Environmental Effect of Parathion Methyl on Bio-chemical Changes and Detoxification Capacity

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Abstract— The aim of this study was to evaluate the environmental effects of the organophosphate pesticides, parathion methyl (PM) at sublethal dosage (1)-on antioxidant defense system such as reduced glutathione (GSH) and glutathione S-transferase (GST) in liver, (2)-on plasma lipid ans glucose constituents: total cholesterol (TC), triglycerides (TG) and plasma glucose (PG) in vertebrate, the Wistar rats and to investigate if taurin was able to modulates the PM-induced changes in plasma levels of TC, TG and PG. All these parameters were measured in rats, which were orally given a single dose of MP (2mg/kg body weight for 10 days) and supplemented or not with taurin (diluted in the drinking water at 2%, for 10 days). In these conditions and after a 24h post-treatment, GSH significantly increased and GST activity decreased in liver and both plasma levels of TC and TG significantly increased in rats treated with PM. These last results were not relieved in taurin treated rats administered PM's. We conclude that (1) the administration of subchronic PM causes changes in the antioxidative systems in liver and (2) the organophosphate, methyl parathion interferes with lipids and glucose metabolism in rat and suggests that taurin, in mammalian animals, could reverse PM-induced lipid and glucose metabolism alterations.

Index Terms— OPC, PM, acethylcholinesterase, antioxidant, cholesterol, triglycerides, glucose, taurin, rat.

1. INTRODUCTION

Organophosphorus compounds (OPC) are an important class of organic chemicals and about 200 OPC are sold on the market in the form of thousands of different products to multiple uses: rodenticides, nematicides, herbicides, additives in plastic and oil industry. They are mainly used as insecticides on plants, animals and men [1]; [2]; [3]; [4]. Used during the Second World War to combat gas production and marketed the first time in 1944, the growth of organophosphorus insecticides or miticides was dated of early 1970, favored by the prohibition of using the compounds organochlorines. Less persistent than these, organophosphates have a highly effective power but pose an acute problem.

Different COP share some lipid solubility and their mode of action on the nervous system as inhibitors of acetylcholinesterase irreversibly, which makes them highly toxic. They block acetylcholinesterase whose role is to degrade acetylcholine (Ach) resulting in an accumulation of this neurotransmitter in the synapses which leads to an excessive stimulation of muscarinic and nicotinic receptors at the central and autonomic nervous systems, and the neuromuscular junction [3].

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Despite prohibitions or restrictions on use and the low persistence of these OPC, they are found in soil, surface and within living organisms in all countries of the world [5]. The origin of such pollution is linked to agricultural activities [6] and aquaculture [7], the Culicidae struggle in Mediterranean and tropical regions [8] and protection of agricultural storage rooms. The low persistence in the environment of the OPC has advantages, but requires that the insecticide is applied frequently, which increases the danger of exposure to wildlife. Significant concentrations of OPC were also measured in urban environments in sewage treatment plant effluents. These urban OPC sources are more difficult to identify, often resulting from domestic uses are difficult to control and assessable (pest control: human and animal and plant health company: houseplants and gardens) or deposits, legal or not, in inadequate storage facilities and permeable [9] which contributes to the persistence of these molecules (water, soil and environment).

This durable persistence is threatening to cause contamination through the respiratory tract, oral or dermal. All contaminated living forms are alterations of the nervous system due to the neurotoxicity of these molecules linked to inhibition of acetylcholinesterase [10].

In vertebrates, mammals contaminated by the OPC have symptoms whose severity depends on the dose on the duration of exposure. Cytotoxic and genotoxic effects were observed in rats after a single exposure to the OPC with an increase in the frequency of chromosome aberrations and the number of micronuclei in the cells of the spinal cord [11], damage to the molecule DNA [12] and irreversible liver disease. The OPC can induce in rats or mice reduced fertility (decreased sperm count, increase in the number of abnormal sperm) and a reduction in haemoglobin [13]. In humans, repeated exposure leads to a higher risk of leukemia and soft tissue sarcoma. The mammalian body is much more efficient than birds in detoxification OPC. Thus, birds are 100 times more sensitive than mammals Diazinon, a common insecticide [14].

Birds that do not die directly may undergo various physio-

logical effects. Weakened, they are more prone to hypothermia and predation and may be unable to attract a sexual partner, defend their territory or to raise their broods. Fish are particularly vulnerable to the OPC. A comparative study shows that the presence of organophosphates in water causes a decrease of 34.2% in the number of common carp (Cyprinus carpio) compared to a clean lake [15]. Also, always with carp, the OPC modify the activity of digestive enzymes [16].

Furthermore, non-cholinergic effects have been observed in humans and laboratory animals; in fact, the OPC, as other xenobiotics, generate reactive oxygen species that induce oxidative stress creating damage to cellular and tissue level when endogenous protective capabilities of the organism are exceeded [17]. The effects of oxidative stress induced by the OPC were studied in vivo in rats [18]; [19]) and in vitro in erythrocyte cultures and rat hepatocytes [20]; [21]) and show significant changes in the activity of antioxidant enzymes and suggest that free radicals and / or reactive oxygen species are involved in the toxicity of OPC.

Among the organophosphates, methyl parathion (MPT) has been extensively studied for its neurotoxic properties in mammals and fish [22]; [23]; [4] but also for its induction of oxidative stress. Recent studies show that exposure to TPM generates a high oxidative stress in fish of America (Brycon cephalus) and rats with induced free radicals, drastic changes in antioxidant enzyme systems and increased lipid peroxidation [24], 2009[25]; [26]. However, little is known of the effects of MPT on lipids and carbohydrates metabolism, although they play an essential role in all aspects of biological processes. Thus, variations of their levels in tissues and plasma are usually associated to the oxidative stress with various diseases (formation of gallstones, atherosclerosis and coronary heart disease cytotoxicity, DNA damage) [27], [28].

The purpose of this study is to evaluate, in the vertebrate, the effects of subchronic exposure to MPT (1) on the components of the antioxidant defense system such as reduced glutathione (GSH) and glutathione S-transferase (GST); (2) on lipids and carbohydrates components of plasma including total cholesterol (TC), triglycerides (TG) and plasma glucose (PG) and (3) to evaluate the protective effect of the taurine on CT, TG and GP using a mammalian, the wistar rat.

2. MATERIAL AND METHODS

The animals used for this experiment were adult male Wistar rats (Pasteur Institute in Algiers, Algeria) weighing 220 ± 20 g at the start of experimentation. They were penned and acclimated for 2 weeks in individual cages in temperature (+ 21 ± 1 ° C), humidity (70%) and lighting (12L / 12D) constant conditions. They were treated in accordance with the principles set out in the guide for the care and use of experimental animals [29]; [30]. The use of animals was reviewed and approved by the animal care committee of the institution where the experiments were conducted.

They receive a standard diet in the form of caps (UAB, Algeria). Food and water were provided ad libitum.

The animals were then divided into three experimental groups: 1 Controls, C (n = 6), 2 per Treaties bone with a solution containing methyl parathion, PM (n = 6), 3 per Treaties bone with a solution containing methyl parathion + taurine, PMT (n = 6).

Methyl parathion (C10H14NO5PS, Sigma-Aldrich, France) is administered orally at 2 mg TPM / kg / day for 10 days in 0.3 ml of olive oil daily by gavage (between 09:00 and 10:00). The dose used in our study is following previous work taken as a reference between 1/6 and 1/15 of the LD50 (LD50 Oral: 13-30 mg / kg [31]; [32]. In adult male rats this dose, conventionally used in experimental subacute and chronic toxicity studies, is between LD 50 and NOEL based on inhibition of acetylcholinesterase, which is 0.1 mg / kg / j [32].

NH2CH2CH2SO3H taurine (2-amino-ethane-sulfonic acid) is a product of the catabolism of cysteine. It is naturally present in all tissues, but particularly at high concentrations in the brain, retina, and myocardium, and exceptionally in leukocytes and platelets. The highest concentrations were found in neutrophils or taurine can be metabolized in N- chlotaurine, the latter according to [33] is an anti-radical agent opposite to carbonyl species. Treatment consists of daily administration of taurine per os diluted in drinking water at a rate of 0.2% [34]; [35] for 10 days.

Control animals received daily gavage with 0.3 ml of olive oil orally for 10 days.

At the end of treatment, the rats were sacrificed by decapitation between 09:00 and 11:00 AM. The arterio-venous blood collected in heparinized tubes is rapidly centrifuged (15 min, 3000 rev / min) and plasma aliquots stored at -30 ° C. For each animal, the liver was weighed, and samples of sucrose were ground in phosphate buffer (pH 7.5), centrifuged and the supernatant was stored at -20 ° C for reduced glutathione (GSH), glutathione S-transferase (GST) and acetylcholinesterase (AChE) assays.

The dosage of the acetylcholinesterase (AChE) in the liver was carried out according to the method [36]. Hepatic reduced glutathione (GSH) was measured according to the method [37] and the glutathione S-transferase (GST) as that of [38]. Glucose, cholesterol and plasma triglycerides were measured by enzymatic assays of glucose oxidase [39], cholesterol oxidase [40] and peroxidase glycerol [41] (kits Randox Laboratories).

The experimental results are expressed as mean ± standard deviation (m ± SD). The comparison between the average is carried out using the Student's T test. The significance of the results is estimated variance analysis (ANOVA). The difference between the experimental groups was considered significant for $p < 0.05$.

3. RESULTS

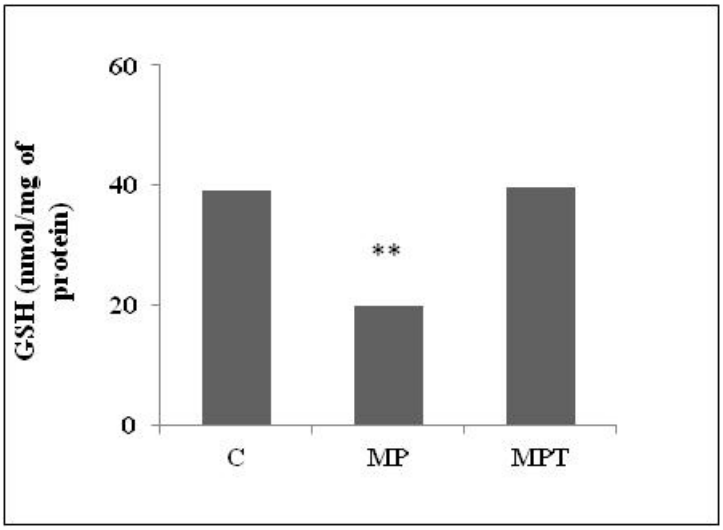
3.1 PM's effect on the liver acetylcholinesterase activity in the rat:

This organophosphorus molecule administered orally for ten days results in a significant decrease in the activity of acetylcholinesterase in treated animals (MP: 1007.40 ± 182.61 IU / l) compared to that measured in control animals (C: 1590.46 ± 71.92 IU / L, MS vs C, $p < 0.001$).

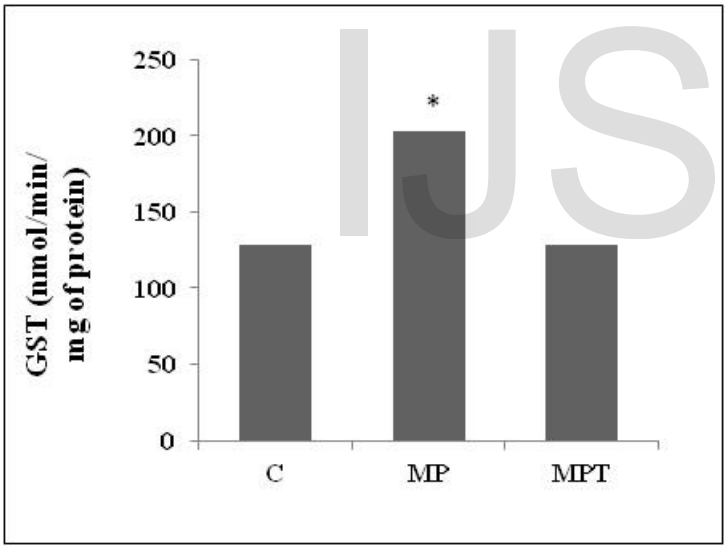
3.2 Effect of MP on biomarkers of oxidative stress and lipid and carbohydrate metabolism:

After 10 days of treatment, the animals fed on MP show a

significant decrease in GSH (MW: 19.70 ± 0.78 nmol / mg protein vs C: 39.04 ± 1.36 nmol / mg protein, p <0.01) associated with a significant increase in GST activity compared to levels measured in the liver of control animals (MW: 202.51 ± 7.13 nmol / min / mg protein vs C: 128 61 ± 1.69 nmol / min / mg protein, p <0.01). (Figure 1)



A



B

Figure 1 Change in GSH nmol / mg protein **(A)** and GST activity in nmol / min / mg protein **(B)** in control animals (C), treated with methyl parathion (MP) and MP supplemented with taurine (MPT). (**: C vs MP, p <0.001; *: C vs MP, p <0.05).

In rats treated orally with MP, significant increases in plasma levels of total cholesterol (TC) triglycerides (TG), and plasma glucose (PG) are measured relative to those measured in control animals (CT: PM 89 ± 09 mg / dl vs C: 77 ± 08 mg / dl, p <0.05 and TG: PM: 152 ± 09 mg / dl vs C: 125 ± 05 mg / dl, p <0.01, GP : 153.00 ± 05 mg / dl vs C: 122.00 ± 03 mg / dl, p <0.001).

Effects of MP administered to rats supplemented with taurine on hepatic antioxidant defense system and rates of plasma lipid and carbohydrate components: total cholesterol (TC), triglycerides (TG), plasma glucose (PG)

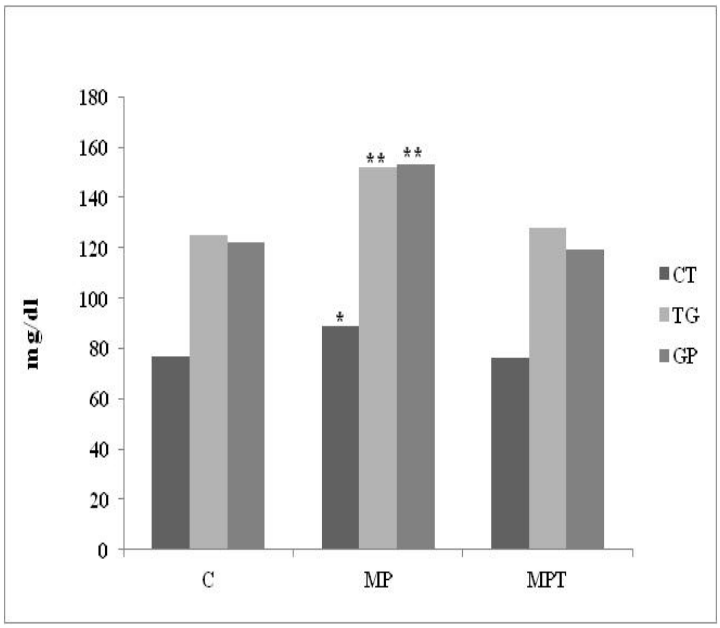


Figure 2 Changes in concentrations of total cholesterol (TC), triglycerides (TG), and plasma glucose (PG) in mg / dl in the control animals (C), treated with methyl parathion (MP) and PM supplemented with taurine (MPT). (**: T vs MPT, p <0.001, *: T vs MPT, p <0.05)

In rats underwent the same treatment to the MP but supplemented with taurine no significant change in GSH levels, activity of GST, TC, TG and plasma G could be measured against the measured rates animals in the control group (GSH: PMT: 39.67 ± 01.81nmol / mg protein vs C: 39.04 ± 01.36 nmol / mg protein, not significant, GST: MPT: 128.09 ± 04.48 nmol / min / mg protein vs C : 128.61 ± 1.69 nmol / min / mg protein, not significant, CT: MPT: 76.00 ± 03.00 mg / dl vs C: 77 ± 08 mg / dl, not significant, TG: PMT: 128.00 ± 07.00 mg / dl vs C: 125 ± 05 mg / dl, not significant and GP: PMT: 119.00 ± 08.00 mg / dl vs C: 122.00 ± 03,00mg / dl not significant). Plasma levels of total cholesterol (TC) and triglycerides (TG) measured in animals of the SME group are significantly lower than those of animals in the PM group (CT: MPT: 83 ± 09 mg / dl vs C: 89 ± 09 mg / dl, not significant and TG: SMEs: 98 ± 15 mg / dl vs PM: 152 ± 09 mg / dl, p <0.01).

A comparison between the different groups (C, MP, MPT) with an analysis of variance ANOVA shows a significant difference between the different parameters (p <0.001).

4. Discussion

While the MP is well known for its neurotoxic, haematotoxic, hepatotoxic, genotoxic and its disruptive effect on the respiratory, cardiovascular, developmental and reproductive system, knowledges about the genesis contribution of oxidative stress in mammals are very few.

In this study, rats were exposed to a single dose of MP determined basing on the bibliographic data. The results of the measurement of hepatic acetylcholinesterase activity show the effectiveness of oral treatment that induces a 30% decrease in

enzyme activity after 10 days. To highlight the toxic nature of the pesticide, the most important biomarkers of antioxidant defense system, GSH and GST were chosen. Our results show that the MP to 2 mg / kg body weight for 10 days affects the functioning of the antioxidant defense system. GSH rate significantly decreased in animals exposed to PM. GSH and its metabolizing enzymes are the best defense against cellular damage induced by reactive oxygen species (ROS) [42]. The decrease in GSH levels in our experimental conditions is due to the use of GSH to counteract the installation of oxidative stress induced by ROS generated by exposure to PM. Moreover, the GST activity increases sharply in the liver in response to treatment. This type of response of antioxidant enzymes was typically used to protect the body by eliminating xenobiotics [43]. So the act of observing the subchronic administration of PM leads to changes in antioxidant defense systems and can have a toxic effect on the body is in agreement with previous work conclusions [44].

Fats and carbohydrates are essential and altered lipid and carbohydrate levels in the blood or tissues can be extremely harmful to the body. Thus the rate of the lipid and carbohydrate components, the total cholesterol (TC), triglycerides (TG) and plasma glucose were measured in our experimental conditions. Our results show a significant increase in cholesterol after treatment with methyl parathion. This is in agreement with previous results obtained with other organophosphate insecticides such as the Ronnel [45], an organochlorine, Dieldrin [46] and carbamates, Furadan [47] and methomyl [48]. However, other studies show a hypocholesterolemia after treatment with various organophosphate insecticides including molecules such as Acephate [49] and dichlorvos [50] and a pyrethroid molecule synthesis, cypermethrin [51]. The reduction in serum TC that was recorded in rats treated with Acephate is attributed to a change in circulating HDL [49]. It has even been suggested that organophosphates could phosphorylate and inhibit the hydroxymethyl glutaryl CoA reductase, the key enzyme in the production of cholesterol [50].

Concerning triglycerides, PM causes a significant increase of plasma levels. These results confirm previous work that showed an increase in plasma TG levels in animals treated with different insecticides organophosphate, Dichlorvos [50] and a carbamate, Furadan is also a neurotoxic insecticide that acts by inhibiting acetylcholinesterase [47]. This elevation of serum or plasma TG could be attributed to the inhibition of enzymes involved in the metabolism lipase hepatic TG and plasma lipoproteins [52]. Other studies, however, reported in rats following treatment with organophosphate insecticide or no effect on plasma TG levels with Diazinon, a decrease of these rates with the Acephate [49]. This decrease may be the result of the reduction induced by acephate all lipoprotein classes of plasma lipid fraction and in particular the lightest of them.

It is not surprising that the effects are so different from one molecule to another because we know that in a class of insecticide, such as the organophosphates, there is a wide variety of molecules behaving differently inside living organisms.

For plasma glucose, the results obtained show that the glucose metabolism is impaired in animals MP and this is reflected by a significant increase in the glucose concentration in the blood. According to Surwit et al. [53], stress increases hepatic glucose production and reducing its peripheral clearance and the involvement of the hypothalamus in the control of hepatic energy metabolism, results on different experimental arguments. Indeed, it has been shown that electrical stimulation of the ventromedial hypothalamus causes hyperglycemia with impaired liver glycogen stores, hyperglucagonemia and an increase in phosphorylase activity, the active form of this enzyme [54]; [55] showed that these metabolic changes are related to the activation of the sympathetic nervous system (release of noradrenaline from nerve endings and adrenaline from the adrenal medulla). Indeed, the activation of the sympathetic nervous system in various stress conditions or during intense exercise causes inhibition of insulin secretion, stimulation of glucagon secretion and the onset of hyperglycemia [56]. In some stressful procedures such as surgical stress or strain, increased glucose is especially related to corticosteroid levels [57]; [58].

In a final experiment, GSH, TC and TG were measured in rats exposed to PM but previously treated with an antioxidant, vitamin E plays an essential role in protecting the membrane of all cells of body. It is an antioxidant, ie, it helps to neutralize free radicals in the body; in addition, it prevents or reduces the oxidation of low density lipoproteins (LDL) [59]. These results suggest that the effects of methyl parathion on plasma lipid components are the result of oxidative stress because vitamin E is able to repay TC and TG induced increases [60]. These results are in perfect harmony with the other work [20] and also show that on an in vitro model, such as rat erythrocytes, vitamin E has a protective effect against oxidative stress induced by two organophosphorus insecticides dimethoate and malathion. So, Uzunhisarcikli and Kalender [61] have showed the effect of vitamin E and C on the hepatotoxicity induced by MPT.

Taurine with antioxidant properties [62] usually shows, in vitro, a defense action in various concentrations depending on the nature of the toxicity and the cells used in the study [63]; [64]). It has been shown that the taurine may be involved in many important functions including: neuro development [65], the stabilization of the membrane, the regulation of proliferation and cell death [66]; [67]; [68]; [69]; [70]. It also has an effect on detoxification [71] and antioxidant [72]. It also has the ability to eliminate reactive species of oxygen, reduces the production MDA, end a final product of lipid peroxidation [66]. It also affects glutathione peroxidase, glutathione reductase and glutathione levels in normal rats [73].

In our experimental conditions, supplemented with taurine rats do not exhibit the strong increases in GSH, CT, TG GP and after exposure to methyl parathion (MP). Such results have been achieved with other chemicals such as fluoride [62], cisplatin [74], methiocarb [75], the arsenic [76]; [77], cadmium [78]), the Cyclosporine A, acetaminophen [79], [80], drugs [81], the aloxan [82]. It was also shown that taurine has effects on lipid metabolism and obesity [83].

5. Conclusion

The results obtained in this study in a vertebrate, the mammal Wistar rat suggest that organophosphate insecticide methyl

parathion, though low, it's persistent in the environment because still used despite the prohibitions, interferes with our lipids and carbohydrates metabolism through induction of oxidative stress exceeding the protective capacity of the body's defense endogenous antioxidants molecular systems and can lead to many diseases. Taurine may alter these changes through its antioxidant properties.

The pollution represents a severe problem for the environment because of discharges in the environment due to the excessive use of insecticides and fertilizers in agriculture.

Finally we recommend:

– An integrated monitoring program should be conducted. Soil and water should be sampled 3–6 times a year for the analysis of anions, cations, heavy metals insecticides and pesticides in groundwaters, rivers and lakes [84], [85], [86],[87]. The data of the environment quality should be centralized in a data bank or a toxicology archive.

– Several studies should be conducted mainly on bioindicator monitoring of risk assessment, water quality changes and toxicology [86], [87], [88], [89].

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