Does exposure to the organophosphate chlorpyrifos induce Parkinson's disease and depression-like behavior similar to rotenone and paraquat?

Master thesis

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Abstract

The cause of Parkinson disease is unknown, but epidemiological studies suggest that there is an association between pesticides and this neurodegenerative disease. Investigations have proven that rotenone and paraquat are emerging Parkinson disease model systems, since they cause nigrostriatal dopaminergic degeneration. Moreover, depression is a prevalent non-motor feature of Parkinson disease. In this paper, we studied to see whether chlorpyrifos is able to elicit Parkinson disease and depression-like behaviors similar to that seen in paraquat and rotenone exposure. Our findings indicate that chlorpyrifos does induce depression-like behavior similar to paraquat and rotenone, but it failed to elicit two hallmarks of PD which are loss of dopamine neurons in the substantia nigra and increased levels of α-synuclein.

Key words: Rotenone, Paraquat, Chlorpyrifos, Parkinson's disease, Depression. Dopamine, Serotonin, Acetylcholine.

Abbreviations: Parkinson's disease PD. substantia nigra SN. dopamine DA. serotonin 5-HT. ventral tegmental area VTA. nucleus accumbens NA, dihydroxyphenylalanine DOPA. dopamine transporter DAT. cerebrospinal fluid CSF. serotonin transporter SERT. vesicular monoamine transporter VMAT, dorsal striatum DSt. Acetylcholine ACh. acetylcholinestrase AChE. Nicotinic acetylcholine receptors nAChR. Muscarinic acetylcholine receptors mAChR. central nervous system CNS. cerebrospinal fluid CSF. positive tyrosine hydroxylase TH^{+}. chlorpyrifos CPF. organophosphate OP. Paraquat PQ. reactive oxygen spices ROS. substantia nigra pars compacta SNpc. homovanillic acid HVA. Organophosphates OP. Chronic OPs induced neuropsychiatric disorders COPIND. chlorpyrifos CPF. chlorpyrofos oxon CPO.

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Introduction:

In recent years, there has been increasing concern regarding developmental exposure to pesticides in agricultural areas, where poor rural population live and work in close contact with these compounds [20]. Moreover, pesticides can be released into soil, air, and water from agricultural settings [20] and disperse on huge scales. Chemical pesticides have been an advantage for farming communities to eradicate pests and endemic disease, as well as to increase agricultural production, but on the other hand they can cause adverse health problems in humans. Current studies strongly supported the hypothesis that pesticides can induce physical and mental problems, thus further increasing the need for investigating the possible health effects of this diverse group of chemicals [22]. Such investigations may aid in revealing the mechanisms underlying many diseases, discovering proper medications for these diseases, and finding a possible solutions for reducing the consequences of large-scale pesticide use.

Paraquat (PQ) and rotenone are commonly used herbicides that induce several health effects among their users [1]. Both of them induce neurodegeneration, especially of dopaminergic nigrostriatal neurons [2][3], but through different mechanisms and pathways. PQ and rotenone are assumed to play an important role in the pathogenesis of Parkinson's disease (PD). Moreover, it has been hypothesized that long-term exposure to these two pesticides may cause behavioral changes [9]. PQ and rotenone cause dopamine (DA) depletion and accumulation of α-synuclein, which are two hallmarks of this disease [4]. In addition, rotenone can induce degeneration of serotonergic neurons as well as dopaminergic neurons in midbrain. Since deficiencies in serotonergic (5-HT) system are a hallmark of human depression [27], it has been assumed that rotenone may also induce depression-like behavior [14].

On the other hand, the primary mechanism of toxicity in chlorpyrifos (CPF) exposure involves inhibition of acetyl cholinesterase (AChE) [22] which is an essential component of nerve transmission in the central and peripheral nervous system [13]. Due to this inhibition the breakdown of the neurotransmitter acetylcholine (ACh) is prevented, resulting in

accumulation of ACh and subsequently cholinergic toxicity, leading to changes in the function of nervous system [22].

Therefore, in this study we investigate the mechanisms of toxicity of rotenone and PQ, that are known to increase the risk of PD and depression-like behavior, to verify whether CPF pesticide which share some toxicological characteristics with rotenone and PQ, act via a similar mechanism of toxicity as rotenone and PQ, or by distinct mechanisms.

2.1 Parkinson disease (PD):

Parkinsonism is a progressive neurodegenerative disease characterized by bradykinesia, muscle rigidity and tremor [(2)]. These symptoms arise when 50-80% of the dopaminergic neurons degenerate in the substantia nigra pars compacta (SNpc) resulting in 80-90% depletion of DA in the putamen and caudate nucleus (Fig. 1) [(2)]. In this disease DA neurons in SN project to the striatum through nigrostriatal pathway, so the loss of DA in SN results in its deficiency in corpus striatum [(36)]. The striatum is responsible for balance, control of movement, and bradykinesia, and the loss of DA in this region then leads to motor dysfunction [(34)(30)]. Although mechanisms underlying death of dopaminergic neurons are still vague, it has been hypothesized that mitochondrial dysfunction may play a significant role [(7)]. The production of noradrenalin and 5-HT in neurons is reduced as well by this disease. This may explain the frequency of depression in these patients [(36)]. Interestingly, the neurons that transport ACh to the cortex of brain are also affected in this disease [(36)].

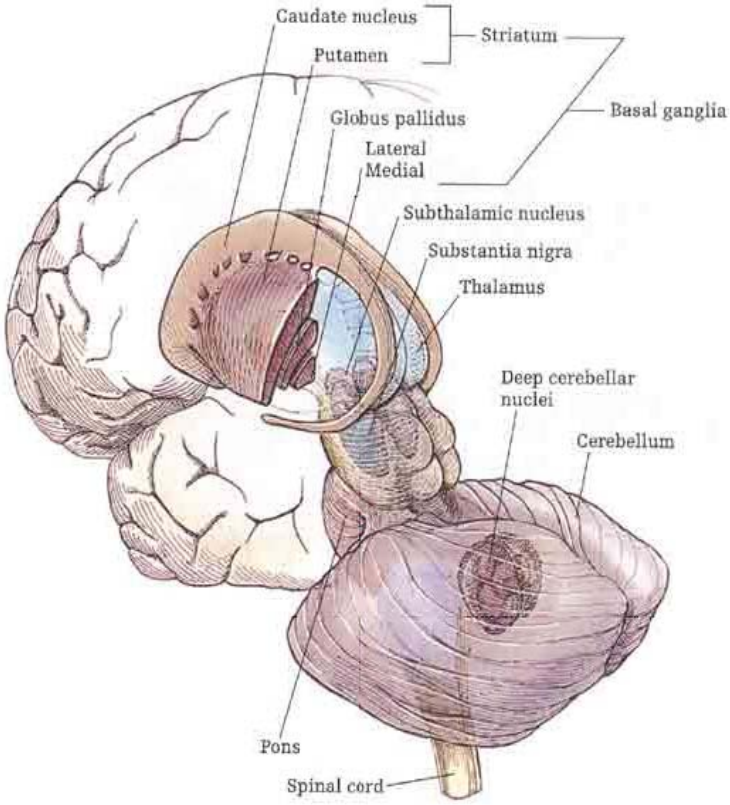

Fig. 1 Basal ganglia and related structures of the brain

The early symptoms of PD are aches, pain, disturbed sleep, anxiety, depression and slower walking [(36)]. The main features of PD are bradykinesia, rigidity and tremor. Non-motor

symptoms include depression (with a prevalence of 40-50%) [32], dementia and disturbed sleep [36].

PD is rare before age 40, but its incidence increases with age, and affects 1-2% of people over 65 years old [36]. Several genes contribute to the incidence of familial PD [36]. Other factors that increase its incidence include environmental factors, like exposure to pesticides [36][1], since these compounds are known to induce oxidative damage and mitochondrial dysfunction in SN and other brain regions [42].

2.1.1 ***Molecular Pathways of Neurodegeneration in PD:***

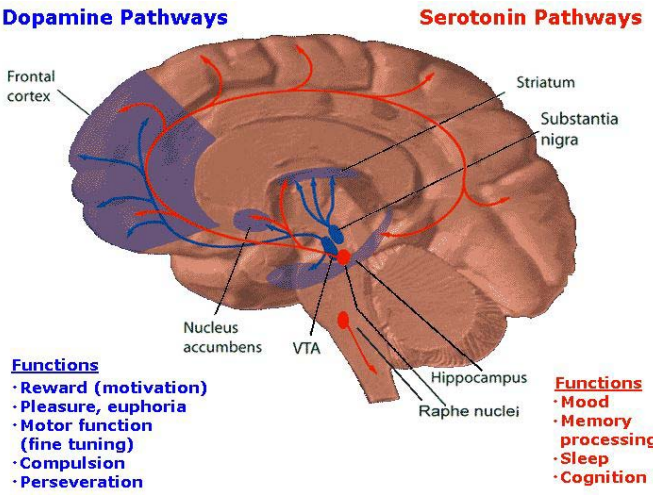

Fig. 2 neuronal pathways that degenerate in PD

Neurodegenerative processes in PD are not limited to dopaminergic nigrostriatal neurons but also include mesolimbic and mesocortical systems as well as serotonergic cells in the raphe nucleus [66]. In PD, DA depletion occurs in three pathways, but it occurs predominantly in the nigrostriatal system and to a lesser extent in mesocortical and mesolimbic pathways [74][73].

The dopaminergic systems in the brain originate from groups of cells that are located in the midbrain and the hypothalamus. The dopaminergic neurons are scattered into 3 groups, A8 and A9 in the substantia nigra pars compacta (SNpc), and A10 in the ventral tegmental area (VTA) [32][33].

The nigrostriatal pathway originates in the substantia nigra, passes through the hypothalamus, and terminates in the caudate nucleus and putamen (see Fig. 2) [68]. The nigrostriatal pathway contains 70% of brain DA and is involved in motor and motivation behavior [32]. Rigidity, tremor and akinesia in PD are due to the degeneration of nigrostriatal dopaminergic pathway. Moreover, post-mortem analysis of brains of PD patients with depression revealed that nigral neuron loss was 7 times greater than in non-depressed PD patients [37], indicating the role of striatum in depression incidence. The mesolimbic pathway originates in the VTA and

projects through the hypothalamus and terminates in the nucleus accumbens (NA) [68]. This pathway is involved in the reward process in brain [40]. It is believed that depression results from a reduction in the activity of reward systems [68]. Some DA neurons in the mesolimbic pathways pass through the NA and terminate in the frontal cortex which forms the mesocortical pathway [68].

Other pathways that are affected in this disease are serotonergic mesostriatal and mesolimbic pathways. The majority of 5-HT neurons in the brain are located in the raphe nucleus. The dorsal raphe nucleus stimulates the striatum (mesostriatal pathway), whereas the median raphe nucleus innervates the hippocampus (mesolimbic pathway) (Fig. 2) [68].

In addition hippocampal atrophy has been reported in PD patients with depression [76]. The hippocampus is known to have a role in memory and learning. Abnormalities in these cognitive behaviors are observed in depressed patients [62]. Although hippocampal DA levels are lower than in the other regions, the loss of these projections likely plays an important role in behavioral alterations [50].

2.1.2 Biochemistry of PD:

Dopamine:

DA dysfunction has always been the centre of attention of researchers as alterations in the levels of this neurotransmitter are involved in many brain dysfunctions, such as PD [43]. DA, a catecholamine neurotransmitter, is synthesized from dihydroxyphenylalanine (DOPA) by DOPA decarboxylase [34]. Since DA neurons are involved in cognition and modulation of behaviors, their degeneration not only causes PD but also induces changes in mood and behavior in these patients [33]. DA is produced in SN, located deep in the brain stem, and transports messages between striatum and SN [30]. DA controls several functions, such as locomotor activity, cognition, emotion, neuroendocrine secretion and coordination of body movements [31][34][43]. These functions of DA are mediated by the activation of 5 receptor subtypes: D1-like (D_1, D_5) and D2-like (D_2, D_3, and D4) [34]. DA is loaded into synaptic vesicles via a vesicular monoamine transporter (VMAT), whereas the duration of its actions in the synaptic cleft depend on re-uptake by the membrane dopamine transporter (DAT) [34]. Homovanillic acid (HVA) is the major DA metabolite, and is present in brain and cerebrospinal fluid (CSF). Nearly all HVA in the CSF originates from the brain, so this reflects DA turn over in the central nervous system (CNS) [32].

Serotonin:

Degeneration of serotonergic cells reduced brain 5-HT content and alterations in the activity of 5-HT receptors are reported in post-mortem studies of PD patients [(69)(77)], illustrating the involvement of 5-HT in PD.

Serotonin or 5-hydroxytriptamine (5-HT), which is synthesized from the amino acid tryptophan, is mainly found in the raphe region of the pons and upper brainstem and projects to the forebrain [(34)]. 5-HT loading into synaptic vesicles occurs via VMAT, whereas re-uptake from the synaptic cleft is regulated by the membrane serotonin transporter (SERT), which is crucial in 5-HT homeostasis [(41)]. Serotonergic cell bodies in the raphe nucleus project to the SNpc and VTA and their terminals project to dorsal striatum (DSt) and NA [(15)]. 5-HT receptors, including 5-HT_{1A} and 5-HT_{2C}, play key role in behaviors and loss of function of these receptors causes widespread psychiatric disorders, such as depression, anxiety, and schizophrenia [(34)].

Acetylcholine:

In addition to the dopaminergic and serotonergic systems, the cholinergic system also undergoes degeneration in PD, resulting in deficits in ACh at synapses [(75)]. ACh is synthesized from acetyl coenzyme A and choline [(34)]. ACh is the neurotransmitter of both sympathetic and parasympathetic paraganglionic neurons [(34)]. The postsynaptic actions of ACh are terminated by acetylcholinesterase (AChE) which cleaves ACh [(34)]. ACh exerts its actions by two types of post synaptic receptors: nicotinic and muscarinic ACh receptors. Muscarinic receptors (mAChR) are highly expanded in the striatum and forebrain regions [(34)]. These receptors are also present in the ganglia peripheral nervous system and mediate cholinergic responses of autonomic organs [(34)]. Nicotinic acetylcholine receptors (nAChR) are expressed on dopaminergic terminals in the DSt and regulate dopamine release in this region [(46)(37)]. Consequently, nAChR are involved in various neurobiological systems including depression [(37)]. Moreover, cholinergic pathways in the brain are associated with a wide range of human and animal functions such as hunger and thirst, thermoregulation, respiration, aggression, and cognition [(22)].

2.1.3 Pathological hallmarks of PD:

Loss of DA neurons in SNpc is one of the pathological hallmarks of PD. Even patients with mild symptoms of PD already lost about 60% of their DA neurons. Although loss of DA

occurs in other regions of CNS, it is the depletion of DA cells in the SNpc that causes motor manifestation of PD [78].

Another pathological hallmark of PD is the presence of Lewy bodies. Lewy bodies have an eosinophilic core, and a surrounding pale halo. They are frequently seen in the brain (mainly in SN, dorsal nucleus of the vagus, and nucleus basalis) of patients with PD [78]. α-synuclein is a dominant protein in Lewy bodies and abnormal accumulation of this protein in Lewy bodies has been detected in PD [35].

α-synuclein is a 140 amino acid neuronal protein that plays an important role in the pathogenesis of some neurodegenerative diseases [35]. In fact it plays a role in degeneration of nigrostriatal dopaminergic neurons seen in PD [17]. Also, α-synuclein is known to be an inhibitor of tyrosine hydroxylase (TH; an essential enzyme for dopamine synthesis). So reduction in the concentration of α-synuclein increases the TH activity and consequently DA synthesis [35]. Another role of α-synuclein is regulation of SERT function [41]. Increases in the levels of this protein gradually reduce 5-HT uptake [41]. Since abnormalities in α-synuclein are related to the developmental of PD, we can assume that changes in α-synuclein induce reduction in SERT levels, which result in the 5-HT abnormalities as observed in PD [41].

2.2 Depression:

According to DSM-IV depressive disorder is "a mental disorder characterized by an all-encompassing low mood accompanied by low self-esteem, and by loss of interest or pleasure in normally enjoyable activities".

Depression is a prevalent non-motor feature of PD, which strongly influences the patients' quality of life. The frequency of depression among patients with PD is around 40-50% [13] indicating that this disorder is highly related to PD. Several studies showed that depression is present in patients many years before developing motor symptoms [38].

In neuroscience it is supposed that CNS controls and defines all aspect of behavior and is responsible for integrating sensory and motor patterning [30]. Many of the behavioral diseases have a neurochemical origin, and have their root in abnormal neurotransmitter activity [30].

The etiology of depression in PD is complex and multifactor. However destruction of dopaminergic neurotransmitters in msocortical and mesolimbic in addition to loss of 5-HT, ACh, and noradrenalin neurons in brain, have been suggested as a hypothetical mechanism of

depression in patients with PD. This hypothesis also has been supported by postmortem data [40].

2.2.1 Animal Models of Depression:

One of the major problems in the research on the neurobiology of depression is the lack of validated animal models [40]. Nevertheless, some of the tests, such as forced swim test, learned helplessness, and elevated plus-maze have been very useful and reliable in assessing depression behaviors.

Forced swim test:

This test is one of the most used animal tests for studying depression, and the depressive like behavior is evaluated by assessing the immobility of the animal. In this test, the animal is placed in a tank containing water, and a researcher measures the duration of immobility in the animal. Antidepressant medicines decrease the time of immobility [40].

Learned helplessness:

Another commonly used test that evaluates depression is this test. In this test rats are exposed to uncontrollable events or shock in which escape is impossible. This test cause several changes that are similar to depression [40].

Elevated plus-maze test:

The apparatus is used in this test is like a 'plus' sign with two opposite open arms and two opposite closed arms and this plus shape is elevated above a platform. During the test, rat is placed in the centre of the plus maze and then is allowed to explore the maze. The number of entries into the open and closed arms is recorded and the percentage of time spent in the open arms and the percentage of open arm entries is calculated based on the total time. Anxiolytic activities are considered as increases in percentage of time spent in the open arms [26].

2.2.2 Pathophysiology of Depression in PD:

Dopamine:

Results from investigations in depression and PD indicate that dopaminergic dysfunction plays a role in causing depression symptoms [32]. Studies demonstrated that there is a high

correlation between depression-like behaviors and dopaminergic neuronal death in basal ganglia. In fact, post-mortem analysis of brains of PD patients with depression demonstrated that PD patients with depression had a strong reduction in DA level in VTA rather than in the nigrostriatal system [39]. Basal ganglia (including striatum), which contain the majority of DA in the brain, play an important role in mood changes and depression besides controlling motor functions. Importantly, since VTA is a part of mesolimbic and mesocortical system and these pathways play a role in depression, we can suggest that depression in PD patients can be the result of DA loss in these regions.

Serotonin:

Studies in 5-HT function in PD revealed that there is a linear relation between depression in PD and a lower concentration of 5-HT [39]. A huge loss of 5-HT neurons in the raphe nucleus was found at autopsy in PD patients with depression [39]. Thus alteration in the 5-HT function likely plays a role in depressed patients with PD [32]. Some investigations revealed that there is a high concentration of 5-HT_2 receptors in the frontal cortex of depressed suicide victims and depressed patients [39]. The other evidence in favor of a correlation between 5-HT and depression is the decreased level of 5-HIAA (a 5-HT metabolite) in the CSF of these patients. Many of these cognitive dysfunctions, such as depression, are associated with abnormalities in the serotonergic system and with a reduction in SERT binding in the mid brain, temporal lobes and thalamus [41].

Although the role of DA in PD is proven, a direct association between DA and depression is still a matter of debate since depression in PD is not highly correlated with HVA levels in CSF (Mayeux 1990) [32]. Moreover, L-dopa, which alleviates the motor function of this disease, does not always reduce depression in these patients [32][47], indicating that just some depression in PD may be dopaminergic in origin [45]. Thus, the available data support the view that although DA depletion may be associated in a way with mood alterations, it is not solely responsible for inducing depression seen in PD, so changes in 5-HT function may play a greater role rather than DA in depression [47].

The last two factors, α-synuclein and AChE, are involved in generating depressive like behavior in PD patients indirectly through influencing DA and 5-HT levels in the brain.

α-synuclein:

α-synuclein is identified as a substance regulating dopaminergic and serotonergic neurotransmissions through modulation of SERT activity and inhibition of TH [41]. An increase in the level of brain α-synuclein decreases 5-HT uptake by human SERT [41] and reduces TH activity leading to decreasing DA synthesis [35]. That α-synuclein can modulate these monoamine transporters suggest that this protein may be involved in psychiatric disorders such as depression [42].

Acetylcholine:

Results from a swim test on rodents demonstrated that a depressive situation has a profound influence on ACh signaling in the nucleus accumbens, and that there is a direct relation between elevated ACh and depression-like behavior [44]. In fact, DA release is regulated by activation of nicotinic receptors in the dorsal striatum, and since dopaminergic activities result in striatal long-term depression [37][46], it can be concluded that ACh is involved in depression-like behavior indirectly.

Pesticides:

The inappropriate use of pesticides is frequent in developing countries, where it leads to extreme exposure and high risk of poisoning [20]. Some of these pesticides may act via a common mechanism of toxicity. Among these agricultural chemicals, rotenone and paraquat are well known pesticides that predominantly affect the dopaminergic systems [3]. Since one of the hallmarks of PD is losing 50 to 80% of DA in the SN, it has been hypothesized that pesticides play an important role in the onset or progression of this disease [16][2].

In this study, we will focus on rotenone and paraquat as reference pesticides to investigate whether organophosphate chlorpyrifos (CPF) uses the same mechanism of toxicity leading to PD and depression-like behavior.

3.1 Rotenone:

Rotenone ($C_{23}H_{22}O_6$) is a widely used pesticide. This substance is a naturally complex ketone, extracted from the roots of Lonchocarpus Cappassa [3]. Rotenone has a tendency to biodegrade within just several days, regardless of on how many acres it has been spread [3]. This pesticide is tremendously lipophilic, thus it can easily cross the cell membranes without any transporter [4]. So, it freely crosses the blood-brain barrier and passes into the brain, where it accumulates in mitochondria [4]. In fact, rotenone treated rats developed many key features of PD, including loss of dopaminergic neurons in SN, increased oxidative stress and α-synuclein aggregation [5][12]. Moreover, several studies reported that rotenone is able to produce depression-like behaviors through reducing the levels of 5-HT and DA in the brain [13].

3.1.1 Mechanisms of rotenone toxicity:

Based on several investigations, the toxic effect of rotenone is multifactorial [3]. Rotenone can induce its toxicity via 1) oxidative stress; 2) induction of apoptosis; 3) accelerating the formation of α-synuclein aggregation and fibrillation [3]; 4) killing serotonergic neurons [13]. It is important to note that apoptosis and killing serotonergic and dopaminergic neurons can be a consequence of oxidative stress.

1) Rotenone mainly exerts its toxicity via oxidative stress in brain. Although the origin of this oxidative damage is not well understood two pathways have been assumed as the main sources of generating reactive oxygen spices (ROS) formations. a) impaired complex 1 activity and b) enhancement of activated microglia.

a) Complex 1 inhibition and oxidative stress: Rotenone has been reported to be a potent mitochondrial complex1 inhibitor [(7)]. Since rotenone is very lipophilic, it can cause homogeneous complex 1 inhibition across brain regions [(6)]. Complex 1 inhibition has several consequences, such as decreased ATP production, and oxidative damage [(12)]. Mitochondria are the main source of ROS. Although ROS formation usually occurs at a low level during mitochondrial respiration [(12)], high ROS generation, seen in the SN of PD, disrupts mitochondria and cellular components [(12)]. In fact, it has been hypothesized that the primary mechanism behind chronic inhibition of complex 1 is cumulative oxidative damage (Sherer et al. 2003b) [(3)]. Rotenone toxicity has been considered to originate from oxidative stress in brain, including decreased levels of lipids, proteins, and oxidative modifications to DNA [(3)]. Chronic complex 1 inhibition also induces damage in TH positive (TH^+) neurons in SNpc and finally decreases TH protein levels in the DSt (nigrostriatal DA system) [(3)(12)].

b) Rotenone, microglia, and the production of ROS: rotenone is known to activate microglia leading to enhanced production of ROS [(79)]. Thus, microglia induces neurodegeneration by the release of ROS [(79)]. Microglia activation is a hallmark of neurodegenerative diseases such as PD [(79)]. Since SN has the highest density of microglia in the brain, an increased level of reactive microglia in the striatum and SN of patients with PD has reported [(3)].

2) Rotenone and apoptosis: rotenone is supposed to exert its cytotoxicity via the initiation of apoptosis [(3)]. Rotenone treatment activates the p38 MAP kinases and JNK pathways which results in apoptosis and death of dopaminergic neurons [(70)]. JNK and p38 MAP are activated by cell-stress such as oxidative stress and toxic chemicals insults [(70)]. In fact, cell apoptosis is again the consequence of oxidative stress since MAP kinases and JNK are activated due to presence of excess ROS. This programmed cell death, apoptosis, contributes to variety of neurodegenerative disease such as Parkinson [(55)].

3) Rotenone and α-synuclein: rotenone infusion induces α-synuclein positive cytoplasmic aggregation in nigral neurons [(6)]. One of the hall marks of PD is presence of Lewy bodies containing α-synuclein [(6)]. It has been reported that fibrillation of α-synuclein highly

increased by rotenone exposure in SN and striatum, as it is one of the most effective pesticides in developing fibril formations [3]. In this part, again it seems that oxidative stress may contribute to α-synuclein aggregation [12].

4) Killing serotonergic neurons: Rotenone disrupts vesicular transportation through depolymerisation of microtubules, which leads to accumulation of vesicles in the soma. The high amount of 5-HT in the cytosol may be associated with the cell death due to oxidative degradation of 5-HT, which generates reactive oxygen [14]. In fact, decreased 5-HT contents in the striatum are not affected unless high dose rotenone or long term low dose rotenone used. This reduction of serotonergic neurons in the striatum is responsible for non-motor symptoms including depression seen in patients with PD [65].

3.1.2 Rotenone and dopamine:

Depolymerisation of microtubules plays a key role in determining the selective toxicity of rotenone on DA neurons [14]. Rotenone, through oxidative stress, causes microtubule depolymerization which disturbs vesicular transport, resulting in accumulation of vesicles in the soma that leads to increased oxidative stress [14].

It is reported that DA neurons of nigrostriatal pathway are more vulnerable to oxidative stress induced by rotenone than DA neurons in mesolimbic or mesocortical pathways [80]. This vulnerability may be associated with the high density of microglia in nigrostriatal pathway, but it is not well defined why DA neurons in VTA are more resistant to rotenone than nigral DA neurons [80].

All in all, rotenone may induce its neurotoxic effects through the combination of some or all of these factors [3].

3.2 Paraquat:

Paraquat (PQ), similar to rotenone, is a commonly used herbicide that induces several health effects among its users [2] such as eye injury, nose bleed, irritation of skin, shortness of breath, nausea and vomiting. Moreover, acute poisoning by high levels of PQ affects lung, liver, kidney and brain [3]. It has been reported that PQ is a potent factor for development of PD [1] since it causes selective degeneration of dopaminergic neurons in the SNpc [19].

Exposure to PQ occurs through inhalation, oral ingestion, and skin [9]. It is poorly absorbed via inhalation, but ingestion of 50 mg/kg can cause death within just few days [9].

3.2.1 Mechanisms of paraquat toxicity:

It has been hypothesized that chronic treatment with PQ induces degenerative effects on mesocortical, mesolimbic and nigrostriatal dopaminergic pathways [(1)(2)(3)(11)], which reduces DA levels in the caudate-putamen, SN and VTA [(16)]. In addition, in PQ-treated mice we can observe that the reduction of DA is usually accompanied by a decline in the amount of HVA, which is one of the main DA's metabolites [(19)].

PQ, as a candidate neurotoxicant, has been verified to induce its neurotoxicity via different mechanisms [(3)]. This herbicide induces its toxicity through 1) generation of ROS, 2) induction of apoptosis, 3) formation of α-synuclein, and 4) reduction of TH^+ neurons [(17)].

1) PQ generates ROS and in this part we will mention two main pathways.
a) PQ is a strong redox cycler and yields a high amount of ROS [(3)(19)]. The process of redox cycling begins with the enzymatic one electron reduction of the herbicide, proceeds with the electron transfer to molecular oxygen and leads to the ROS [(18)(86)]. Generation of high amount of ROS is known to play a key role in PQ cytotoxicity [(3)]. ROS production, and consequently oxidative stress is directly associated with dopaminergic injury and selective dopaminergic neurodegeneration [(3)(18)(19)]. However, the exact mechanism behind this selective dopaminergic neuronal death by oxidative stress is unknown [(18)]. Based on one study, PQ-induced oxidative stress is mainly cytosolic, while rotenone-induced oxidative stress usually occurs in the mitochondria [(10)].
b) PQ has been shown to generate ROS in microglia via activation of NADPH oxidase. In fact, microglia are among the main producers of ROS, leading to damage in neurons [(81)]. It is important to note that although activation of microglia is assumed to be a consequence of the process of degeneration in PD, the toxic substances released by glia could considerably contribute to the progression and propagation of neuronal degeneration even when the primary cause of the neurodegeneration has disappeared [(84)].

2) Activation of JNK, and to a lesser extent p38, mediates the PQ-induced apoptosis of dopaminergic neurons [(82)]. Oxidative stress again plays an important role in inducing apoptotic cell death since ROS, produced as a consequence of PQ exposure, will activate

signal transduction pathways, such as JNK. Thus, JNK MAP kinase mediates the apoptotic death of dopaminergic neurons after PQ exposure [82][18].

3) Recent studies on mice revealed that exposure to PQ leads to a significant increase of α-synuclein in brain, which is followed by the accumulation of α-synuclein-containing lesions among neurons of the SNpc [3].

4) Finally, PQ decreases the number of TH^+ neurons in the SN [16], which equals the reduction in dopaminergic neurons [19].

3.2.2 PQ and dopamine:

PQ does not inhibit DAT which excludes the potential role of DAT for transporting PQ and thereby selectively inducing damage to DA neurons through DAT [4]. Recent studies suggest that dopaminergic neurons may be more vulnerable to oxidative injury. Thus, ROS generation as the consequence of PQ exposure could at least in part answer the selective degeneration of DA observed in PQ exposure. Moreover, PQ causes a dose-dependent decline in dopaminergic neurons [9]. Also, the toxic effect of PQ on dopaminergic neurons has been found to be increased by α-synuclein aggregation [16].

It is important to note that no significant decrease in the level of 5-HT were observed after PQ treatment [66].

3.3 Organophosphates (OP):

OPs have been widely used in agricultural setting in developing countries [20]. One of the major concerns for individuals exposed to this compound is that OPs elicit developmental neurotoxicity, even when exposure is very low [25]. Some of the most used OP pesticides are chlorpyrifos, diazinon and parathion. Some investigations reported that toxicity was found not only among farmers who directly were exposed to OPs, but also among fishermen who were indirectly exposed to this compound and living within a 25 km radius of the cultivated lands [20].

OPs exert toxicity to target and non-target species [23] through a common mechanism of toxicity, which is inhibition of AChE. AChE is a widely distributed serine esterase that is inhibited by phosphorylation [22]. AChE is present throughout the central and peripheral nervous system of vertebrates and its regular physiological function is to hydrolyze the

neurotransmitter ACh [22]. Measurement of ChE inhibition is the most sensitive index of exposure since toxicity is not evident unless there is more than 20% of ChE inhibition [23].

OPs induce toxicity through several exposure pathways, such as absorption from skin, mucous membranes, respiratory tract and gastrointestinal tract; the latter is usually the result of suicidal ingestion [21].

Exposure to Ops can result in two well-defined toxicities:

3.3.1 Acute OP toxicity [20]:

The adverse effects of acute exposure are considered as an acute cholinergic crisis. These effects include paralysis of the neck, limb, bradycardia, ataxia, lethargy, and respiratory muscles [22][20].

OP compounds are electrophilic compounds with potency of phosphorylating the serine hydroxyl group, which is located in the active site of AChE [22]. Thus, OPs cause acute systemic toxicity by inhibiting AChE through phosphorylation, which results in accumulation of ACh at nerve endings all over the body, which is a sign of cholinergic toxicity [20][21] [22]. ACh binds to, and stimulates two types of cholinergic receptors, mAChR and AChR, which are located in both brain and spinal cord [22]. Accumulation of ACh and its action on these two receptors changes the function of the autonomic nervous system, the somatic motor neurons and the brain [23].

3.3.2 Chronic OP toxicity [20]:

Some of the adverse effects of chronic toxicity are not related to ChE inhibition, as they occur below the threshold of AChE inhibition [20]. The symptoms of chronic exposure include nausea, vomiting, diarrhea, salivation, lacrimination, trembling of hands, numbness and cramps of face, neck, arms, legs, and irritability. Moreover, it causes some respiratory problems such as cough, runny nose, wheezing, and shortness of breath, and irritability [20]. Toxicity in these situations is mediated by other mechanisms rather than inhibition of AChE, and it may be due to the ROS formation as a result of OPs exposure [20].

3.3.3 Neurobehavioral effects of chronic OP exposure:

Chronic toxicity of OPs has been linked to impaired neurobehavioral performances [20]. Chronic OPs-induced neuropsychiatric disorders (COPIND) are not associated with AChE

inhibition, but are related to destructions in other systems such as serotonergic and dopaminergic pathways [24]. Clinical symptoms of COPIND include confusion, lethargy, anxiety disorder, depression, psychotic symptoms; problems with short term memory, learning, attention, mood instability, and suicidal thinking [20][21][24]. Also, chronic neuropsychiatric disorders like anxiety, depression, and problems with memory have been reported in workers who have been exposed to OPs [21]. In fact, numerous studies have asserted that exposure to OPs is associated with emotional disorders, such as depression and anxiety [26].

3.4 Chlorpyrifos:

For further studying about the toxicity in OPs we concentrate on chlorpyrifos (CPF) as it is the most studied, used, and highly effective OPs [25][26] due to its stability and persistence [57]. Its main target of toxicity is the CNS. Recent studies revealed that exposure to CPF not only causes adverse effects on cholinergic system, but also in other neurotransmitter systems, particularly the serotonin 5-HT [26] and DA systems [29].

3.4.1 Mechanism of CPF toxicity:

CPF exerts its toxicity through different mechanisms including:

1) inhibition of AChE, 2) reducing monoamine levels, 3) activation of glia and enhanced ROS formation, 4) initiation of apoptosis, and 5) perturbation in mitochondrial function [57][55].

1) Cholinergic toxicity:

Some OPs inhibit AChE directly, while some of them require bioactivation, i.e., metabolisation to the active oxon-form. CPF needs to be metabolized to its active metabolite, chlorpyrofos oxon (CPO) [58]. CPF was originally thought to exert its neurotoxic effects through inhibition of AChE by CPO [25][50]. In fact, AChE inhibition results in accumulation of ACh in cholinergic synaptic and consequently over-stimulation of cholinergic receptors, mAChR and nAChR, in striatum and NA [48][49][57].

Although CPF is known to be an AChE inhibitor, inhibition of AChE is inadequate to explain all observed adverse effects of this pesticide on CNS [56]. It is therefore postulated that CPF also exerts its toxicity via reducing the level of monoamines that are known to be major contributors to neurobehavioral defects [26][27].

2) Monoaminergic toxicity:

CPF is able to reduce levels of monoamines, such as DA and 5-HT, via oxidative stress in the striatal pathway (striatum), mesolimbic (nucleus accumbens) pathway and hippocampus [52][50][49]. Even when CPF exposure is below the threshold for cholinesterase inhibition, CPF disrupts the function of monoamines [50]. Apparently, non-cholinesterase actions of CPF contribute to the emergence of neurobehavioral deficits [50].

5-HT: Exposure to CPF induces oxidative stress, which results in persistent changes in the 5-HT system [27][51]. 5-HT is a very sensitive system, even if the exposure is below the threshold for cholinesterase inhibition [25][26]. Studies on rats exposed to CPF revealed that they had an increase in plus-maze open arm activity that may be attributed to the loss of 5-HT synaptic function, and the role of 5-HT in depression [27]. Investigations revealed the evident effects of CPF on cellular damage and 5-HT level in hippocampus, the highly targeted region by CPF [50]. Moreover, measurements were conducted for three 5-HT synaptic proteins: 5-HT1A and 5-HT2C as postsynaptic receptors, and SERT as the presynaptic transporter. All of these proteins were reduced as a consequence of exposure to CPF [25][27]. The two receptors of 5-HT, 5-HT1A and 5HT2, play an important role in 5-HT related mental disorders [25]. Moreover, SERT regulates synaptic concentration of 5-HT, and is the main target for antidepressant drugs [25].

DA: oxidative stress contributes to CPF-induced neurotoxicity, but oxidative damage in the brain due to CPF exposure occurs only at doses above the threshold for systemic toxicity [50][51]. Recent studies showed minor effects on DA levels with CPF treatment [50]. Although CPF is able to alter DA metabolism, it does not cause frank degeneration of dopaminergic nigrostriatal neurons [52]. In fact, it is reported that DA content was unaffected in most brain regions except considerable depletion of DA in the hippocampus at either low or high dose of CPF [50]. In addition, acute CPF intoxication decreased DA content in nucleus accumbens [49]. One recent study reported that DA in striatum only affected at higher doses [50]. In contrast, another investigation found that CPF failed to influence striatal dopaminergic titer even when relatively high doses administered [60]. So, it is still a matter of debate whether CPF is potent enough to induce dopaminergic neurodegeneration in the striatal pathway. The available data shows that further studies should be undertaken to reveal the consequences of long-term exposure to CPF on striatal dopaminergic pathway.

3) Alteration of glia and enhanced ROS formation:

Glia are necessary for architectural modeling of the brain. CPF itself, rather than CPO, directly targets glia and alters their differentiation in synaptic formation. Moreover, CPF enhances ROS formation resulting in generation of oxidative stress [57][58][59]. As the brain is particularly vulnerable to oxidative stress due to its high rate of oxygen consumption and lipid content [59], production of ROS easily results in lipid peroxidation, protein fragmentation, and DNA strand breaks [59].

4) Apoptosis:

Studies showed that CPF and CPO induce neuronal apoptosis in CNS. CPF activates the three families of MAP kinases, the ERK1/2, the JNK, and the p38 which induce apoptosis. MAP kinases become activated by toxic chemicals and oxidative stress. Activated ERK1/2 and JNK have a pro-apoptotic role after CPF exposure [55]. Apoptosis is toxic endpoint of CPF toxicity in CNS that occurs independently of AChE inhibition [55]. Apoptosis is one of the leadings factors in neurodegenerative diseases such as PD [55].

5) Perturbation in mitochondrial function:

Another toxic end point of CPF is destruction of mitochondria. Recent studies suggested that mitochondrial inhibition results from CPF exposure. In fact, CPF is able to interfere with mitochondrial function and reduce it even at its low concentrations [55][52].

In addition, two investigations on permethrin and CPF studied the effect of these two compounds on TH and α-synuclein. They revealed that CPF had no influence on α-synuclein expression by itself, while permethrin was able to elevate level of α-synuclein [85][52]. Furthermore, CPF had no effect on TH and DAT expression.

DAT and TH are frequently expressed in dopaminergic terminals, thus reduction in DAT and TH cause dopaminergic depletion in nerve terminals [85]. Previous studies strongly supported the view that reduction in DAT and TH expression occurs in the brain of individuals with PD [85].

Effects of exposure to pesticides have been extensively documented both on behavior and neural systems. In this section we assess the differences and similarities in rotenone, PQ, and CPF to find out the main reasons underlying PD and depressive like behaviors in these patients, and conclude whether CPF is able to elicit PD and depressive-like behavior similar to PQ and rotenone.

4.1 CPF and rotenone:

Despite many differences between CPF and rotenone, both compounds mainly exert their toxicity via oxidative stress, but they induce it through several pathways (see table 1).

Firstly, rotenone and CPF cause oxidative damage through inhibition of mitochondrial complex 1. Impaired complex 1 activity in turn increases the generation of ROS. Although destruction of mitochondrial function induces ROS formation, it is assumed that the mechanism by which inhibition complex 1 kills neurons is cumulative oxidative damage.

Then, CPF activates glial cells which results in ROS formation and finally oxidative stress. Similarly rotenone is capable of stimulating microglia in SN and striatum to release ROS. The presence of activated microglia in PD is well documented, but it is important to note that although glial activation is generally considered to be a consequence of the process of degeneration in PD, the toxic substances released by glial cells could significantly contribute to the progression and propagation of neuronal degeneration even when the initial cause of neuronal degeneration has disappeared [(84)].

The consequence of oxidative stress which is produced through complex 1 inhibition and targeting glial cells due to CPF and rotenone exposure is very extensive.

Apoptosis in CPF is modulated by ERK1/2, JNK, and p38. Similarly, rotenone treatment induces posphorilation of JNK and p38 MAP kinases which are activated by oxidative stress. These pathways finally cause dopaminergic and serotonergic death [(70)].

Thus, CPF and rotenone by inhibition of complex 1, modulation of JNK and p38 signaling pathways are known to cause cell apoptosis, and finally death of DA and 5-HT neurons.

The most important differences between these two compounds are reducing TH^+ neurons and increasing α-synuclein expression in SN that are caused by rotenone exposure. In contrast,

CPF had no influence on inducing damage to TH^+ neurons and increase in α-synuclein which are the hallmarks of PD [85][52].

4.2 CPF and PQ:

Both herbicides exert their toxicity via oxidative stress, but there are some differences between their mechanisms (see table 1).

CPF, unlike PQ, induces ROS formation through destruction of mitochondrial function [55][52], while PQ differs from CPF and rotenone by its lack of effect on mitochondria. It is believed that PQ is a potent redox agent, and in this manner it mainly induces ROS formation.

CPF and PQ both stimulate glial cells, and since stimulated glia generate ROS, they cause damage in neighboring cells. glial activation is a consequence of the process of degeneration, but stimulated glial cells can contribute to the progression of neuronal degeneration [84].

Finally PQ is able to increase formation of α-synuclein in SN and reduce the amount of TH^+ neurons in the brain. While, CPF failed to increase expressions of α-synuclein or reduce the amount of TH^+ neurons.

Another way that CPF reduces the amount of dopaminergic neurons is via activating MAP kinase. Similarly, PQ has shown to induce dopaminergic neuron death through JNK, and in a lesser extent via p38 activation.

All in all, regardless of the differences in mechanisms all these pesticides are associated with neurodegeneration and share common features, including oxidative damage and subsequent oxidative damage and cell apoptosis.

	complex1 inhibition	Targeting glia cells	Activation of JNK and p38	Increasing α.synuclein	Reduction of TH^+ neurons	Inducing ROS formation	Cell apoptosis
Rotenone	✓	✓	✓	✓	✓	✓	✓
PQ	✗	✓	✓	✓	✓	✓	✓
CPF	✓	✓	✓	✗	✗	✓	✓

(Table 2)

Behavioral deficits are associated with adverse effects of PD. The frequency of depression among patients with PD is around 40-50% indicating that this disorder is highly related to PD [13].

The phenomenology of depression is complex and abnormalities in several areas of brain are involved in the depression etiology. Therefore, it is natural to observe different aspects of depression due to pesticides exposure since they exert their toxicity in different brain areas [71].

Rotenone and PQ exposure induce reduction in dopaminergic neurotransmitter. In addition, rotenone has reported to reduce 5-HT content. Since these neurotransmitters are associated with depression-like behavior, it has been suggested that exposure to these components induce depression in individuals in addition to PD [13].

Also, exposure to CPF was shown to result in depletion of 5-HT as well as ACh. Therefore, it is not unlikely that this compound is also able to induce depression-like behavior. Thus, in this part we again compare these three pesticides to illustrate how and where these pesticide treatments decrease the level of DA, 5-HT, and ACh (Table 2).

The most important difference between these compounds is that neither rotenone nor PQ alters the amount of AChE, while CPF is known to inhibit AChE in striatum, nucleus accumbens, and hippocampus [50][49]. One study reported that cholinergic cell groups undergo degeneration in PD, and are responsible for cognitive impairment in PD [75]. The cholinergic system has long been recognized to be involved in depressive disorders. It is proven that AChE inhibitors that increase the level of ACh in synapses can cause depression. This consequently sensitizes the muscarinic cholinergic receptors in the striatum, caudate nucleus, and frontal cortex [44]. Increased muscarinic receptors in these regions have been reported in patients with PD [75]. Therefore, it reinforces the concept that individuals exposed to CPF are vulnerable to manifest depressive behaviors.

CPF induces a significant decrease in DA content in NA (mesolimbic pathway) and hippocampus at either the low or high doses of CPF [50][52][49]. In contrast, CPF is not able to cause degeneration in nigrostriatal DA [52][50]. PQ inhibits dopaminergic neurons in three systems, nigrostriatal (SN and caudate putamen), mesolimbic and mesocortical pathways [16]. Rotenone also disrupts the amount of DA neurotransmitters in nigrostriatal system, but not in mesolimbic or mesocortical systems. It is not well defined why DA neurons in VTA are more resistance to rotenone than nigral neurons [80].

Rotenone and PQ reduce the amount of DA in several pathways, and each pathway causes different behavioral manifestations.

Rotenone and PQ destroy the DA in nigrostriatal pathway, so we expect to observe the same behavioral deficit in patients exposed to each of these pesticides. Post-mortem examination of brains of PD patients with depression revealed that nigral neuron loss was 7 times greater than in non-depressed PD patients [(37)]. So depression is associated to striatal dopaminergic depletion [(72)]. Thus, rotenone and PQ are likely to induce depression in the same way. In addition, one study indicated that the striatum plays an important role in learning and memory [(37)]. So we can assume that patients with PD also manifest some problems in learning and memory.

Furthermore, both CPF and PQ inhibit dopaminergic neurons in mesolimbic pathway. Mesolimbic DA system appears to be responsible for the maintenance of rewarded responding [(40)]. It has been hypothesized that depression results from a reduction in the activity of reward systems [(68)].

Although DA content was unaffected in most brain regions, CPF showed to decrease the DA level in hippocampus, but the other pesticides failed to reduce DA in this region [(50)]. Patients with PD were shown to have hippocampal atrophy, which is the result of neurodegeneration, indicating that hippocampus is also affected in this disease [(76)]. The hippocampus mediates cognitive features of depression such as memory impairment, thought of hopelessness, guilt, and suicidality [(45)].

Significant decreases in 5-HT were found in NA, striatum and hippocampus due to CPF exposure [(49)(50)]. In contrast, rotenone does not affect the number of 5HT neurons, except the highest dose used, which decreased 5-HT contents in the striatum and hippocampus [(65)(63)(64)(13)]. Also, no significant changes in 5-HT level were observed after PQ treatment [(66)].

Previous studies demonstrated that accelerated degeneration of 5-HT is more frequent in PD patients with depression than in PD patients without depression [(14)]. Besides, reduction of this neurotransmitter causes some other behaviors such as suicidal acts [(49)]. All these data support the idea that a reduction in 5-HT levels is a hall mark of depression in PD. So, CPF and rotenone exposure induce the same behavioral deficits as a result of 5-HT depletion. More studies on the role of 5-HT destruction in each 5-HT pathway would be helpful to improving

our knowledge in understanding the different aspects of depression caused by 5-HT depletion.

	Inhibition of AChE	Reduction 5-HT (hippocampus) Mesolimbic	Reduction 5-HT (Striatum) Mesostriatal	Reduction in DA striatal	Reduction in DA hippocampus	Reduction in DA mesolimbic	Reduction in DA mesocortical
Rotenone	✗	✓ conditional	✓ conditional	✓	✗	✗	✗
PQ	✗	✗	✗	✓	✓	✓	✓
CPF	✓	✓	✓	✗	✓	✓	✗

(Table 2)

Although the role of DA is well documented in PD, a direct relationship between DA and depression is still a matter of debate, as no correlation between HVA in CSF and depression in PD has been observed. In addition, L-dopa does not always alleviate depression in patients with PD [32] which can be interpreted that just some depression in PD may be dopaminergic in origin [45]. Therefore, DA depletion does not solely play a role in generation of depression in patients, and changes in 5HT may have a more prominent role in PD depression.

5. Conclusion:

The objectives of this study were to investigate whether CPF induces PD and depression-like behavior similar to that encountered in PD. To test this rational we studied two more pesticides, rotenone and PQ, as the reference pesticides mainly because many investigations have proven that rotenone and PQ are emerging PD model systems.

PD etiology has a multifactorial nature, so it is hard to conclude which factor exactly induces this disease. Therefore, one factor is not responsible for neurodegeneration in PD. Therefore, we compared the mechanism of toxicity of CPF with rotenone and PQ to investigate what are the main reasons underlying PD, and does CPF share any mechanism of toxicity as the other two pesticides.

One study reported that cholinergic cell groups undergo degeneration in PD, but since rotenone and PQ, as two emerging PD model systems, have no influence on the content of ACh, we can conclude that alteration in the level of this neurotransmitter is not the major contributor to this neurodegenerative disease.

It is well established that not only nigrostriatal but also mesocortical and mesolimbic pathways are involved in the degeneration of dopaminergic neurons in PD, but their projections are far less severely affected than the nigrostriatal DA system [(73)]. CPF, similar to PQ, was shown to target the mesolimbic pathway. It has been assumed that loss of mesocortical and mesolimbic DA has a role in PD patients with depression [(40)]. Thus, we can conclude that CPF can initiate depression-like behavior similar to PQ in patients.

Serotonergic system is also affected in PD, but its main role is increasing the risk of depression in patients with PD. CPF similar to rotenone degenerates serotonergic neurons. It is well documented that accelerated degeneration of serotonergic neurons is more frequently seen in PD patients with depression than in PD patients without it [(14)]. Moreover, there are some evidences that suggest 5-HT evokes DA release in the NA (involved in mesolimbic pathway) which is down regulated by $5HT_{2c}$. Thus, a decrease in the 5-HT level or increase in the $5\text{-}HT_{2c}$ inhibitory activity may be related to extra reduction in DA neurotransmitter.

Since this down regulation occurs in the mesolimbic pathway, it results in worsening of depression symptoms [13].

Thus, decrease in the amount of 5-HT synaptic elicited by CPF exposure resembling that seen in rotenone exposure. So we can conclude that CPF exposure, similar to rotenone, does induce depression-like behaviors. Furthermore, exposure to CPF like PQ decreases the DA content in NA, involving in mesolimbic pathway [49], which leads to depression [40][68]. Thus, we can support the hypothesis that CPF can cause depression behaviors similar to that seen in PD due to PQ exposure. Furthermore, in contrast to rotenone and PQ, CPF showed to decrease the DA level in hippocampus. Since PD patients with depression were shown to have hippocampal atrophy, it is tempting to speculate that CPF may even induce more severe depression in comparison with rotenone or PQ exposure.

All these neurotransmitters, involved in the pathogenesis of PD, are depleted in the brain of PD patients, but we are not aware what is exactly going on in the brain of PD patients, and what is the main reason for neurodegeneration and cell apoptosis in their brains.

Inhibition of complex 1 could be one of the main factors generating PD [3], but since recent studies revealed that the toxicity of PQ is not dependent on complex 1, unlike CPF and rotenone, we can conclude that complex 1 inhibition is not the main reason of this disease. Moreover, it is strongly suggested that JNK and p38 MAP kinases play a role in nigrostriatal dopaminergic death in PD, and as we previously showed, all three pesticides activate these proteins [70]. Another model of neurodegeneration which has a role in pathogenesis of PD is activated microglia. Again, all three pesticides have been shown to stimulate these cells, resulting in generation of oxidative stress.

The three factors that CPF failed to cause and has not in common with rotenone and PQ are inducing dopaminergic neurodegeneration in nigrostriatal pathway, reducing the number of TH^+ neurons and increasing the level of α-synuclein protein [85][52].

The major question that remained unanswered up to now is whether CPF is an emerging PD model system similar to rotenone and PQ or not. As previously mentioned, CPF did not induce frank degeneration of dopaminergic nigrostriatal neurons which is one of the cardinal sign of PD [49][52][50][60]. Only one study reported that DA was affected in the striatum when higher doses of CPF administered [50], while two other studies refuted it [52][60]. From these data we can postulate that CPF is not potent enough to degenerate DA in the striatum.

In addition, CPF neither increases the level of α-synuclein nor affects the number of TH^+ neurons. Recent studies strongly supported the view that α-synuclein has a novel role in degeneration of nigrostriatal dopaminergic neurons seen in PD [17]. Moreover, it reduces TH expression occurs in the brain of individuals with PD. In fact, TH expression is one of the biological markers for striatal dopaminergic neurotoxicity [85].

Our results point to the need for more investigations to see whether high dose long-time exposure to CPF is able to cause loss of SNpc DA neurons and increased levels of α-synuclein or at least to induce one of them. In addition, it should be examined in that case high dose of CPF induces just one of those factors, i.e. degeneration of DA in nigrostriatal pathway, is it potent enough to elicit the signs of PD. If it is not so, we can conclude that increased levels of α-synuclein, as well as DA depletion in SN, is central in the pathogenesis of PD to exacerbate the neurodegeneration of DA in nigrostriatal pathway.

Taken all the evidence together, CPF is able to induce depression-like behaviors similar to that seen in PD. But, whether it can induce PD symptoms is a matter of debate since it failed to elicit two hallmarks of PD, i.e., loss of SNpc DA neurons [78] and increased levels of α-synuclein [17]. As mentioned above long-term exposure studies on CPF should be undertaken to assess whether is it able to elicit hallmarks of PD or not.

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