EVALUATION OF THE NEPHROTOXIC EFFECTS OF INSECTICIDE DIAZINON IN RATS

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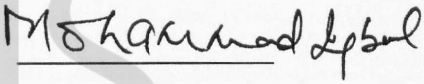

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ABSTRACT

EVALUATION OF THE NEPHROTOXIC EFFECTS OF INSECTICIDE DIAZINON IN RATS

Oxidative damage of biomolecules is implicated in the pathogenesis of various renal injuries. Diazinon (O,O-diethyl-O-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphorothioate), an organophosphate insecticide, has been used worldwide in agriculture and domestically for several years, which has led to a variety of negative effects in non-target species including humans and therefore, are cause of concern. There are few studies on diazinon with reference to its toxicity in kidney on exposure to low doses based on LD50. The possible toxicity of diazinon is assumed to be as a result of induction of oxidative stress, however, there are not enough studies to confirm this as a result of exposure to low doses of diazinon for acute, subacute and chronic periods. Therefore, the present study was conducted to analyze the direct toxic effects of diazinon which caused biochemical and ultrastructural changes and to evaluate its mechanism of action with special reference to its possible reactive oxygen species generating potential (ROS) in kidney with acute, subacute and chronic exposure in rat models. Adult Sprague Dawley male rats were treated with diazinon in corn oil orally (gavage) according to the selected doses (10 mg/kg body weight, 15 mg/kg body weight and 30 mg/kg body weight) for 7, 14 and 56 consecutive days. The selection of dose regimen of diazinon was based on previously published data which indicate substantial alterations in many biochemical parameters. All of these animals were sacrificed 24 h after the last dose of diazinon or saline within a period of 1 h. Blood and kidney tissues of these animals were taken quickly. Kidneys were cleaned free of extraneous material and perfused immediately with ice cold saline (0.85% w/v, sodium chloride) for biochemical and histopathological studies to assess the derangement in the functioning of kidney. Body weight decreased significantly in diazinon treated group compared to the saline treated control. Treatment of rats with diazinon induces oxidative stress in kidney, as evident by significant induction in lipid peroxidation (TBARS) which is accompanied by depletion of enzymatic and non-enzymatic antioxidant molecules (viz. GPx-glutathione peroxidase; GR-glutathione reductase; GST-glutathione S-transferase; G6PD-glucose 6-phosphate dehydrogenase; CAT-catalase; GSH-reduced glutathione). In contrast, activities of renal γ-glutamyl transpeptidase (γGGT) and quinone reductase (QR) were increased significantly. Parallel to these changes, diazinon treatment enhances renal damage as evidenced by sharp increase in blood urea nitrogen (BUN) and serum creatinine (CRN). Additionally, histopathological examinations showed extensive renal injuries, characterized by nuclear pycnosis, kidney swelling with obliteration of space in Bowman's capsule, degeneration of tubular epithelial cells, necrosis of proximal tubules, flattened epithelium and congested blood vessels. Reviewing all observations, our results indicate that diazinon treatment eventuates in decreased renal glutathione, a fall in the activities of antioxidant enzymes including the enzymes involved in glutathione metabolism and excessive production of oxidants with concomitant renal damage, all of which are involved in the cascade of events leading to diazinon-mediated renal oxidative stress and toxicity. We concluded that in diazinon exposure, depletion of antioxidant enzymes is accompanied by induction of oxidative stress that might be beneficial in monitoring diazinon toxicity.

ABSTRAK

Kerosakan oksidatif biomolekul adalah terlibat dalam patogenesis pelbagai kecederaan di ginjal. Diazinon (O, O-dietil-O-[2-isopropil-6-metil-4-pirimidinil fosforo thioate]) adalah sejenis insektisid organo-fosfat yang telah digunakan secara meluas di seluruh dunia selama beberapa tahun dalam bidang pertanian dan juga secara domestik. Ia telah mendapat perhatian disebabkan oleh pelbagai kesan negatifnya terhadap spesies bukan sasaran termasuk manusia. Terdapat beberapa kajian telah dijalankan keatas diazinon dengan merujuk kepada ketoksikannya dalam ginjal terhadap pendedahan kepada dos rendah berdasarkan LD50. Ketoksikan diazinon berkemungkinan disebabkan oleh pengaruhan tekanan oksidatif, tetapi tiada kajian yang mencukupi untuk mengesahkan ini adalah disebabkan pendedahan kepada dos rendah diazinon untuk tempoh akut, subakut dan kronik. Oleh itu, kajian ini dijalankan untuk menganalisis kesan toksik secara lansung diazinon yang menyebabkan perubahan biokimia dan ultrastruktur dan menilai mekanisme kerjanya dengan rujukan khusus terhadap kemungkinan potensi penghasilan spesies oksigen reaktif (ROS) di dalam ginjal model tikus dengan pendedahan akut, subakut dan kronik. Tikus jantan dewasa Sprague Dawley telah dirawat dengan minyak jagung diazinon secara oral (gavage) berdasarkan dos yang telah dipilih (10 mg/kg berat badan, 15 mg/kg berat badan dan 30 mg/kg berat badan) untuk 7, 14 dan 56 hari berturut-turut. Pemilihan kumpulan dos diazinon adalah berdasarkan kepada data yang telah diterbit sebelum ini yang mana menunjukkan perubahan yang menyakinkan dalam kebanyakan parameter biokimia. Semua haiwan ini dibunuh selepas 24 jam dos terakhir diazinon atau salina dalam jangka masa 1 jam. Darah dan tisu ginjal daripada haiwan ini diambil dengan serta-merta. Ginjal dibersih untuk mebebaskannya daripada bahan asing dan segera direndam dengan salina yang sejuk (0.85% w/v, natrium klorida) untuk kajian biokimia dan histopatologi dengan tujuan menilai kecacatan dalam fungsi ginjal. Berat badan menurun secara signifikan bagi kumpulan yang dirawat dengan diazinon berbanding dengan kumpulan kawalan yang dirawat dengan salina. Rawatan keatas tikus dengan diazinon mengaruhkan tekanan oxidatif di dalam ginjal. Ini terbukti dengan pengaruhan yang signifikan dalam peroksidaan lipid (TBARS) yang disertai dengan penyusutan molekul antioksidan enzim dan tanpa enzim (iaitu GPx-glutathione peroksidase; GR-glutathione reduktase; GST-glutathione S-trasferase; G6PD-glukosa 6-fosfat dehidrogenase; CAT-katalase, GSH-reduced glutathione). Sebaliknya, aktiviti transpeptidase γ-glutamil (γGGT) dan kuinon reduktase (QR) ginjal meningkat secara signifikan. Seiring dengan perubahan ini, rawatan diazinon menambahkan lagi kerosakan ginjal dan dibuktikan dengan peningkatan yang mendadak dalam nitrogen urea darah (BUN) dan kreatinin serum (CRN). Selain itu, pemeriksaan histopatologi juga menunjukkan kecederaan ginjal yang teruk dicirikan oleh piknosis nuklear, pembengkakkan ginjal dengan penyempitan ruang dalam kapsul Bowman, degenerasi sel epitelium tubul, nekrosis tubul proksimal, epitelium leper dan kongesi salur darah. Secara keseluruhannya, keputusan kami menunjukkan bahawa rawatan diazinon menyebabkan penurunan glutathione ginjal, penurunan aktiviti enzim antioksidan termasuk enzim yang terlibat dalam metabolisme glutathione dan penghasilan oksidan secara berlebihan diikuti dengan kerosakan ginjal. Kesemua yang terlibat berlaku secara berturutan dan menyebabkan tekanan oksidatif dan ketoksikan di ginjal akibat daripada rawatan diazinon. Kami menyimpulkan bahawa dalam pendedahan kepada diazinon, pengurangan enzim antioksidan akan disertai dengan pengaruhan tekanan oksidatif yang mungkin bermanfaat dalam pemantauan ketoksikan diazinon.

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LIST OF ABBREVIATIONS

DZ	Diazinon
UV/ VIS	Ultraviolet/visible
GSH	Reduced glutathione
CAT	Catalase
Eq	Equation
$NADP^+$	Oxidized nicotinamide adenine dinucleotide phosphate.
EDTA	Ethylene diamine tetra acetic acid.
LD_{50}	Lethal dose which cause the death of 50% of experimental animals
BSA	Bovine serum Albumin
MDA	Malondialdehyde
b.w.	Body weight
Kg	Kilogram
l	Liter
nmol	Nano mole
g	Gram
pH	Potential of Hydrogen
µmole	Micro mole
ml	Milli liter
mg	Milli gram
°C	Degree Celsius
OPs	Organophosphates
M	Molar
cm	Centimeter
USA	United State of America
LH and FSH	Lutenising Hormone and Follicle Stimulating Hormone
NADPH	β-nicotoniamide adenine dinucleotide 2 phosphate reduced tetrasodium salt
Min	Minimum
mM	Milli molar

QR	Quinone reductase
GGT	γ-Glutamyl transpeptidase
Vol	Volume
ROS	Reactive oxygen species
NCI	National cancer institute
DDT	Diphenyl trichloroethane
GR	Glutathion reductase
EC	Enzyme classification

CHAPTER 1

INTRODUCTION

1.1 Introduction

Production of sufficient amount of agricultural crops to meet the need mankind results in the use of pesticides. Pesticides are intended to remove harmful and unwanted organisms but it can also harm human, wildlife and the environment. The Environmental Protection Agency estimates that 10,000-20,000 physician-diagnosed pesticide poisonings occur each year among the approximately 2 million agricultural workers in the U.S. agricultural workers. Agricultural workers, groundskeepers, pet groomers, fumigators, and a variety of other workers are at risk of exposure to pesticides including fungicides, herbicides, insecticides, and rodenticides

The use of pesticides is not new but the types of the pesticide used by humans changed with the passege of time. In the past i.e substances such us salts, metals and sulfur were used as pesticides. After World War II the modern era of pest control began and synthetic organic chemical industries developed, compunds such as orghanoclorine chemicals (DDT and Lindane), carbamates, pyrethroids, phenolic compounds and organophosphates.

Among the pesticides, organophosphates (OPs), are commonly used as insecticides, and are regarded the most toxic of all pesticides to vertebrates. OPs inhibit the activity of cholinesterase (ChE) within the body (Kappers *et al.*, 2001). The toxicity of OPs cause adverse effects on many organs, (Sultatos, 1994) among others the immune system (Handy *et al.*, 2002; Neishabouri *et al.*, 2004), reproductive system (Joshi *et al.*, 2003), haematological and biochemical changes (De Blaquiere *et al.*, 2000).The people exposed to OPs and especially those working with or in contact with the pesticide are monitored routinely by measuring the plasma ChE activity before the appearance of the clinical symptoms. OPs cantian components of phosphoric acid, phosphorothioic acid, while another group of OPs

that is widely used and has been shown to have toxic effect on human is phosphoroamidothioate (De-Bleecker *et al.,* 1993). Important members of OPs are malathion, methyl parathion and diazinon.

Diazinon (O,O-diethyl–O-[2-isopropyl-6-methyl–4-pyrimidinyl] phosphorothioate) is an OP insecticide with a broad range of activities. It has been widely and effectively used throughout the world with applications in agriculture and horticulture for controlling insects in crops, ornamentals, lawns, fruit and vegetables (Garfitt *et al.*, 2002). Diazinon is classified as a moderately hazardous class-II organophosphorus insecticide by WHO. Treatment of rats with diazinon also resulted in hyperglycaemia, depletion of glycogen from the brain and increased activity of the hepatic gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (Matin *et al.*, 1990). Diazinon affects mitochondrial membrane transportation in rat liver (Nakagawa *et al.*, 1999). Furthermore, it disturbs cytochrome P450 system in human liver (Kappers *et al.*, 2001; Sams *et al.*, 2003). Meanwhile, diazinon causes toxic effects on other organisms (Keizer *et al.*, 1995).

An imbalance between free radicals (reactive oxygen species) and anti-oxidant mechanisms in cells cause oxidative stress. High quantities of reactive oxygen species (ROS) can result in the indication of lipid peroxidation in the cellular, mitochondrial and nuclear membranes, along with degradation of cytosolic proteins and damage to DNA. Antioxidant enzymes play an important role in minimizing the damaging effect of elevated ROS. Therefore, depletion of these defense elements further promotes oxidative stress (Olgun *et al.*, 2006). The enzymes involved in reducing the harmful effect of oxidative stress include catalase, Glutathione s-transferase, glutathione peroxidase, glutathione reductase and reduced glutathione. (Defeng *et al.*, 2003). Pesticides, organophosphate and organochlorines are involved in the production of free radicals to initiate lipid peroxidation and disturb the antioxidant status (Abdollahi *et al.*, 2004). ROS were known to be the mediators of oxidative stress formed on exposure to pesticides and were known to disrupt cell functions and morphology resulting in apoptosis and necrosis.

There are a few studies on diazinon with reference to its toxicity to the kidney on exposure to low doses based on LD_{50}. The possible toxicity of diazinon is assumed to be duo to of induction of oxidative stress and there are not enough studies to confirm this on exposure to low doses of diazinon for acute, subacute and chronic period. It is worth studying the direct toxic effects of diazinon with acute, subacute and chronic exposure in rat models with minute doses of diazinon -10, 15 and 30 mg/kg body weight based on 1/30, 1/20 and 1/10 of LD_{50} as most of the time these minute levels are common in environment. Hence the present study were designed to confirm the acute, subacute and chronic exposure models of low doses of diazinon in adult male rats with special reference to its possible reactive oxygen species generating potential in kidney. The possible biochemical and histopathological changes that were involved in the renal toxicity of diazinon exposure were confirmed in this study

1.2 Hypothesis

1. Long term and short term exposure to low doses of insecticide diazinon leads to renal toxicity.
2. Long term and short term exposure of low doses of diazinon may result inhibition in the activity of renal antioxidant enzymes.

1.3 Objectives

To investigate the acute, subacute and chronic toxicity of diazinon exposure on kidney functions and to determine the possible biochemical and histopathological changes, that are involved in the renal toxicity of rats.

1.3.1 Specific Objectives

We have aimed to investigate the oxidative stress inducing effects of low doses of diazinon in kidneys.

3.1.1 Lipid peroxidation

3.1.2 Reduced glutathione

3.1.3 Glutathione peroxidase

3.1.4 Catalase

3.1.5 Glutathione reductase

3.1.6 Glucose-6-phosphate dehydrogenase

3.1.7 Qunione reductase

3.1.8 Glutathione S-transferase

3.1.9 γ-Glutamyl transpeptidase

3.1.10 Serum creatinine

3.1.11 Blood urea nitrogen

3.1.12 Histopathological changes in kidney-Qualitative analysis of kidney-necrosis.

1.5 Significance of the Study

Since diazinon has been widely used in the world with applications in agriculture and horticulture the study of the mechanism of action of this compound will provide an insight into the prevention of toxic manifestation of diazinon. By understanding the mechanism of action of diazinon it will be easier to determine the prophylactic measures against the deleterious effects of such chemicals which are commonly being utilized in day to day life.

CHAPTER 2

REVIEW OF LITERATURE

2.1 General

Pesticides are chemicals that are used to control the pests which harm crops, human and transmit diseases and they still play an important role in agriculture by increasing the crop production and reducing food cost. The pesticide use the effectiveness of the chemicals on the wide range of insects such us arthopode and pathogens . But at the same time the extensive use of the pesticide introduce undesirable changes which cannot be ignored (Bolognesi, 2003).

The use of pesticides has increased sharply in both developed and developing countries during the last few decades. Pesticide use is not only common in agricultural areas but also in homes, yards, public buildings, stores, schools, parks, and other places, resulting in per-acre pesticide intensity in some urban areas that exceeds agricultural use. Annually between 1945 and 1985 about 600,000 tons of pesticides are exported to and used in developing countries; about 50,000 of these were used for public health problems (Dich *et al.*, 1997). In 1985, the estimated world production of formulated pesticides was three million tons corresponding to a market value of 15,900 million US dollars (World Health Report, 1990). Currently there are more than 1,600 pesticides available (Hayes & Lawes, 1991) and its world wide use is still increasing (Edwards, 1977) about 4.4 million tons of pesticide are used every year with the value of $20 billion (Environmental Protection Report, 1989).

The extensive use of pesticides in public health and agricultural programs has caused severe environmental pollution and health hazards, including cases of severe acute and chronic human poisoning (Abdollahi *et al.*, 1995). Pesticides in some cases not only kill the harmful pests but also the beneficial insects that can kill the unwanted pests and also decrease the biodiversity of aquatic insects and fishes like trout (Edwards, 1977). Some pesticides can persists in an aquatic system

usually become absorbed onto floating particles and eventually ends up in bottom sediment and some of these pesticides can persist in the sediments for many years and are periodically recycled into water when the sediment is disturbed (Edwards, 1977).

2.2 Pesticides in Agriculture

Pesticides are the common way of controlling pest worldwide in agriculture and it is difficult to imagine enough yields without the use of pesticide. However, the irrational use of these products has led to serious problems and the costs of pesticide use are already higher than the benefits (Rola & Pingali, 1993). Pesticides are usually used worse in developing countries where many products of the WHO category I are still used. Those products are highly or even extremely toxic and lead to a considerable amount of poisoning (World Health Report, 1992). Some sources have reported up to 25,000,000 cases per year (Knirsch, 1994). But still it is believed that many agricultural poisoning cases go unreported. The many barriers to accurate reporting include lack of access to medical care and fear of reprisal and job loss (Reeves *et al.*, 1999).

Pesticides have a major impact in reducing the agricultural ecosystem biodiversity. In soil, they have major effects on decreasing the diversity of soil inhabiting organism since they selectively kill a particular group of organism (Edward & Thompson, 1973). From 1991 to 1998, pesticides use increased 40% in California, from 153 million to 215 million pounds; approximately 90% of the reported use occurs in agricultural production (Galt, 2008). Many pesticides are also used in non-agricultural sectors, particularly in homes and landscape management, where the herbicide 2, 4-dichlorophenoxyacetic acid is the most widely employed (California Department of Pesticide Regulation, 2000).

2.3 Classes of Pesticides

The term pesticides include many chemicals such as insecticide (organophosphate, organochlorine, carbamates and pyrethroid), and herbicide. According to the statistical analysis total 890 active ingredients are registered as pesticides in USA and currently marketed in some 20,700 pesticide products (Bolognesi, 2003)

2.3.1 Organochlorines

Insecticides are also referred to as chlorinated hydrocarbons, containing carbon, hydrogen and chlorine. The insecticides are very toxic and are also considered to be persistent organic pollutants, persist in the environment through food chain and accumulate in human tissues, fluids and are excreted in breast milk. They have been prohibited in most countries, but in India some organochlorines like lindane is still used extensively in agriculture and malaria eradication program (Siddiqui *et al.*, 2002).The compounds are lipid soluble and stored in the fatty tissue and small repeated exposure may results in clinical toxicity (Sonawane, 1995).

Organochlorines, such as dieldrin and aldrin, induce derangement of certain antioxidant mechanisms, including alterations in antioxidant enzymes and the glutathione redox system (Bagchi *et al.*, 1993). They are suspected to disrupt the endocrine system and increasing the risk of hormone dependent disorders such as breast and prostate cancer in humans (Siddiqui, 2005). Organochlorines stimulate the central nervous system (CNS); these compounds show variation in the mechanism of action. Organochlorines like cyclodienes, chlorocyclohexanes and other related compounds cause the inhibition of gamma amino butyric acid (Ellenhorn *et al.*, 1997).

Organochlorines such as DDT were extensively used as insecticide and it was banned in 1960s due to harmful effects on the environment. It induces oxidative stress and lipid peroxidation (Gultekin, 2000). It also exerts effects on the antioxidant enzyme on the sperm within epididymis of goats (Gangadharan, 2001) and rats (Latchoumycandane, 2002). Vector-borne diseases such as malaria, onchocerciasis (river blindness), schistosomiasis and African trypanosomiasis are controlled by pesticides, DDT, dieldrin.

2.3.2 Carbamates

Carbamates are important insecticides that are introduced in 1956; they are derivatives of carbamic acid, persistent and broad spectrum toxicant affecting large group of organism and having different mammalian toxicity. They are used as dusts or sprays and are absorbed through skin as well as by ingestion and inhalation.