



**STUDY OF THE EFFECT OF DICHLORVOS TOXICITY ON FORMALIN
INDUCED PAIN**

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ABSTRACT

Organophosphorus compounds are widely used to control insects and environmental pollution. The aim of this study is to determine the effect of toxicity to dichlorvos on formalin pain responses and changes of some biochemical parameters. In this study, 70 male NMRI mice were used (7 groups and 10 mice in each group). The first group received drinking water + plantar normal saline, the second group received free water + plantar formalin, the third, fourth, fifth, sixth and seventh groups received dichlorvos (20 mg/ kg) orally in drinking water + Formalin respectively for one, two, three, four and five weeks. Plantar subcutaneous injection of formalin 5% was used in order to induce pain and inflammation. Duration of licking and biting of the injected foot were recorded at intervals of 5 minutes to an hour by a chronometer. Plantar injection of normal saline in the control group caused a significant pain ($p < 0.05$) only in the first five minutes. Plantar injection of formalin in normal rats caused a significant pain ($p < 0.05$) at first, fourth, fifth, sixth, seventh and eighth 5 minutes. As a result, formalin produces a two-stage pain (the first phase: 0-5 min and the second phase: 15-40 min after injection). Dichlorvos increase significantly the first phase of formalin-induced pain as time dependant ($p < 0.05$), whereas decreased the second phase of pain significantly ($p < 0.05$). But, the proof of this claim needs further studies in other species and humans.

INTRODUCTION

Organophosphorus compounds are widely used to control insects and environmental pollution (Ahmadi, 1390; Salehi, 1391). Because of the easy access and high toxicity

of these compounds, the accidental and intentional poisoning with these compounds is extensive and each year thousands cases of poisoning have been reported. In Iran organophosphorus poisoning causes of death in people exposed to these compounds (Ahmadi, 1390; Salehi, 1391). These compounds cause inactivation of the AChE enzyme through phosphorylation of serine amino acids, leading to an accumulation of acetylcholine at nerve synapses (Ahmadi, 1390).

Dichlorvos is of the Organophosphorus insecticides widely used in agriculture, veterinary, and medicine (Ahmadi, 1390). Studies have shown that some organophosphate poisons produce free radicals causing oxidative stress and lipid peroxidation in living organisms (Ahmadi, 1390; Salehi, 1391). Also, studies confirm the incidence of neuropathy and the pain changes resulted of these compounds (Ahmadi, 1390; Salehi, 1391). Due to the wide use of these compounds as well as species diversity and different substituent of organophosphate poisons and different effects of them on tissues, conducting supplementary studies is necessary. Pain is one of the most important disorders in the body and is one of the main problems observed in different medical status as well as a clinical demonstration in most of diseases (Rang, 2003). Pain prevalence is high, so that 20% of people experience pain

in their lifetime. More than 20 percent of adults suffer from chronic pain, and in most cases it treatment, confront with some problems (Delroy, 2004; What, 2005). Despite the great advances in the field of medicine and pharmacy, the treatment of pain and its associated disorders, is one of the challenges for doctors and researchers (Rang, 200; Katzung, 2004). Since pain and inflammation, today, is the most common neurological disease, and despite the widespread use of organophosphate poisons there is no report on the toxic effects of pesticides on humans and animals pain and inflammation,

The purpose of this study was to determine the effect of dichlorvos toxicity on formalin pain response in mice.

Model of nerve injury

A variety of disease processes can affect the nervous system (Table 1) based on the target lesion (Schwann cells and axons) there are two kinds of peripheral nerve responses to injury. Diseases that primarily affect Schwann cells cause to loss the myelin and lead to fragments demyelination. On the contrary, its early neuronal and axonal involvement leads to degeneration. In some diseases, axonal degeneration followed by axonal degeneration (Robbins, 2007).

Fragmental demyelination

It occurs when dysfunction or death of Schwann cells, or damage to the myelin

sheath occur. In this condition, there is no primary axon abnormality. The process involves a number of Schwann cells and their inter-nodes, but others remain protected. The damaged myelin surrounded by Schwann cells and macrophages. The naked axons Stimulate remyelination and the population of cells in endometrial become differentiated to replace damaged Schwann cells. These cells multiply and surround axons and by the time the remyelination is done in naked parts of axons, myelin is made again. The resulted Inter-nodes of remyelination are shorter

than normal, and some of them are needed to cover the unmyelinated area. The resulted Inter-nodes of remyelination besides being shorter have smaller diameter compared to normal mode. Followed by repeated periods of demyelination and rebuilding of myelin, layers of Schwann cells are accumulated which in cross-cut are seen as concentric layers of Schwann cell cytoplasm and a large amount of a basement membrane that surround a fine myelinated axons (bulbous shape buds). Over time, many chronic demyelinating neuropathies cause somewhat axonal damage (Robbins, 2007).

Table 1: Causes and types of peripheral neuropathies

<p>Nutritional and metabolic neuropathies</p> <p>Diabetes, thiamine deficiency, pyridoxine deficiency, alcoholism, renal failure</p> <p>Toxic neuropathies</p> <p>Lead, arsenic, cisplatin, vincristine, organic solvents and organophosphate toxins</p> <p>Inflammatory neuropathies</p> <p>Guillain-Barre syndrome, chronic demyelinating inflammatory neuropathies, vasculitic neuropathy, leprosy, sarcoidosis</p> <p>Congenital neuropathies</p> <p>Congenital motor and sensory neuropathy (disease Charcot-Marie, Blackberry, Refsum disease), congenital sensory neuropathy, Leukodystrophy</p> <p>Miscellaneous</p> <p>Amyloid neuropathy, paraneoplastic neuropathy, neuropathy associated with abnormalities of immunoglobulin</p>
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Axonal degeneration

Axonal degeneration due to early axon destruction causes the secondary damage of the myelin sheath. Axon damage may be due to local events that have been developed in the nerve in a moment of time (e.g., Trauma or ischemia) or due to general abnormalities affects neuronal cell bodies

(neuropathy) or its axon (axonopathy) initiated. When axonal damage is due to localized lesions, such as denervation due to injury, distal nerve will suffer from Wallerian degenerative. In one day, axon will be degenerated and Schwann cells begin to degrade myelin and axonal fragments will be surrounded by them and small egg-

shaped components will be created (oval-shape myelin). Macrophages are called in the area and participate in axon and myelin debris phagocytosis. In neuropathies or axonopathies with slow progression evidence of myelin damage is negligible, because at any time, only a small number of nerve fibers are in degeneration. Degenerative changes are created in two or three of the distal nodes in base of proximal severed nerves and then are reconstructed. With axons re-growth, the base of the proximal degenerated axons, finds a new growth buds. The growth buds use Schwann cells, which have been empty due to axonal degeneration, as a guide to their movement, if properly placed in the distal nerve segment. The presence of a large number of fine, small-diameter axons in the form dense communities is suggestive of degeneration (reconstruction groups). The regrowth of axons is a slow process (1 to 2 mm/d) and is limited simply by the low velocity of components in axonal transport, tubulin, actin and intermediate filament movement. Despite its slowness, the process leads to the potential for improving the function following peripheral axon injury (**Robbins, 2007**).

METHODS AND MATERIALS

In this study, 70 male NMRI mice were used. The selected animals had equal conditions in terms of weight (25-30 g) and food. The animals were randomly assigned

to 7 treatment groups (each group, n = 10) and were kept in poly-bicarbonate cages under identical conditions of fed and free water. The first group received drinking water + plantar normal saline, the second group received free water + plantar formalin, the third, fourth, fifth, sixth and seventh groups received dichlorvos (20 mg/kg) (**Pesticides, 2011**) orally in drinking water (after calculating the daily water use by each group rats) + Formalin respectively for one, two, three, four and five weeks. Plantar subcutaneous injection of formalin 5% was used in order to induce pain and inflammation. Then the animal behavior was recorded by camera for an hour. Duration of licking and biting of the injected foot were recorded at intervals of 5 minutes to an hour by a chronometer (**Khayat nouri, 1382**). To induce pain and inflammation the plantar subcutaneous injection of formalin 5% by size 28 syringes (20 microliter) was used. The animal was then placed into a cylindrical container in the formalin test model and the animal behavior was filmed from mirror for an hour. The duration of licking and biting of the injected paw was recorded at intervals of 5 minutes to an hour by a chronometer (**Khayat Nouri, 1382**). Data were expressed as mean \pm SD and ANOVA and Tukey test at a significance level of $P < 0.05$ were used for data analysis.

RESULTS

Plantar injection of normal saline into the control group causes a significant pain ($P < 0.05$) only in the first five minutes. Plantar injection of formalin in normal rats caused significant pain ($P < 0.05$) at first, fourth, fifth, sixth, seventh and eighth 5 minutes compared to the rest 5 minutes. As a result

the formalin induced a two-stage pain (stage I: 0-5 and the second phase: 15-40 min after injection). Dichlorvos significantly increase ($P < 0.05$) the first phase of formalin-dependent pain, whereas decreased significantly the second phase pain ($P < 0.05$) (Figure 1-3).

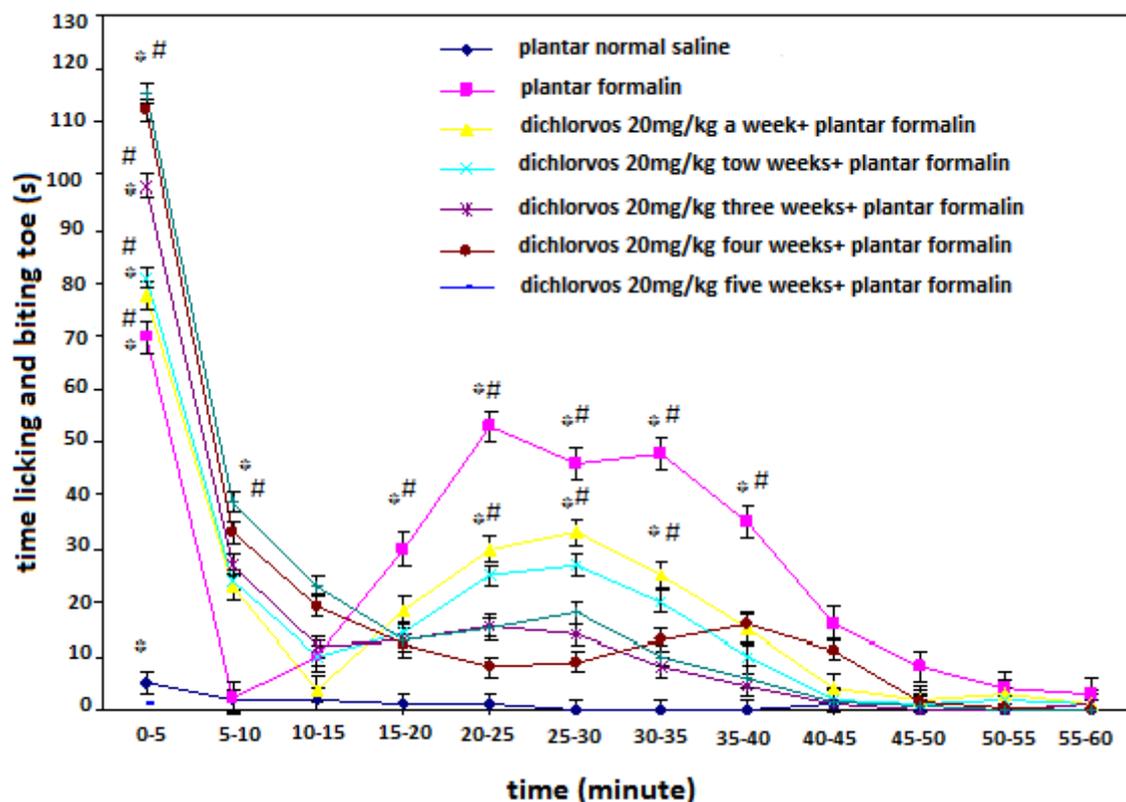


Figure 1-3: Effect of dichlorvos on formalin-induced pain (duration of licking and biting of the injected paw) at various times. ($P < 0.05$) * In comparison with other five minutes groups in intra groups. ($P < 0.05$) # in comparison with the first and second phase pain in other groups.

DISCUSSION AND CONCLUSION

In the present study after 20 μ l plantar injection of formalin 5% the licking and biting reactions were seen in the injected foot in first, fourth, fifth, sixth, seventh and eighth five- minutes. Given that the pain was very severe in theses five minutes compared to other times, it can be

concluded that the pain appears in two stages (the first stage: 0-5 m and second stage: 15-40 m after injection). In researches on Pain, some pain-relieving substances such as acetic acid, bradykinin, prostaglandins, potassium ions, histamine, serotonin, and nicotine were used to create pain and examine Behavioral, hormonal,

visceral and electrophysiological reactions. In this regard, formalin was used widely which creates normal behavioral reactions and known as formalin test (Tjolsen, 1992). In this test, the test different concentrations of formalin from 0.1 to 5%, as well as different volumes from 10 to 50 μ l, in various parts of the body, including the palms and soles, upper lip, into the peritoneum and colon are injected to create and study the behavioral and hormonal as well as somatic and visceral pain responses (Diaz, 1997; Ness, 1999; Ren, 1999; Shutov, 2006).

Following the injection of formalin, behavioral responses such as licking, biting, chewing and keeping the member motionless in two-stage form, were recorded. The first phase was started immediately 5-10 minutes after injection (called acute or neurogenic pain) and the second phase was started 15-20 minutes after injection for 20-40 minutes in the form of increase in behavioral reactions (known as chronic or inflammatory pain). Between the two phases, there is a 5-15 minute interval as a decrease in pain response (Tamadonfard, 1383; Ness, 1999; Tjolsen, 1992). In the present study, 20 μ l formalin 5% was used in order to induce pain and examine the reactions in rats. As mentioned before, plantar injection of different concentrations of formalin induces pain in rats (Tamadonfard, 1383; Nass, 1999;

Tjolsen, 1992). On the other hand, the pain responses were recorded based on measuring the duration of licking and biting the injected foot that based on the experiences, this approach of the recording is better than scoring (Ness, 1999; Porro, 1993; Tjolsen, 1992).

In one study, the duration of licking and biting of the injected paw with formalin (5%) was intense at intervals of 0-5 and 20-40 minutes after injection (Tamadonfard, 1383). Consequently, the two-phase formalin pain in this study did not conflict with previous reports and fully reveals that formaldehyde causes a two-phases pain. The first phase of formalin-induced pain is a neurogenic pain by direct stimulation of pain receptors and any chemical intermediate does not involved in this phase (Porro, 1993; Tjolsen, 1992). The second phase of formalin-induced pain is an inflammatory pain and occurs by involving the inflammatory mediators such as prostaglandins, bradykinin, histamine and enzymes (Porro, 1993; Tjolsen, 1992). Given that Dichlorvos, in the present study, increased the pain response in the first phase, it can be suggested that Dichlorvos might have a direct and indirect pain-relieving effect by impacting the release of pain and inflammatory mediators. However, due to the damaging effects of organophosphate toxins on nerve and induced neuropathy caused by these

compounds that have been reported in previous studies (Ahmadi, 1390; Salehi, 1391), it is expected that the pain increase in the first phase is due to nerve damage caused by Dichlorvos. Also, in the second phase the formalin-induced pain was reduced observed after consumption of Dichlorvos. However, further studies are needed to understand whether or not this poison has inflammatory effects. Several studies have shown that acute and sub-acute exposure to certain organophosphate toxins caused cholinesterase inhibition as well as increased production of free radicals and oxidative stress (Ahmadi, 1390).

Another study shows that following oral administration of malathion in rats the activity of antioxidant enzymes was changed and lipid peroxidation in erythrocytes and plasma was increased (Mohammad Nezhad, 1390; Salehi, 1391). It has been found that non-lethal doses of organophosphates caused some restructuring of the reproductive system and production of abnormal sperm by direct damage of DNA (Fattahi, 1386). Poisoning caused by exposure to organic insecticides, is one of the key problems in developing and developed countries. A wide range of acute poisoning occurs by pesticides, particularly organophosphate compounds (Choudhari, 1984). The mechanism of toxicity of organophosphorus compounds is to inhibit the acetylcholinesterase enzyme

(Sayim, 2005). When the enzyme is inactivated, acetylcholine accumulates in the nervous system, resulting in nerve stimulation (Kerem, 2007). Organophosphate pesticides can cause adverse effects on the tissues and blood factors (Antal, 1979). Oxidative stress in the blood and in different organs after exposure to organophosphates has been described in many articles. In summary, this study showed that Dichlorvos causes pain increase (first phase) and inflammation reduce (second phase) in mice. However, its confirmation needs for further studies of other species and humans.

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