in NCP, TCC, NAS, NFS and mfb.

Table 1. 1: EFFECT OF ENDOSULFAN AND ANTIOXIDANTS ON THE DISTRIBUTION OF AChE IN THE DIFFERENT NUCLEI AND FIBRE TRACTS OF CEREBRUM

Nuceli and Fibre Tracts	Groups									
	C	E	R	R+E	L	L+E	V	V+E	R+L+V	R+L+ V+E
Cerebral Cortex (CC)	- -	- -	+ -	+ -	+ -	+-	+ -	+ - /±	+ + -	- -
Cerebral Cortex Area Cinguli (CCA)	- -	- -	+ -	+ -	±	±	±	±	+ + -	- -
Cingulum (Cg)	±	- -	+ -	+ -	±	±	+ + -	- -	+ + -	±
Claustrum (Cl)	±	-	±	±	±	±	+ -	±	+ -	±
Commisure Anterior (CA)	- -	±	±	+ -	+ -	±	+ + -	+ -	+ -	±
Truncus Corpus Callosum	+ -	± /- -	+ + -	+ -	+ + -	+ -	+ + -	+ - / - -	+ + +	+ -
N. Lateralis Septi (LS)	+ -	±	+ + -	+ -	+ -	+ -	+ -	+ + -	+ + -	+ -
Nucleus Dorsalis Sepi (DS)	- -	- -	±	- -	- -	- -	- -	±	+ -	±
N. Medialis Septi MS	+ + -	+ -	+ -	+ + -	+ -	±	+ -	+ -	+ + -	+ -

Nuceli and Fibre Tracts	Groups									
	C	E	R	R+E	L	L+E	V	V+E	R+L+V	R+L+V+E
Nucleus Diagonalis Band of Broca (NFDB)	+ + -	+ -	+ + -	+ + +	+ + -	+ -	+ -	+ -	+ + -	+ -
Nucleus Accumbens Septi (NAS)	±	- -	+ + +	+ + -	+ + -	±	+ -	±	+ + +	±
Nucleus Fimbrialis Septi (NFS)	±	- -	±	±	+ -	±	±	+ -	+ + -	+ -
Nucleus Lateralis Septi (NLS)	+ + -	- -	+ -	±	+ -	+ -	±	±	+ -	±
Nucleus Triangularis Septi (nts)	±	- -	±	±	±	+ -	±	+ -	+ -	±

External Capsule (EC)	+ -	- -	±	+ -	±	±	+ -	±	+ + -	+ -
Nucleus Caudate Putamen (NCP)	+ + +	+ + -	+ + +	+ + + +	+ + +	+ + -	+ + +	+ + -	+ + + +	+ + +
Median Forebrain Bundle (mfb)	±	- -	±	±	±	±	±	- -	+ -	±
Commisure Anterior Pars Anterior (CAP)	±	**- -**	**+ -**	±	±	±	**+ -**	±	+ -	±

Histoenzymological index taken for enzymatic activity was as follows:
++++ (very strong activity) > +++ (strong) > + + - (moderate) > + - (mild) > ± (negligible) > - (no activity), NA= Not Attempted

TABLE 2.1 : EFFECT OF ENDOSULFAN AND ANTIOXIDANTS ON THE DISTRIBUTION OF BCHE IN THE DIFFERENT NUCLEI AND FIBRE TRACTS OF CEREBRUM

Nuceli and Fibre Tracts	**Groups**									
	C	**E**	**R**	**R+E**	**L**	**L+E**	**V**	**V+E**	**R+L+V**	**R+L+V+E**
Cerebral Cortex (CC)	+ - /±	+ -/±	±	+ -	+ -	+ -	+ -	+ -	+ -	+ -
Cerebral Cortex Area Cinguli (CCA)	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
Cingulum (Cg)	+ + +	+ + -	+ + -	+ -	+ -	+ -	+ + -	+ -	+ + -	+ + -
Claustrum (Cl)	+ + -	+ -	+ + -	+ -	+ + +	±	+ + +	+ + -	+ + + +	+ + -
Commisure Anterior (CA)	+ + +	+ + - /+ -	+ + + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + + +	+ + +
Truncus Corpus Callosum	+ + +	+ + -	+ + + +	+ + +	+ + +	+ + -	+ + +	+ + +	+ + + +	+ + +

Nuceli and Fibre Tracts	**Groups**									
	C	**E**	**R**	**R+E**	**L**	**L+E**	**V**	**V+E**	**R+L+V**	**R+L+V+E**
Nucleus Triangularis Septi (NTS)	+ + -	+ -	+ + +	+ + -	+ + +	+ + -	+ + +	+ + -	+ + +	+ + -
External Capsule (EC)	+ + -	+-	+ + + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + + +	+ + -
Nucleus Caudate Putamen (NCP)	+++	++-	++++	+++	++++	+++	++++	+++	+++	++ -
Median Forebrain Bundle (mfb)	+ + + +	+ + -	+ + + +	+ + +	+ + + +	+ + +	+ + + +	+ + +	+ + + +	+ + +
Commisure Anterior Pars Anterior (CAP)	+ + + +	+ + -	+ + +	+ + -	+ + + +	+ + +	+ + +	+ + +	+ + + +	+ + +

Histoenzymological index taken for enzymatic activity was as follows:
++++ (very strong activity) > +++ (strong) > + + - (moderate) > + - (mild) > ± (negligible) > - (no activity)

Nuceli and Fibre Tracts	**Groups**									
	C	**E**	**R**	**R+E**	**L**	**L+E**	**V**	**V+E**	**R+L+V**	**R+L+V+E**
Nucleus Triangularis Septi (NTS)	+ + -	+ -	+ + +	+ + -	+ + +	+ + -	+ + +	+ + -	+ + +	+ + -

External Capsule (EC)	+ + -	+-	+ + + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + + +	+ + -
Nucleus Caudate Putamen (NCP)	+++	++-	++++	+++	++++	+++	++++	+++	+++	++ -
Median Forebrain Bundle (mfb)	+ + + +	+ + -	+ + + +	+ + +	+ + + +	+ + +	+ + + +	+ + +	+ + + +	+ + +
Commisure Anterior Pars Anterior (CAP)	+ + + +	+ + -	+ + +	+ + -	+ + + +	+ + +	+ + +	+ + +	+ + + +	+ + +

Histoenzymological index taken for enzymatic activity was as follows:

++++ (very strong activity) > +++ (strong) > + + - (moderate) > + - (mild) > ± (negligible) > - (no activity)

4. DISCUSSION:

For a long period of time there has been an extensive, intensive and invasive utilization of pesticides all over the world. This exposure has resulted in impaired health which has been a subject of multifaceted studies. In human subjects, exposure to pesticides like endosulfan has been linked with a number of neurological disorders and ailments such as Parkinson disease, dementia, amyotrophic lateral sclerosis, Alzheimer disease, cognitive dysfunctions etc (15**,** 16). The manifestations of endosulfan exposure, has led to the need of effective neuroprotective agents which can serve as potential therapeutic drugs in combating these disorders.

In the present investigation, an attempt was made to evaluate the protective role of antioxidants viz., trans-resveratrol, alpha –lipoic acid and vitamin E against neurotoxic effects induced by endosulfan in the brain of Swiss albino mice. These antioxidants were administered solitarily and in combination, along with and without endosulfan. The results and observations of the present study significantly depicted the adverse effects of endosulfan as manifested by alterations in the histoenzymological distribution profile of neurotransmitter enzymes like AChE, and BChE. The neuroprotection rendered by the antioxidants resveratrol, alpha-lipoic acid and vitamin E did not allowed the pesticide to cause disturbances and alterations in the histochemical profile of these enzymes, which was rather similar to that of control group.

Endosulfan, an organochlorine pesticide has been reported to influence the distribution pattern of neurotransmitter enzymes present in the brain. It has been related with a number of complications of central nervous system (CNS) such as stress, trauma, convulsions, and tremors etc. There are several reports establishing the role of endosulfan in inhibiting the release of neurotransmitters like cholines, present in the brain (17,18). As endosulfan has been reported to cross blood brain barrier, any alterations in the permeability of blood brain barrier (BBB) due to its accumulation may result into altered variations in activity of neurotransmitter enzymes, which were duly observed in the present study.

As cholinesterase's (ChE's) are also found to be localized in blood brain barrier, any alterations in the permeability of blood brain barrier can manipulate the activity of cholinesterase's (19). In the present study, it was observed that endosulfan exposure led to significant variations in the distributional pattern of AChE activity. It was observed to be reduced in different nuclei and fiber tracts of cerebrum. . Highest concentration of endsoulfan has been observed in the cerebrum (white matter) followed by the remaining parts of the brain (20). Thus, any alterations in the neurotransmitter enzymes in this region may be linked to variations in the main functions of learning, memory, language and communication etc associated with it. Substantial data suggest a correlation between AChE and these functions, therefore, any disruption in the cholinergic system results into mutilation of various functions associated with different regions like gross impairment of visual-motor coordination, cognitive and emotional deterioration, severe impairment of memory and inability to perform most daily tasks (21,22,23). A reduction in cholinergic enzyme activity (both AChE and BChE) was observed in the present study on endosulfan intoxication in different nuclei and fiber tracts of cerebrum of mice brain, which suggests that organochlorine pesticides deteriorate the normal functioning of CNS. These observations are in concurrence with the findings of earlier reports which indicate the possible role of AChE in neurotransmitter system and its involvement in learning, memory and mood processes etc (24, 25, 26, 27).

There has been paucity of related information pertaining to the endosulfan induced variations in the distributional pattern of AChE activity in different regions of brain. However, numerous other biochemical studies have been conducted on endosulfan induced alterations in the levels of neurotransmitter enzymes which are in concurrence with the results of present investigation. These studies significantly show a decline in AChE levels. The study conducted by

Gupta,1978 demonstrated that a dose of endosulfan greater than 30 mg/kg body weight administered to rats and mice resulted in decreased brain acetyl cholinesterase levels(28). Similarly, Anand *et al.,* 1980 studied the electrical activity in cat brain treated with endosulfan and observed a decline in acetylcholine levels(29).

Assis *et al.,* 2011also investigated the *in vivo* and *in vitro* effects of the pesticide endosulfan on the cholinesterase (ChE) activity in the rats (30). The results of all the above mentioned research studies are in concurrence with present investigation which clearly delineates the hazardous potential of endosulfan with an ability to inhibit the AChE activity.

Butyrylcholinesterase, known to be predominantly distributed in the white matter of the central nervous system, is a non-specific cholinesterase enzyme involved in neural function. It is mainly localized in capillary endothelial cells, glial cells and neurons. It is has also been reported to serve as an important tool to monitor the toxic effects of pesticides (31). As expected , in endosulfan treated experimental group, lower BChE activity was observed in the regions of TCC, NCP,CC and mfb of cerebrum where this enzyme is abundantly found.

Declined BChE activity in this region may cause difference in ionic balance. Similarly, a decline in butyrylcholinesterase was also observed in the nuclei and fiber tracts on endosulfan administration. As these regions are linked to cholinergic and non cholinergic neurotransmission, lowered enzyme reaction may perturb the process of neuronal conduction. Looking at the important role of BChE in the central cholinergic system it can be hypothesized that interruption of this enzyme and its altered profile in cerebrum would be harmful to normal functioning of the brain and may cause severe neurological disabilities.

Reduction in plasma BChE has also been reported in many studies on exposure of farm workers to endosulfan and other pesticides (32). Attademo *et al.,* 2011 also reported intoxication of endosulfan in form of reduced BChE levels in the blood plasma of frog *Leptodactylus chaquensis* (33).

The study pertaining to histochemical localization of all these neurotransmitter enzymes is of prime importance as different studies related to their biochemical , haematological and other aspects have been conducted earlier however, there is lack of substantial data regarding the qualitative distributional pattern of these enzymes. The findings of present investigation clearly demarcate the distribution of these enzymes in different regions of brain under the impact of endosulfan and antioxidants, administered in combination and solitarily along with and without endosulfan respectively.

Antioxidants terminate the chain reaction before vital molecules are damaged. These are intimately involved in the prevention of cellular damage. They have been reported to protect neurons against damage and many neurological disorders including Alzheimer's, Parkinson's and neuropathy etc. Therefore, antioxidants such as trans-resveratrol, α-lipoic acid and vitamin E were used in the present study to counterbalance the hazardous effects of endosulfan. It was observed that all these antioxidants were able to render protection against endosulfan induced alterations in the distributional profile of neurotransmitter enzymes. The altered activities of AChE, BChE, on endosulfan administration were modulated and brought back to normal profile quite similar to that of control on administration of antioxidants. It is thus assumed that application of these antioxidants as probable preventive agents could be targeted in therapeutic amelioration of endosulfan induced neurological abnormalities.

Many scientists have reported the neuroprotective potential of resveratrol, among which the reports of Zhang *et al.,*2010 and Liu *et al.,* 2011 substantiate the beneficial properties of resveratrol in rendering protection against various neurological disorders including brain ischemia, seizures, and Parkinson's disease, Huntington's disease, Alzheimer's disease and strokes etc (34, 35). Numerous *in vitro* and cell culture studies of Kennedy *et al.,* (2010) and Gupta *et al.,* (2012) delineate neuroprotective effects of resveratrol in the treatment of Alzheimer's disease (AD) (36, 37).

Similarly, in the present investigation alpha - lipoic has been reported to be beneficial in neurodegenerative disorders like age related cognitive decline, Alzheimer's and Parkinsons disease (38,39).

This study also showed that vitamin E moderately attenuated the toxic manifestations of endosulfan. Vitamin E has been proven to encompass antioxidant properties and has an important role in protecting biological systems (40). Butterfield *et al.,* (2002) has reported it as one of the best therapeutic strategies in neurodegenerative disorders associated with oxidative stress (41). The results of present study are in concurrence with the above mentioned reports where supplementation of vitamin E augmented the altered histoenzymological profile induced by endosulfan exposure.

All these reports validate the results of present investigation where an increase in AChE, BChE localization was observed on administration of antioxidants.

Hence, it can safely be concluded that antioxidants such as trans- resveratrol, alpha- lipoic acid and vitamin E are most effective neuroprotective agents. Nevertheless, combination of all the three antioxidants rendered maximum

protection as when administered in combination than given solitarily along with endosulfan. This may be due to the intrinsic properties that these antioxidants possess. Thus, it can be hypothesized that the diet rich in multiple antioxidants can act as an effective neuroprotective source minimizing the various neurological anomalies associated with pesticide toxicity.

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