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Oxidative injury caused by individual and combined exposure of neonicotinoid, organophosphate and herbicide in zebrafish

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ABSTRACT

The greatest challenge in environmental toxicology is to understand the effects of mixture toxicity as environmental pollutants co-exist and exhibit combined effects. Thus, it is necessary to evaluate the mixture toxicity associated with two or more co-existing compounds. Pesticides are widely used to control pest, they are ubiquitous in nature and present in all environmental components. Pesticide residue can be detected in almost all components of environment and food samples. Imidacloprid (IMD) (neonicotinoid), dichlorvos (DIC) (organophosphate) and atrazine (ATZ) are three widely used pesticides for commercial uses. Present work includes the assessment of effects of individual exposure of IMD (27.5 mg/L), DIC (15 mg/L), and ATZ (03 mg/L) and in combination of three (CMD) (13.75 + 7.5 + 1.5 mg/L IMD, DIC & ATZ, respectively) in terms of LPO, GSH content and antioxidant enzymes activities (superoxide dismutase, catalase and glutathione peroxidase) in zebrafish (*Danio rerio*), exposed for 24 h. CMD group exhibits highest lipid peroxidation than other individually exposed groups. Similarly, the activities of antioxidant enzymes were highest in CMD group with reduced GSH content. Results indicate that exposure to mixture of pesticides develops synergistic effects which were more toxic in compare to individual exposure and also produce toxicity in all examined tissues rather than selective organ toxicity.

1. Introduction

In natural environment, human being and other living organisms get exposed to the mixture of pesticides, unlike single pesticide. Pesticides are widely used to increase agricultural yield and consequently, raised environmental concern and frequently detected in water bodies, soil, food and vegetables [1]. Pesticides are known to induce reactive oxygen species (ROS) and cause oxidative stress leading to inactivation of antioxidant enzymes and reduction of free radical scavengers. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are important endogenous antioxidative enzymes that protect cell from oxidative damage [2]. Zebrafish, an important model organism having high predictivity with human involved in various research activities including toxicological research [3,4].

Imidacloprid (IMD), a neonicotinoid pesticide widely used for protecting crops from insects and comparatively safer, thus, considered as a replacement of organophosphate pesticide. It affects insect's postsynaptic nicotinic acetylcholine receptor and act as an agonist resulting in death [5]. A dose of 10 mg/kg/day for 90 days is not sufficient to produce significant biochemical alterations in experimentally exposed rats [6]. However, research has raised concern over the use of imidacloprid as it affects egg-shell thinning, reduction in egg production and hatching time in birds [7]. Dichlorvos (DIC) is an organophosphate pesticide, widely used for agriculture purpose, household and veterinary uses. Significant concern was raised over the use of DIC due to its acute and chronic toxicity because it is stable in water, soil and air, with a half-life ranging from two (air) to seven days (water and soil) [8]. DIC is extremely toxic to aquatic animals especially fish and known to cause oxidative stress, injury to cell, lipid peroxidation and other events consistent with elevated ROS levels [9]. Atrazine (ATZ) is an s- triazine, commonly used herbicide that inhibits photosynthesis in broadleaf and grass on crops. It is commonly applied to corn, sugarcane, sorghum and turf grass. The widespread use of atrazine has resulted in the contamination of surface and ground water. Atrazine is resistant to degradation and acts as an endocrine disruptor in zebrafish and other animal. Atrazine is also known to cause oxidative stress, injury to cell, lipid peroxidation and other events consistent with

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elevated ROS levels [6,10,16].

In mixture toxicity, toxicokinetic and toxicodynamic exchange can occur among toxicants resulting in potentiating and addition effects [11]. Thus, the major concern for this work is to understand the combined effects of mixture toxicity caused by above mentioned pesticides because of their high use and detected in river water samples. Reported minimum concentration of imidacloprid in Ebro river, Spain is 1.64 ng/L and maximum concentration is 14.96 ng/L. Similarly the minimum concentration of atrazine is 8.13 ng/L and maximum is 12.22 ng/L. The study provides huge data on different organophosphate pesticides concentration, however the results of dichlorvos are not reported [12]. Ample studies were conducted on chronic toxicity with these pesticides [6,10,15–17], however, literature is not available on the acute toxicity caused by said pesticides. Therefore, investigations were carried out for the evaluation of the individual and combined effects of acute exposure of imidacloprid, dichlorvos and atrazine in terms of biochemical alterations in zebrafish.

2. Materials and methods

2.1. Chemicals and reagents

Commercial product of Imidacloprid (PubChem CID: 86418), Dichlorvos (PubChem CID: 3039) and Atrazine (PubChem CID: 2256) were procured from pesticide testing laboratory, Gandhinagar, India. All standards and other reagents used were of analytical grade and highest purity.

2.2. Animals, treatment and collection

Adult (4–5 months) zebrafish of length 2.8 ± 0.5 cm and weight 0.295–0.395 gms were procured from pet shop certified as wild species and handled in accordance with good animal practice as defined by the animal welfare bodies and the study was approved by the university committee No. PhD/FS/RA/02. Zebrafish were kept under controlled condition with temperature (25 ± 1 °C), pH 7.0 ± 0.2, conductance 0.2 µohm, dissolved oxygen 7.2 ± 0.3 mg/L, hardness 110.0 mg/L, alkalinity 0.25 µg/L and 12 h light and dark cycles. Animals were not fed 24 h prior to experimentation. The rationale for pesticides concentration was based on pilot experiments and literature. For, imidacloprid, no data is available for zebrafish. However, the dose of 10 mg/kg/day for rats was observed as no observed effect level (NOEL) [6]. Thus, an experiment was conducted and no mortality was observed up to the concentration of 25 mg/L for 24 h. However, at 30 mg/L, 30% mortality was observed. Therefore, in present experiment the 27.5 mg/L concentration of imidacloprid for 24 h was used. For, dichlorvos, the reported concentration is 05 mg/L for acetyl cholinesterase inhibition study in zebrafish [13]. No mortality was found up to the concentration of 15 mg/L for 24 h. However, at 20 mg/L, 100% mortality was observed accordingly, 15 mg/L concentration was used in present experiment for dichlorvos. For atrazine, LC_{50} of atrazine in zebrafish is 9.56 mg/L for 96 h [14]. No mortality was observed up to the concentration of 03 mg/L for 24 h. However, at 04 mg/L 50% mortality was observed in 24 h so, 03 mg/L concentration was used in present experiment for atrazine. For combined exposure, half concentrations of individual pesticides were used. The experiment was conducted in accordance with organisation for economic co-operation and development (OECD) guidelines.

Total 90 adults (4–5 months) zebrafish were divided into five group (one control + four exposed) viz. Group: 1 (de-ionized water as control), Group: 2 (IMD, 27.5 mg/L), Group: 3 (DIC 15 mg/L), Group 4 (ATZ, 03 mg/L) and Group 5 (IMD 13.75 mg/L + DIC 7.5 mg/L + ATZ 1.5 mg/L) under semi-static conditions. At the end of the exposure period (24 h), the fish were sacrificed under a stereo microscope. Liver, kidney and brain were collected, minced and homogenized (2.5% w/v) with ice-cold 0.15% KCl-0.1 M phosphate buffer (pH 7.4).

2.3. Biochemical assays

An end product of lipid peroxidation is MDA (Malondialdehyde), were measured in tissue homogenates on the basis of the reaction with thiobarbituric acid (TBA) to form a pink colour complex, MDA produced was determined with the absorbance coefficient of the MDA-TBA complex at 550 nm using 1, 1, 3,3-tetraethoxypropane as the standard [18]. Glutathione levels were determined using 5,5′-dithio-bis (2-nitrobenzoic acid) [DTNB] for colour development at 420 nm. A standard curve using reduced glutathione was used for calibration [19]. The activity of superoxide dismutase was determined in the tissue homogenates by the modified method of NADH-phenazine methosulphate-nitroblue tetrazolium formazan inhibition reaction spectrophotometrically, measured at 550 nm [20]. The activity of catalase was determined at 550 nm [21]. The activity of glutathione peroxidase was determined using Glutathione as substrate and DTNB as standard at 420 nm [22].

2.4. Statistical analysis

Statistical significance of mean value of different biochemical parameters (LPO and GSH), antioxidant enzyme (SOD, CAT and GPx) in different tissue (liver, kidney and brain) for different groups (control, IMD, DIC, ATZ and CMD) tested by two-way analysis of variance (ANOVA).

3. Results

3.1. Lipid peroxidation

In liver, significant induction of MDA levels was observed in IMD, DIC and CMD group, while no change was observed in ATZ in compare to control (Fig. 1). In kidney, significant induction of MDA levels observed in the DIC and CMD group, while IMD and ATZ shows no change in compare to control. Significant alterations were not observed in MDA levels in brain of individually exposed groups. Interestingly, in combined exposure (at a half concentration of individual pesticides), the brain showed significant elevation in MDA levels in compare to control and all individually exposed group.

3.2. Glutathione

In liver, elevation of GSH content was observed in individually exposed IMD and DIC groups (Fig. 2). In contrast, GSH content depleted significantly in CMD group. In kidney, significant increase in GSH content observed in all three individually exposed groups. However, CMD group shows significant decrease in GSH content when compared with all individually exposed groups. Significant elevation observed in brain of IMD and DIC exposed fish however, no changes were observed in ATZ and CMD exposed group in compare to control group. Decrease in GSH content observed in CMD group when compared with IMD and DIC groups.

3.3. Superoxide dismutase activity

In liver, significant increase in SOD activity was observed in DIC and CMD group in compare to control, IMD and ATZ group (Fig. 3). No change was observed in ATZ group, while, IMD group shows decrease in SOD activity in compare to control. In kidney, significant decrease in SOD activity was observed in IMD and ATZ exposed groups in compare to control. In brain, depletion in SOD activity observed in IMD and DIC exposed groups in compare to control. While, no changes were observed in individual exposed ATZ group. Interestingly, in CMD exposed group (at a half dose of individual pesticide), the brain tissue showed significant elevation in SOD activity in compare to control and all individually exposed groups.