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Ameliorative Role of Certain Antioxidants Against Endosulfan Induced Neurotoxicity on GABA and Serotonin Levels in Mice Brain

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Abstract: The use of pesticides is devastating the fitness of nature as well as of mankind. Endosulfan, is one such organochlorine pesticide that possess harmful impact on the health of human beings. In the present study, an attempt has been made to investigate the toxic effects of endosulfan on the neurotransmitter levels of GABA and serotonin in the brain of mice. Endosulfan was administered at a dose of 2.45 mg/kg body weight to cause neurotoxicity. The mice were divided into 10 different groups. The effects of endosulfan exposure necessitated the development of effective neuroprotective medicines that may be used as possible therapeutic treatments to treat brain problems caused by pesticides. In order to compare the level of protection offered by resveratrol, alpha-lipoic acid, and vitamin E, three neuroprotective antioxidants were given to the mice individually, in combination, and alongside endosulfan in an effort to counteract the toxicity caused by endosulfan. The study concludes, that antioxidants possess neuroprotective properties and when they are given in combination are more beneficial in preventing neurological disorders.

Keywords: Endosulfan, Neurotoxicity, Antioxidants, Neuroprotection, GABA, Serotonin, Resveratrol, Alpha-lipoic acid, Vitamin E

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Introduction

Pesticides have been used extensively, intensely, and intrusively all over the world for a very long time. Multiple neurological issues and deteriorated health have been brought on by this exposure. Endosulfan belongs to the group of organochlorine pesticides, chemically it is 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3 benzodioxathiepin-3-oxide. It poses an insidious threat to human health. Human exposure to endosulfan is a universal problem. Endosulfan can enter the body through inhalation route by breathing contaminated air, from eating

and drinking contaminated products through oral route or through skin (dermal route). Human beings primarily get exposed to this dreaded pesticide through dietary exposure by consuming food or tobacco products that have been sprayed with endosulfan (Ely *et al*., 1967; Lazaro *et al*., 1999; Carreno *et al.,* 2007; Menezes *et al.,* 2017; Ghorbani *et al.,* 2021). Vegetables, seafood, spices, wine corks, infant formula milk, and many other items from the supermarket have been found to contain traces of endosulfan (Strandberg and Hites, 2001; Mezcua *et al*., 2007)**.** Drinking water from contaminated ground or surface water provisions is also a mode of exposure (Mukherjee and Gopal, 2002; Kaushik *et al*., 2012). In humans and animals, endosulfan intoxication primarily affects the central nervous system. Endosulfan can cross the blood brain barrier (BBB) and get accumulated in the brain to cause its functional mutilation, it can also induce autophagy and apoptosis in mice neuronal cells (Chan *et al*., 2006; Song *et al*., 2019). Endosulfan exposure has also been related to a higher prevalence of Parkinson's disease, cerebral palsy, and epilepsy (Joshi, 2001; Jia and Mishra, 2007). Endosulfan has negative effects on non-targeted species such as aquatic organisms, terrestrial organisms, plants, algae, mammals, and humans too. Notably, the toxicity of endosulfan can become more severe when it interacts with other chemicals present in ecosystem (Sathishkumar *et al*., 2021).

The effects of endosulfan exposure have made it necessary to develop strong neuroprotective medicines that might be used as possible therapeutic treatments to treat endosulfan induced illnesses. For that, three neuroprotective antioxidants namely resveratrol, alpha-lipoic acid, and vitamin E have been used in this study to mitigate the toxicity caused by endosulfan.

A wide range of substances known as antioxidants are available that work by squelching free radicals. They have a reputation for guarding against the harm that free radicals and other oxidizing agents can do to cells. As a result, they are often referred to as ROS sweepers or scavengers (Halliwell and Gutteridge, 1989).

Resveratrol's anti-inflammatory, antioxidant, estrogenic, and hypolipemic qualities have been linked to its neuroprotective abilities against ischemia, seizures, and Parkinson's disease (Benecke *et al*., 1993; Tredici *et al*., 1999; Robb and Stuart, 2010; Price *et al*., 2011). It has made a tremendous positive significant impact on Alzheimer`s disease (Karuppagounder *et al*., 2009; Li *et al*., 2012).

A large decrease in the accumulation of heavy metals like copper and iron is one of the many favourable impacts of vitamin E's ability to quickly enter the brain (Midaoui and Champlain, 2002). It has been claimed to benefit patients suffering from Alzheimer's disease and other types of memory loss caused by trauma or a cerebral vascular accident (Munch *et al.,* 2010). Alpha-Lipoic Acid has been associated with the decline in the severity of disorders of Central Nervous System by decreasing oxidative damage in the CNS (Packer *et al*., 1995; Lynch, 2001).

The current study's main objective was to assess and evaluate the different qualitative and quantitative distribution of neurotransmitters in different brain regions under the influence of harmful exogenous agents like endosulfan and to determine whether certain beneficial agents, like resveratrol, alpha-lipoic acid, and vitamin E, can shield the brain from exogenous influences. The biomarker considered are Gamma-Aminobutyric Acid (GABA) and serotonin. Gamma-Aminobutyric Acid is the most predominant inhibitory neurotransmitter in the mammalian CNS (Central Nervous System), it acts like a brake to the excitatory neurotransmitters that lead to anxiety (Lydiard, 2003). It has been reported to boost mental alertness by elevating the production of alpha waves associated with relaxation. Serotonin, a neurotransmitter, involved in the transmission of nerve impulses is known to play a distinct role in brain function and mental health, mood, emotion, blood pressure, body temperature, neuroendocrine regulation, sleep, sexual activity, and appetite and thus is implicated in the control

of numerous behavioural and physiological functions, personality traits, mood, aggression etc. (Takano *et al.,* 2007; Frokjaer *et al*., 2008).

Materials and Methods

Swiss albino adult healthy male mice (7□8 weeks, 28±7 g) were chosen as the study's test animals. For the current experiment, endosulfan at a dose of 2.45 mg/kg body weight was used after conducting pilot experiments. Administration of antioxidants APA (alpha- lipoic acid), resveratrol, and vitamin E were determined in accordance with their recommended daily dietary intake. Endosulfan was administered after one hour of antioxidants administration.

Endosulfan of 99% purity, vitamin E, alpha-lipoic acid, Stilbene trans-resveratrol and Gamma-aminobutyric acid were procured in their standard forms from reputed and trusted suppliers.

Antioxidant administration was as follows:

- Resveratrol (5 kg/kg body weight)
- Alpha- Lipoic Acid (20 mg/kg body weight)
- Vitamin E (50 mg/kg body weight)

Antioxidants and endosulfan were all provided orally and in accordance with individual's body weight after being prepared by dissolving them in olive oil.

Experimental Groups:

The mice were divided into 10 groups and treated as follow:

Group 1: Control (served only the vehicle)

Group 2: Endosulfan

Group 3: Resveratrol

Group 4: Endosulfan + Resveratrol

Group 5: Alpha- lipoic acid

Group 6: Endosulfan+ Alpha- lipoic acid

Group 7: Vitamin E

Group 8: Endosulfan+ Vitamin E

Group 9: Resveratrol+ Alpha-lipoic acid+ Vitamin E

Group 10: Endosulfan+ Resveratrol+ Alpha-lipoic acid + Vitamin E

Estimation of gamma amino butyric acid:

Estimation of GABA was conducted by chromatographic technique. The original method developed by Sadasivudu and Murthy (1978) was adapted with some modifications.

Estimation of serotonin:

Estimation of serotonin was conducted by direct precipitation method which is developed by Weissbach *et al*. (1958).

Statistical analysis:

Values were expressed as mean ± SEM (n=6). The values of GABA are in mmoles/g weight wet tissue. The results obtained with biochemical experiments were subjected to statistical analysis by using one way ANOVA (analysis of variance) to evaluate the significance difference if any among the groups and $P<0.05$, $P<0.01$, was considered as significant.

Results

In the present study, a significant decrease in the GABA level was observed in endosulfan treated group as compared to control group and antioxidant (trans-resveratrol, alpha-lipoic acid, and vitamin E) treated groups. Changes in GABA in whole brain of variously treated groups was found significant at 1% CD ($P<0.01$) (Table 1, Fig. 1). In group 2 (endosulfan treated group), a clear significant decrease (28.411± 1.53961; $p < 0.01$) was noted with respect to control (50.113 ± 1.53961) and the observed decrease was significantly ($p <0.01$) elevated by pre-administration of antioxidants as observed in group 4, 6, 8 and 10. The best protection was rendered by combination of antioxidants (group 10; 48.74167 ± 0.59824) followed by resveratrol (group 4; 45.008 ± 1.53961), lipoic acid (group 6; 43.62667± 1.53961) and vitamin E (group 8; 40.15± 1.53961). GABA levels were maximum in

Table 1: Effect of endosulfan and antioxidants (trans-resveratrol, alpha lipoic acid, and vitamin E) on GABA and serotonin levels in the brain of *Swiss* albino mice)

S. No.	Groups	Concentration of GABA (in mmoles/g weight wet tissue)	Concentration of Serotonin (in µg)
1	Control	50.113 ± 1.53961	32.66 ±1.0328
2	Endosulfan	28.411 ± 1.53961 ^{a**}	27.33 ±0.5164 ^{a**}
3	Resveratrol	57.668 ± 1.53961 ^{a**}	41.66 ±0.5164 ^{a**}
4	Resveratrol + Endosulfan	45.008 ± 1.53961 $^{a** b**}$	34.66 ±0.5164 $^{a** b**}$
5	Alpha-Lipoic Acid	57.375 ± 1.53961 ^{a**}	39.66 ±0.8165 ^{a**}
6	Alpha-Lipoic Acid + Endosulfan	43.62667 ± 1.53961 $^{a** b**}$	30.33 ±0.5164 $^{a** b**}$
7	Vitamin E	53.69833 ± 1.53961 ^{a**}	36.5 ±0.5477 ^{a**}
8	Vitamin E Acid + Endosulfan	40.15 ± 1.53961 $^{a** b**}$	32.66 ± 0.5164 $^{a* b**}$
9	Resveratrol + Alpha-Lipoic Acid + Vitamin E	64.56333 ± 0.90159 ^{a**}	48.33 ±0.5164 ^{a**}
10	Resveratrol + Alpha-Lipoic Acid + Vitamin E +Endosulfan	48.74167 ± 0.59824 $^{a** b**}$	36.88 ± 0.4083 $^{a** b**}$

Results are expressed as mean ± SD; ** = significant at 1 % (i.e., p < 0.01); * = significant at 5 % (i.e., p < 0.05; a = as compared to control (group 1); b = as compared to endosulfan (group 2)

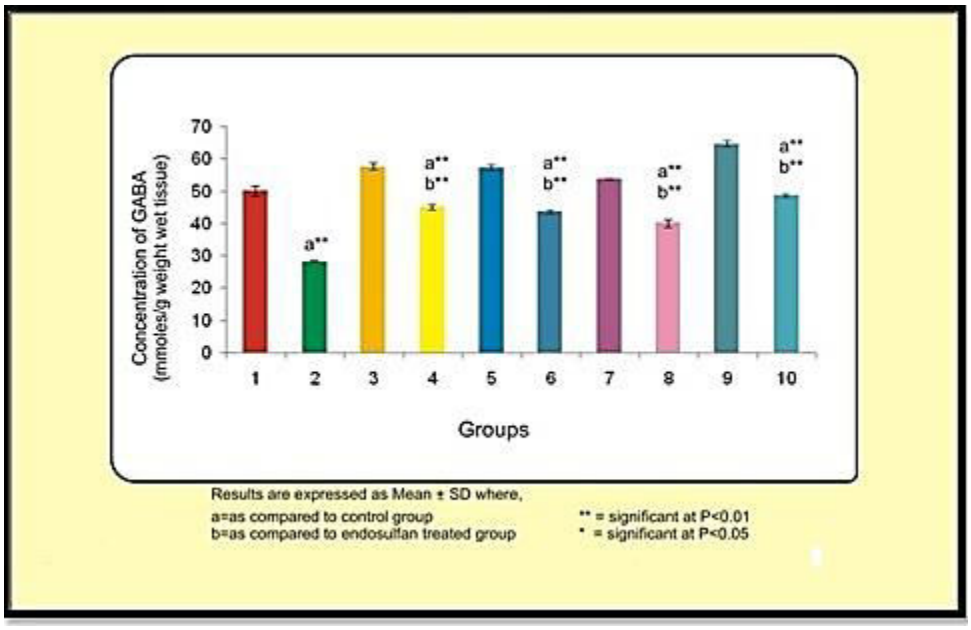

Fig. 1: GABA levels in the brain of mice induced by endosulfan and antioxidants treated groups.

groups administered combination of antioxidants (group 9) followed by resveratrol (group 3), alpha- lipoic acid (group 5) and vitamin E (group 7) at 64.5633 ± 0.9014, 57.6683 ± 0.9840, 57.3750 ± 0.7478 and 53.6983 ± 0.2308, respectively. These variations in the GABA levels suggest that antioxidants under investigation ameliorate the endosulfan induced toxicity in the brain of mice. The overall levels of GABA in descending order of groups are as follows; Group 9 > Group 3 > Group5 > Group 7 > Group 1 > Group 10 > Group 4 > Group 6 > Group 7 > Group 2.

The content of serotonin in the brain was calculated by plotting standard curve. The values

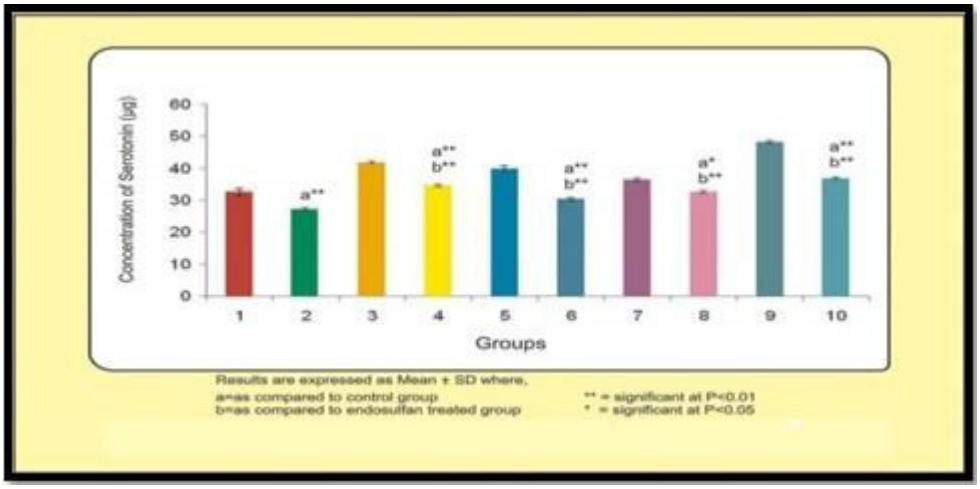

Fig. 2: Serotonin levels in the brain of mice induced by endosulfan and antioxidants treated groups.

of serotonin are expressed in micrograms. Results are expressed as mean ± SEM from 6 animals. Biochemical estimation of serotonin in the brain of mice revealed significant variations among all the groups (Table 1, Fig. 2). The serotonin content significantly declined ($p< 0.01$) after endosulfan treatment (group 2; 27.33 ±0.5164) as compared to control (group 1; 32.66 ± 1.032). However, in the antioxidant pre-treated groups a significant increase was observed among which combination of antioxidants (group 10) rendered best protection (36.88 ± 0.4083) followed by resveratrol (group 4) (34.66 ± 0.5164) followed by vitamin E (group 8) (32.66 ±0.5164) and alpha-lipoic acid (group 6) (30.33 ± 0.5164). Serotonin levels were maximum in groups administered antioxidants in combination or solitarily. Maximum serotonin concentration among these groups were observed in combined antioxidant treated group (group 9; 48.33±0.5164, $p<0.01$) followed by resveratrol (group 3; 41.66±0.5164, $p<0.01$) alpha- lipoic acid (group 5; 39.66 ±0.8165, $p<0.01$) and vitamin E (group 7; 36.5 ± 0.5477, $p<0.01$). The variations in the serotonin content of brain on endosulfan exposure suggest its neurotoxic potential while the administration of antioxidants revealed a significant elevation in serotonin levels quite similar to the control group.

The overall levels of serotonin in descending order of groups are as follows: Group 9 > Group 3> Group 5 > Group 10> Group 7 > Group 4> Group 1 and 8 >Group 6 > Group 2.

Discussion

In the current study, an effort was made to assess the effectiveness of antioxidants trans-resveratrol, alpha-lipoic acid, and vitamin E in protecting Swiss albino mice's brains against the neurotoxic effects of endosulfan. Estimation of GABA served as a biomarker to study its toxic effects on the brain.

Gamma-aminobutyric acid (GABA) is a key inhibitory neurotransmitter in the central nervous system of mammals (Baxter, 1972; Puts and Edden, 2012). Dysfunction of the GABAergic system has been linked with Parkinson's disease, temporal lobe epilepsy, schizophrenia, cerebral stroke, Huntington's disease, and anxiety disorders etc. (Pascual *et al*., 1998; Kleppner and Tobin, 2001; Lydiard, 2003; Babu *et al.,* 2011; Teixeira *et al.,* 2012). GABA receptors may have a role in endosulfan-induced neurotoxicity, according to several earlier investigations (Abalis *et al.*, 1986; Cole and Casida, 1986; Ozoe and Matsumura, 1986; Markey *et al.,* 2007). Endosulfan acted as a noncompetitive Gamma-Aminobutyric Acid (GABA) antagonist at the chloride channel within the GABA receptor in brain synaptosomes in a series of *in vitro* studies using 3H-dihydropicrotoxinin (Abalis *et al*., 1986;

Cole and Casida 1986; Ozoe and Matsumura, 1986; Gant *et al*., 1987). Similarly, Ansari *et al*. (1987), after witnessing convulsions, tremors, and hyperactivity in rats exposed to endosulfan, hypothesised that alterations in neurotransmitter levels (particularly, serotonin, GABA, and dopamine) in the brain could be caused by endosulfan's neurotoxicity.

A study conducted by Pomes *et al.* (1994) reported that α-endosulfan is endorsed with the capacity to block the chloride uptake induced by GABA in primary cultures of cortical neurons from 15-day old mice fetuses by interacting with the t-butylbicyclophosphorothionate (a GABA antagonist) binding site. Cabaleiro *et al*. (2008) detected reduced levels of GABA in the offspring of female rats who were fed endosulfan during pregnancy. Endosulfan reduces the levels and metabolism of norepinephrine, serotonin, and dopamine, which suppresses the striatal aminergic systems in rats (adult male) (Lafuente and Pereiro, 2013). Depending on the type of diet (low or high fat) taken, endosulfan sulphate treatment during fetal development affects mice glucose homeostasis, hepatic lipid metabolism, and the microbes in their gut (Yan *et al*., 2021). Evidences from available literature correlates with the findings of present study where endosulfan administration caused a significant decline in GABA levels in the brain of mice. This fall in the concentration of GABA is a consequence of inhibition of glutamic acid decarboxylase and formation of 2-keto-4-pentenoic acid (Bist and Bhatt, 2009). In the present study augmentation in GABA levels was also observed in pre-treated antioxidant groups along with endosulfan supplementation. These observations are in concurrence with the above-mentioned studies.

The mesencephalon, pons, and medulla oblongata all contain serotonin neurons, which are mostly found in the Dorsal Raphe Nucleus (DRN) or Nucleus Supra Trochlearis (Olszewski and Baxter, 1982). The substantia nigra, numerous thalamic centres, nucleus caudatus putamen, nucleus accumbens, cortex, and hippocampus are innervated by efferent fibres. Previous studies have suggested that serotonergic systems may be involved in the neurotoxicity of endosulfan. According to studies by Agrawal *et al*. (1983) and Zaidi *et al*. (1985), serotonin has been linked to an increase in aggressive behaviour (foot-shock-induced fighting) in rats after several exposures to endosulfan. In albino rats, endosulfan treatment resulted in the maximum number of dopamine binding sites as well as an increase in serotonin receptor sites without any change in binding affinity (Batra *et al*., 2018).

In the present investigation, a significant decrease in serotonin content in the brain of mice was observed which are in concurrence with the studies demonstrating the role of this neurotransmitter in endosulfan-induced neurotoxic effects. Anand *et al., (*1985) reported a marked reduction in the serotonin content of brain in rats fed with endosulfan 3 mg/kg for 10 subsequent days. Gopal *et al*. (1985) also noticed a marked decline in the brain serotonin levels in *Channa punctatus* exposed to sublethal and lethal concentrations of endosulfan. As organochlorine pesticides inhibit the conversion of tryptophan into serotonin, low levels of serotonin may be observed (Xu *et al.,* 2012). Serotonin is involved in cognitive deficits therefore any variations in 5-HT levels elicit changes in emotional states, appetite, and sleep patterns and any perturbations in this system cause errors in the brain architecture (Aldridge *et al*., 2005; Slotkin *et al.,* 2006). Endosulfan has also been previously reported to cause fluctuations in the serotonin levels of brain (Kutluhan *et al.,* 2003; Koca *et al*., 2006).

In adult rats, Agrawal *et al*. (1983) found that a single intraperitoneal dose of 3 mg/kg of endosulfan administered daily for 30 days significantly increased the 3H-serotonin binding affinity and foot-shock-induced fighting. Endosulfan was administered intravenously to rat pups for 25 days, and the rats' frontal cortical 3H-serotonin binding and foot-shock-induced fighting behaviour both significantly increased (Zaidi *et al*., 1985). While endosulfan-treated rats showed an

increase in dopamine (DA) concentration and a decrease in serotonin (5-HT) in amygdaloid, septal, and nigral brain-lesioned rats, Anand *et al*. (1985, 1986) reported that endosulfan-induced inhibition of 3H-5-hydroxytryptamine uptake in platelets. Serotonergic receptor sensitivity was altered in rats subjected to repeated high doses of endosulfan, and this was accompanied by an increase in aggressive behaviour (Seth *et al*., 1986).

Repeated exposure to endosulfan can lead to changes in the balance of neurochemicals, decreased activity of Na^+/K^+, $Ca2^+$ and Mg^{2+}-ATPase enzymes, increased acetylcholinesterase activity, elevated ammonia levels, and increased oxidative/nitrosative stress in the brain of rats (Oyovwi *et al*., 2021). Endosulfan isomers and the sulfate metabolite both caused germ cell cycle arrest (Du *et al*., 2015). Lakshmana and Raju (1994) reported small but significant changes in the levels of noradrenaline, dopamine, and serotonin in the developing brain of rat when rat was fed endosulfan. In another study with endosulfan, Wistar rat pups were dosed by gastric intubation with 6 mg/kg of endosulfan from postnatal days 2-25 and increase in serotonin levels was observed (Anguilar,2004). Paul *et al*. (1994) observed a correlation between the increase in serotonin and inhibition of a learning paradigm on endosulfan exposure in rats. They showed increased serotonin levels in the cerebrum and midbrain regions. Cabaleiro *et al.* (2008) reported increase in serotonin concentration in the prefrontal cortex of male offspring rats exposed to endosulfan to a dose of 0.61 mg or 6.12 mg endosulfan/kg/ day. In the present study, an increase in serotonin content may be to counterbalance the increased depression due to oxidative damage caused by endosulfan. Endosulfan inhibited AChE and BChE in various nuclei and fiber tracts of various brain regions involved in learning, perception of pain, muscle stretch, intelligence, organizational skills, tactile responses, memory, thought processing and perception, interpretation of sensory impulses, motor function, planning, emotion, language comprehension, coordination, eye movements, and regulation of other normal bodily functions (Bano, 2020). Administration of antioxidants in endosulfan treated groups considerably augmented the levels of serotonin in mice. Our results are in concurrence with above studies.

Conclusion

It can be concluded from the above studies that antioxidants possess neuroprotective properties and when they are given in combination are more beneficial in preventing neurological disorders.

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