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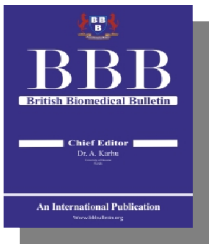

Original

Obliteration in Oxidative Stress and Ca^{++} Uptake in Brain Mitochondria Leads to Impairment of Cholinergic System: A Possible Mechanism Underlying Neurotoxicity Induced by Dichlorvos

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A B S T R A C T

Dichlorvos is an organophosphate insecticide effectively used against mushroom flies, aphids, spiders, mites, caterpillars and thrips. Toxicity of dichlorvos is confirmed by air, water and food via inhalation, dermal, absorption and ingestion. Earlier studies velate the dichlorvos administration with the toxicity of the reproductive system, respiratory system, cardiovascular system and nervous system. Primarily, it affects the nervous system through cholinesterase inhibition or anticholinesterase effect and also leads to increased intracellular calcium level, oxidative stress and the rate of lipid peroxidation in the brain mitochondria. Mitochondria are pleomorphic organelles that generate the ATP supply for the cells. Disturbance in the electron transport chain (ETC) of mitochondria by the dichlorvos ultimately increases the ROS production, thereby leading to an increase of oxygen consumption and decrease of ATP synthesis which is the hallmark of the neuronal lesions. The increased calcium level is reported to be associated with neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Since, the brain is a controlling and coordinating organ in the body, therefore toxic effects of dichlorvos on it will also be deleterious to other organ-systems indirectly. Current review deals with possible implications of impairment of cholinergic circuit and brain mitochondrial functions carried by dichlorvos which may be the cause of potential neurotoxicity.

Introduction

Dichlorvos is an organophosphate insecticide which is reported to cause toxicity in animals[1-7] and humans[8-10]. It is known as a classical acetylcholinesterase (AChE) inhibitor[11]. Trade and other names of dichlorvos include 2, 2-dimethyl-dichlorovinyl phosphate (DDVP) (Fig.1), Apavap, Benfos, Cekusan, Cypona, Derriban, Derribante, Devikol, Didivane, Duo-Kill, Duravos, Elastrel, Fly-Bate, Fly-Die, Fly-Fighter, Herkol, Marvex, No-Pest, Prentox, Vaponite, Vapona, Verdican, Verdipor and Verdisol.

Dichlorvos is a colorless synthetic pesticide which is used to protect plants, fruits and vegetables from insects. It is effective against mushroom flies, aphids, spiders, mites, caterpillars and thrips[12,13]. Dichlorvos enters into the environment during its manufacture, use and human activities. Exposure of dichlorvos to humans may occur via air, water and food. Dichlorvos is reported to be highly toxic via inhalation, dermal absorption and ingestion[14,15]. Dichlorvos is highly toxic and persistent in nature. The half-life of dichlorvos in the soil is more than the atmosphere[16-18]. It does not absorb to the soil particle because of its high solubility in water (Table 1) therefore leading to groundwater contamination. Table 1 shows other physic-chemical properties of dichlorvos.

Commercial manufacture of dichlorvos started in 1961. In the USA and Europe, dichlorvos is available in the market as dusts, granules, pellets/tablets, impregnated resin strips, emulsifiable concentrates, soluble concentrates, wettable powders and pressurized formulations[29,30]. Dichlorvos strips were first marketed in 1967 to control flies[31].

Dichlorvos requires a minimum purity of 97% for use of public health[32,33]. In agriculture dichlorvos is used to protect stored crops from insect damage. It is effective at very low concentrations and used as a fumigant against insects. Dichlorvos strips are widely used as a component in pet flea and tick collars in livestock industry[34-36]. It has veterinary and human medicinal uses in the control of severe internal and external parasite infestations[35]. It is used in animal feed as an antihelmintic agent for swine, horse and dogs[36-40]. In the aquaculture, dichlorvos is mixed directly in to the water for controlling fish parasites[33,41-44].

Individuals involved in the manufacture, formulation and application of dichlorvos in agriculture and households could be exposed by it through direct dermal contact or other treated surfaces[26,34,36,45]. Once, it is applied only a mere fraction reacts to target pest, rest of dichlorvos enters the food chain consequently leading to its biomagnifications to each tropic level.

Dichlorvos may cause toxicity of reproductive system[46], pancreas[47], kidney, liver, spleen[48,49], immune system[50] and brain[51,52]. Brain is among the most metabolically active organs in the body which requires large amounts of energy for proper functioning. Mitochondria are significant source as well as targets of reactive oxygen species. Neurons depend on the mitochondrial functions, especially for their energy supply. Therefore, impairment in mitochondrial functions of neurons may cause neurodegenerative disorder. Dichlorvos affects the brain neurotransmitter level abruptly and primarily affects the nervous system through the anticholinesterase effects.

Neurotoxic effects of dichlorvos

Dichlorvos is a reported neurotoxicant which creates several anomalies in the brain. The brain is one among the most complex organs which serves as controlling and coordinating center in the vertebrate body. Brain injuries can

occur due to internal and external factors which are responsible for the neurodegenerative diseases. Glucose is the primary fuel for the brain, which if, in scanty amount may adversely affect the neurons. Dichlorvos leads to defects in the ability to metabolize glucose and inhibit the activity of the neurotransmitters like acetyl-cholinesterse[53,54]. The metabolic maintenance of brain tissue needs the glucose as a major fuel. Dichlorvos exposure affects the alteration in neuronal glucose. Significant depletion in the glycogen content, activity of hexokinase, phospho-fructokinase, cytochrome oxidase, AChE and memory dysfunction in the brain parts (cerebrum, brain stem, cerebellum) were reported following exposure of dichlorvos[2]. Lactate dehydrogenase (LDH) activity also decreased in the cerebellum after administration of dichlorvos. An increase in the glucose level leads to hyperglycemia that is responsible for the brain hypometabolism means reduction in metabolic rate. Brain hypometabolism may lead to synaptic dysfunction, which involves loss of hormonal support, loss of nerve supply to the target organ and excessive amount of apoptosis of a cell. Dichlorvos exposure, thus may lead to synaptic atrophy in which neurons and the connections between them are degenerated. Reduction of neuronal volume may also occur following it. There is an endogenous antioxidant system (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathion (GSH) of the brain which also becomes weak due to dichlorvos exposure.

Neurons affected by the exposure of dichlorvos are reported with decreased mRNA expression of NF-68 and GABA (A) receptors[55]. Dichlorvos administration increased malondialdehyde (MDA) content, SOD and GSH activity in the brain[56]. Exposure of dichlorvos may cause nigrostriatal neurodegenaration and significant behavioral impairments[57]. There is also evidence that repeated exposure of dichlorvos depletes both oxidized and reduced glutathione levels in rat brain[58]. Lowered glutathione levels may decrease the rate of detoxification of dichlorvos by the glutathione dependent metabolic pathways.

Generation of oxidative stress in mitochondria is the first step towards neurotoxicity

The human brain is a fascinating, enigmatic and most complicated controlling organ. It is responsible for mood, behavior, memory, learning and thought processes[59]. Brain is considered highly vulnerable to oxidative stress than any other organ of the body as it consumes a high amount of oxygen, contains high amount of polyunsaturated fatty acids (PUFA) and low level of antioxidant enzymes[60]. Brain is extremely vulnerable to oxidative stress because it is highly enriched with non-heme iron, which is catalytically involved in the production of oxygen free radicals[61,62]. Oxidative stress is defined as a disproportion in pro-oxidants and antioxidants[63,64]. The disproportion between radical-generating and radical scavenging systems may cause an increase in free radical production or reduced activity of antioxidant defense[65].

Treatment with dichlorvos causes an increase in the levels of MDA and reactive oxygen species (ROS)[66,67] and a decrease in the activities of antioxidant enzymes[68]. Administration of dichlorvos increased lipid peroxidation (LPO) and decreased levels of GSH, SOD, CAT, GPx, glutathione-S-transferase (GST), glutathione reductase (GR)[60].

Enhanced oxidative stress and decreased Mn-SOD activity in the brain mitochondria is reported due to dichlorvos exposure (Table.2). Dichlorvos exposure

causes a significant increase in the activities of antioxidant enzymes viz SOD and CAT which is accompanied by a decrease in the values of and GPx activity[58]. Dichlorvos induced ROS production triggers caspase dependent cell death in rat brain[69,70]. Chronic dichlorvos exposure lead to a decrease in the activity of glucose-6-phosphate dehydrogenase, which was assayed in the brain as an index of oxidative stress[71].

Exposure of dichlorvos inhibits the mitochondrial complex I and cytochrome oxidase, which generates the ROS production[72]. Enhancement in ROS production leads to disturbance of cellular antioxidant defense systems and release of cytochrome c (cyt c) from mitochondria to cytosol resulting in apoptotic cell death.

Mitochondrial impairment leads to changes in DNA and proteins

Dichlorvos has a strong DNA alkylation effect which is responsible for the brain hypoplasia[73]. The toxicity of dichlorvos have also been related to alterations in DNA replication, which causes mutations[74] and cellular hyperproliferation[75-77]. Dichlorvos leads to increase in MDA, protein carbonyl and 8-hydroxydeoxyguanosine as well as protein and mitochondrial DNA (mt DNA) oxidation. Exposure of dichlorvos leads to formation of 8-hydroxydeoxyguanosine as a result protein and mtDNA oxidation and oligonucleosomal DNA fragmentation, a hallmark of impaired mitochondrial bioenergetics and apoptotic neuronal degeneration[5]. Dichlorvos is also responsible for the protein adduction in the brain[78]. It leads to increase in calpain activity[79]. Calcium activated neutral proteinases (calpain or CANPs) is an intracellular neutral cystein proteinase. Catalytic activity of calplain depends on the level of Ca^{++}. Increased activity of calpain leads to neurodegeneration[80]. Dichlorvos stimulates hyperphosphorylation of tubulin and MAP-2 and may ultimately affect axonal degeneration leading to delayed neurotoxicity[81].

Role of Ca++ in the promotion of neurotoxicity

The alteration in cellular Ca^{++} homeostasis is one of the key mechanisms contributing to mitochondrial damage in neurons. Excessive Ca^{++} uptake by the mitochondria leads to mitochondrial dysfunction resulting in neuronal lesions[82]. Dichlorvos leads to increase in the plasma glucose level[2], rate of lipid peroxidation[60,83], protein carbonyl content (PCC)[69] and intrasynaptosomal calcium level through voltage operated calcium channels (VOCC)[3]. Dichlorvos is reported to cause decreased cytochrome oxidase (COX) activity in rat brain, which could be responsible for decreased oxygen utilized by tissue[84]. Dichlorvos induced increase in calcium uptake through VOCC may evoke the release of neurotransmitters (NTs), impairment of mitochondrial oxidative phosphorylation, decrease in ATP synthesis and generation of free radicals (Fig 2)[85]. The increased calcium level is also associated with neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.

Dichlorvos leads to decrease in cytochrome c reductase (complexes I–III), cytochrome oxidase (complex IV), Mn-SOD activities, ATP level and increased ROS accumulation in mitochondria of the hippocampus region of rat brain[61]. Dichlorvos is recorded to make an increase in intrasynaptosomal Ca^{++} and inhibition of calcium ATPase[79].

Many histopathological observations are also in the agreement with the above mentioned facts. The mitochondria of the hippocampus were significantly enlarged

and swollen into spherical shapes following exposure of dichlorvos subsequent to it. Hippocampus showed pyknosis, eosinophilia, karyorrhexia, and chromosome condensation in neurons[61]. Cognitive impairments are also induced by dichlorvos exposure.

Impairment of cholinergic circuit induced by dichlorvos

Cholinergic system is a neurotransmitter system in the brain refers to acetylcholine in the neurological sense. It is a system of nerve cells that uses acetylcholine in transmitting nerve impulses. It regulates higher cognitive functions such as memory, learning, dendrite arborization, neuronal development, and differentiation[86-89]. Its receptors are subdivided into nicotinic receptors and muscarinic receptors[89]. Cholinergic toxicosis[11] and central apnea[90] are the key features of dichlorvos induced alterations in the nervous system (Table.2). It primarily affects the nervous system through cholinesterase inhibition or anticholinesterase effect[91,92]. Brain as a tissue is dissimilar from other body tissues due to its high metabolic rate and complete dependence on glucose for the maintenance of neural activity[93]. Hyperglycemia has been known to occur as a consequence of increased accumulation of acetylcholine (ACh) at the nerve endings following AChE inhibition[14].

Dichlorvos inhibits the AChE[92,94-98] and glutathione reductase (GR) activities in the brain, which are biomarkers of neurotoxicity and oxidative stress[99]. Exposure of the dichlorvos leads to a reduction of the cholinesterase activities in the brain of the chicks[100]. Activities of cytochrome oxidase and AChE are declined by the exposure of dichlorvos which may lead to an increased ACh level[101]. Dichlorvos also shows delayed neurotoxicity potential in animals and humans[4,102-105].

Dichlorvos exerts its toxicity by inhibiting neural acetylcholinesterase. The dichlorovinyloxy group of this molecule withdraws electrons from the phosphorus atom, leaving it susceptible to nucleophilic attack [106]. One potential nucleophile is the serine hydroxyl group located at the active site of acetylcholinesterase. The product of this reaction is a dimethoxy-phosphorylated acetylcholinesterase molecule and dichloroacetaldehyde. The phosphorylated form of acetylcholinesterase is incapable of hydrolyzing acetylcholine. If this enzyme is inhibited, acetylcholine accumulates in the synapse and can interfere with neuron functioning. Disturbed cholinergic neurotransmission in the parasympathetic peripheral nervous system can include lacrimation, perspiration, meiosis, nausea; vomiting, diarrhoea, excessive bronchial secretions, bradycardia, increased salivation and increased urinary frequency and incontinence. Dichlorvos effects on motor nerve fibers in the skeletal muscles can include muscle fasciculations, cramps, muscle weakness and flaccidity. Effects on cholinergic synapses in the central nervous system result in drowsiness, fatigue, mental confusion, headache, convulsions and coma.

Fig.3 shows that dichlorvos leads to an increase in ACh levels and inhibits AChE activities in the brain. It also induces an increase in the calcium uptake which further leads to disruption of ETC. Elevated mt Ca^{++} uptake decreases mt electron transfer activities of cytochrome oxidase (complex IV) along with altered mt complex I and II activity. MDA, PCC and hydroxyl guanosine are formed as a result of enhanced LPO of proteins and oxidation of mt DNA due to alteration in mt Ca^{++} uptake. This is a consequence of enhanced oxidative stress, decrease GSH level and a decrease in mt SOD activity. Chronic exposure of dichlorvos triggers the release of cyt c from mt cytosol and activates caspase-3 which

leads to neuronal cell death, meurodegeneration and deficits in learning and memory.

Dichlorvos reported to affect the brain NTs level abruptly on the brain. Besides affecting cholinesterase dichlorvos induced degeneration of dopaminergic neurons is attributed to an impairment of neuronal mitochondrial complex I activity[57]. A marked change in the dopaminergic neurotransmitter system in terms of increased levels of both dopamine and norepinephrine along with a significant increase in the activity of both the catecholamine synthesizing enzymes tyrosine hydroxylase and dopamine-beta-hydroxylase was reported by dichlorvos exposure[107]. It leads to decrease in a number of the muscarinic acetylcholine receptor binding sites[108,109] and in the expression of M (1), M (2) and M (3) muscarinic receptor subtypes[109].

Conclusion

Dichlorvos is vicious for the humans and animals due to its highly toxic and persistent nature. When applied, a fraction of dichlorvos reaches to target pest, rest is entered in to the food chain and leads to toxicity of different organs of the body. Dichlorvos leads to hypometabolism and destruction of mitochondrial functions in the brain like impairment of oxidative phosphorylation, increased Ca^{++} uptake, decreased ATP synthesis, ROS production and reduced oxygen consumption. Oxidative phosphorylation is a vital part of metabolism. Dichlorvos leads to increase of glucose level which is responsible for the hypometabolism. Impairment of oxidative phosphorylation may leads to hypometabolism and ROS production, which ultimately lead to propagation of free radicals, damage to cells and contributing to disease. Thus, impairment in the mitochondrial functions leads to neurodegenerative diseases. Brain is a vital organ which governs the important physiological activities such as information processing, homeostasis, learning, memory etc. Dichlorvos affects the neurotransmitter level abruptly. It primarily, affects the nervous system through the anticholinesterase effects and leads to neuronal cell death, neurodegeneration and deficits in learning and memory. The dichlorovinyloxy group of the dichlorvos leads to inhibition of the acetylcholinesterase activity. Dichlorvos also has mutagenic and carcinogenic effects. The toxicity of the dichlorvos is related to alterations in the DNA replication which causes mutations. It leads to oxidation of proteins and mitochondrial DNA.

Present review highlights that dichlorvos causes neurotoxicity through the disturbances in the cholinergic circuit and brain mitochondria. Inhibition of acetylcholinesterase is playing a central role for dichlorvos toxicity which ultimately leads to an interruption in the ETC and generation of free radicals. It leads to cell apoptosis and neurodegeneration.

Abbreviations

ACh acetylcholine
AChE acetylcholinesterase
CANPs calcium activated neutral proteinases.
CAT catalase
COX cytochrome oxidase
DDVP 2, 2-dimethyl-dichlorovinyl phosphate.
GPx glutathione peroxidase
GR glutathione reductase
GSH reduced glutathione
GST glutathione-S-transferase
LDH lactate dehydrogenase
LPO lipid peroxidation
MDA malondialdehyde
NTs neurotransmitters
PCC protein carbonyl content

PUFA polyunsaturated fatty acids
ROS reactive oxygen species
SOD superoxide dismutase
VOCC voltage operated calcium channels

Author's contributions

Bharti Chaudhary and Sonam Agrawal are doing wet lab experiments on toxic effects of dichlorvos on brain, kidney and haematological profile of mice. Simultaneously Bharti is the main author of the manuscript. Both the students are working on the experiments designed by DR. Renu Bist who becomes to be their supervisor.

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Table 1. Physicochemical properties of dichlorvos

S. N.	Physicochemical properties		Reference
1	Appearance	Dichlorvos is a colorless to amber liquid with a mild chemical odor	19
2	Chemical name	2,2-dichlorovinyldimethyl phosphate	19
3	Chemical formula	CCl_2= CHO.P0.(OCH3)	
4	Molecular weight	220.98	20
5	Water solubility	10,000 mg/L	19
6	Solubility in other solvents	Miscible in nonpolar solvents such as dichloromethane, 2-propanol, toluene and soluble in ethanol, chloroform, acetone, kerosene	19, 21, 22, 23
7	Vapor pressure	0.01 mm Hg at 30° C	24
8	Adsorption coefficient	30	25
9	Boiling point	140°C at 20 mm Hg, 117° C at 11 mm Hg, 35° C at 0.05 mm Hg	23, 26
10	Melting point	< -60°C	27
11	LD_{50}	70.4 to 250 mg/kg in rats, 206 mg/kg in mice, and 107 mg/kg in rabbits	14, 28

Table 2. Hazardous effects of dichlorvos on the central nervous system

S. N.	Dose (mg kg $^{-1}$bw)	Exposure duration and mode of dichlorvos intoxication	Organ	Animal	Reference
1	1.8	Acute, intraperitoneally	Nitro-oxidative stress	Rat	110
2	25	Single dose, orally	Neuronal loss	Rat	111
3	2	21 days, subcutaneously	Imbalance in antioxidant enzymes, brain AChE	Rat	64
4	8.8	14 days, orally	Oxidative stress	Rat	60
5	57.5	Single dose, feeding tube	Fewer seizures and deaths	Mice	1
6	6	Chronic, 8 weeks	Alteration in cholinergic metabolism	Rat	108
7	2.50	12weeks, subcutaneously	Nervous system	Rat	57
8	200	8 weeks, subcutaneously	Nervous system	Rat	112
9	6	8 weeks, subcutaneously	Nervous system	Rat	2
10	200	Acute, subcutaneously	Nervous system	Rat	4
11	5	Intraperitoneally	Nervous system	Rat	58
12	100	Single dose exposed for 2 weeks, subcutaneously	Nervous system	Hens	102
13	0.6, 1.5, 3.0	10 days, intraperitoneally	Brain	Rat	81
14	2.5	4 weeks, subcutaneously	Brain	Rat	52
15	6	12weeks, subcutaneously	Impairment in mitochondrial energy metabolism	Rat	67
16	6.30	Acute, orally	Cholinesterase inhibition in brain	Chicks	113
17	20	Acute, subcutaneously	Delayed neuropathy	Rat	114

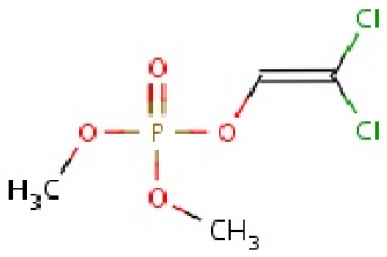

Figure 1. Chemical structure of dichlorvos

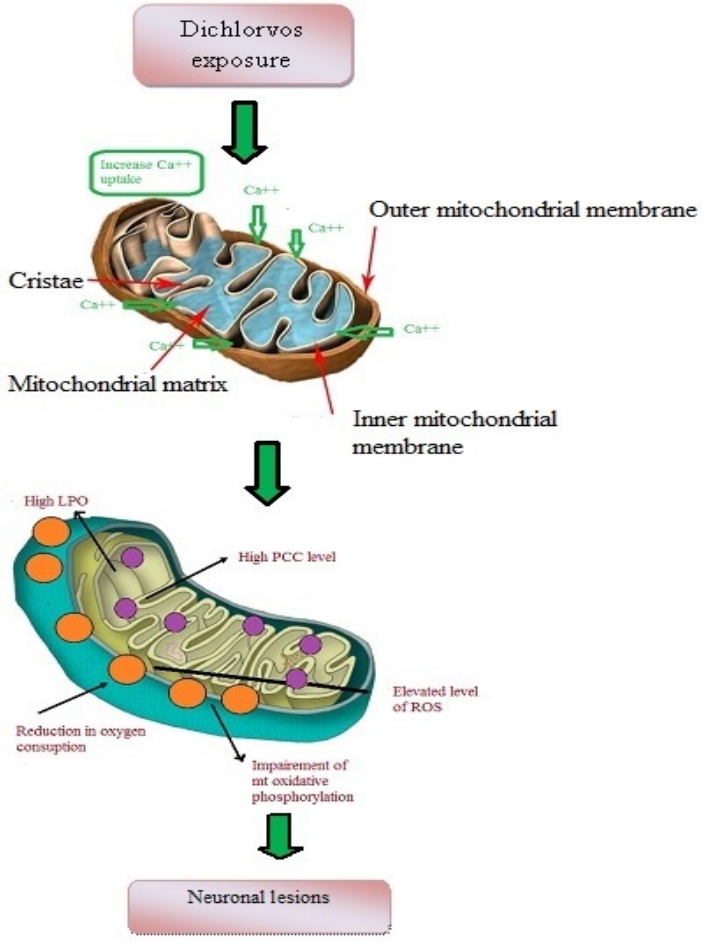

Figure 2. ROS production in mitochondria leading to neuronal dysfunction following dichlorvos exposure

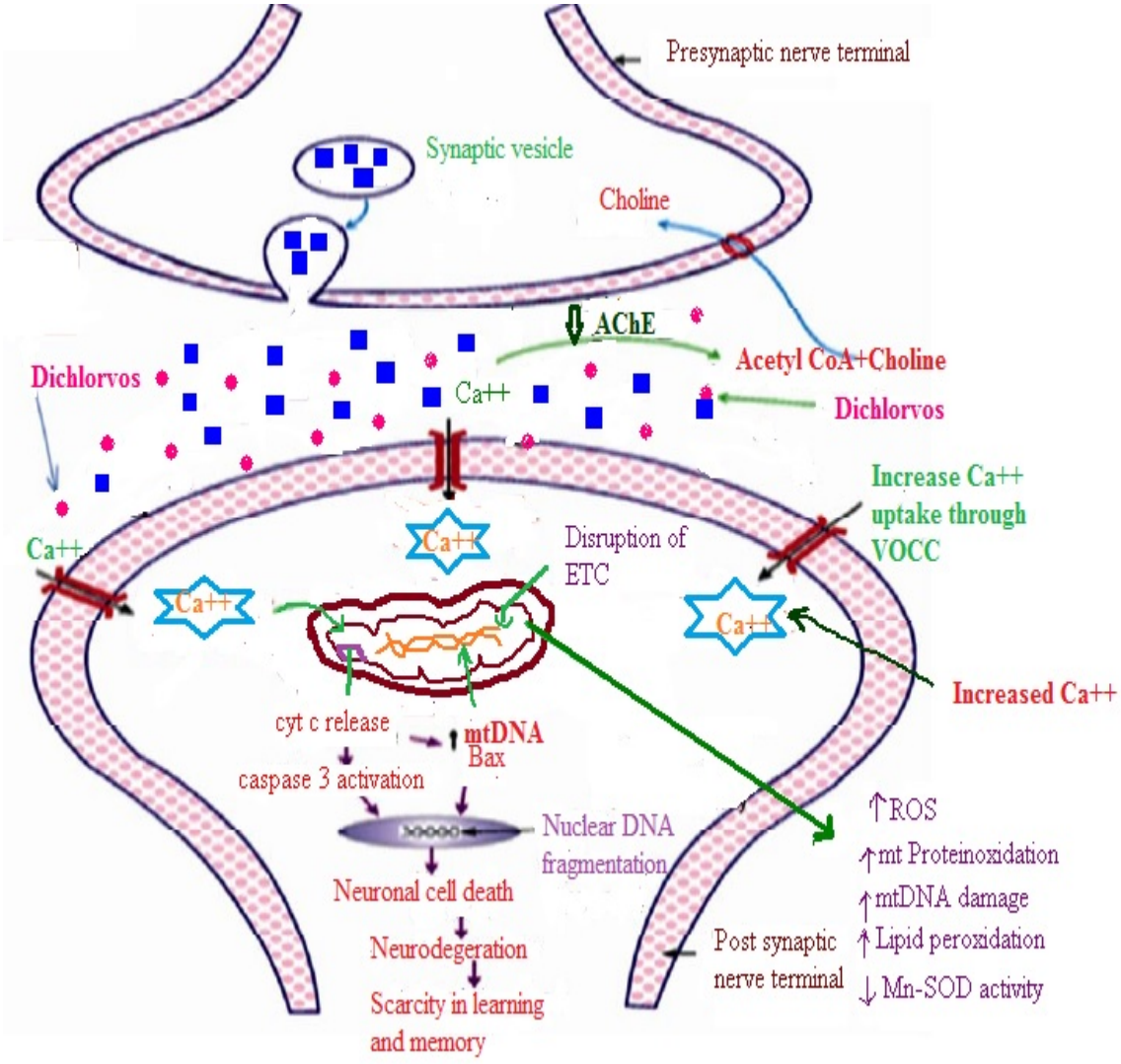

Figure 3. Dichlorvos induced disturbance in cholinergic circuit