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RESEARCH ARTICLE

A STUDY OF LIPID PEROXIDATION, LIVER ENZYMES AND ANTIOXIDANT STATUS IN LUFENURON TREATED MICE, *Mus Musculus species*

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ABSTRACT

An attempt was made to investigate the effect of Lufenuron in liver tissue of mice, *Musculus species.* The sublethal dose of Lufenuron (0.1520 mg/kg) administered to mice. In the present study the level of Lipid Peroxidation (LPO), Glutathione (GSH), Glutathione Peroxidase (GPx), Catalase (CAT) and Superoxide Dismutase (SOD) were observed. Also as a liver function marker serum, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase was estimated The present study suggests that the level of lipid peroxidation was increased and glutathione, catalase and superoxide dismutase were significantly decreased in the liver tissue of Lufenuron exposed mice. The liver enzymes ALT, AST and ALP were also increased in the treated animals The present study concludes that the Lufenuron damages the liver tissue of mice.

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INTRODUCTION

Lufenuron is an active ingredient in the veterinary flea control medication, heartworm prevention, and anthelminthic medicine, sentinel. Lufenuron is stored in the animal's body fat and in transferred to adult fleas through their bite. Adult fleas transfer it to their eggs by its presence in the mother flea's blood, or by the larva feeding on pre-digested blood. Lufenuron, a benzoylurea pesticide, inhibits the production of chitin in larval fleas. Without chitin, a larval flea will never develop an exoskeleton. Attacking the ability to create chitin may make lufenuron a due to its inhibition of Chitin, which makes up roughly 33% of the typical fungal cell wall. Lufenuron was included in a biocide ban proposed by the Swedish Chemicals Agency because it is toxic to fresh water zooplankton (Swedish Chemicals Agency (KemI, 2008-09-23.) Lufenuron mimics the juvenile hormone and inhibits the specific esterases, it inhibits metamorphosis to the adult stage and interferes The moulting of early instar larvae, active by contact and ingestion;

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lufenuron (RS) - 1- [2,5- dichloro -4- (1,1,2,3, 3,3- exafluoropropoxy) phenyl] -3- (2,6-difluorobenzoyl) urea, chitin synthesis inhibitor, active by ingestion; chitin synthesis inhibitor, active by ingestion and contact;, juvenile hormone mimic. All these products were from Dr. Ehrenstorfer (Ausgburg, Germany), with a purity of 98.8, and 99.5% for lufenuron, and flufenoxuron, respectively. The standard solutions were prepared in acetonitrile : water 50:50 (V:V), from 0.05 to 100 mg/l. Most extensively used Pesticides in Brazil belong to the organophosphorus, lufenuron, carbofuran, and thiabendazole. Following application, pesticide residues may remain in the crop and constitute a health risk due to their toxicity. The determination of pesticides in foodstuffs is usually accomplished using chromatographic techniques and involves many preliminary steps including sampling, extraction and clean-up (*K.* Ridway *et al.,* 1978). Lufenuron (antimoulting compound) add EPA report is one of the most newly introduced synthetic insect growth regulators. It is used for control of Lepidoptera and Coleoptera larvae on cotton, maize and vegetables; and citrus white fly and rust mites on citrus fruits (Anonymous, 2004;) Organo phosphorus insecticides are among the most frequently used pesticides. They are used in agriculture, forestry, horticulture, public health (i.e., hospitals) (World Health Organisation. 1986).

Lufenuron is an over-the-counter veterinary remedy available in pet shops under the name "Program". It is given to dogs or cats once a month, and it prevents flea larvae from growing into adult fleas by interfering with their Chitin synthesis. Chitin is the hard substance the remedy against fungal infections, such as ringworm (a dermatophyte infection and not a worm at all). It has no known toxic effects at any dosage on humans or other animals in the environment that do not depend on chitin, though the orally administered pills can sometimes cause an upset stomach with acid reflux. Lufenuron is also sold as a crop protection product (pesticide) by Syngenta for use against lepidoptera, eriophid mites, and Western flower thrips; it has approval in a number of countries for use on a variety of crops, including soybeans and maize. Lufenuron is thought to be an effective anti-fungal in plants. It is safe because Lufenuron is biochemically inert to mammals. Lufenuron is not broken down by the liver or kidneys. Lufenuron's antifungal property may be exoskeleton of insects is made of. The interesting fact about Chitin is that it is not just used by insects and arthropods; it also makes up about half of the fungal cell wall. And fungi - also Candida albicans - can't survive with half of their cell wall gone. The effect it has on Chitin production (Chitin is not found in humans) makes this "off-label" use of Lufenuron an excellent broad-spectrum antifungal, successfully tested on a variety of animals in many countries around the world. It is not approved for use as an antifungal medicine in humans. This is not because of side effects, but simply because the manufacturer is not interested in getting this drug certified for use as an antifungal in humans.

MATERIALS AND METHODS

Chemical

Lufenuron ei 5.4% (w/w) ec (Cigna) Chemical composition of Lufenuron 540% w/w Emulsifying agents caster of polyglcal, ether 36.40.6.00 w/w. Emulsifying agents linear alkylbenzone sulfonic acid. Calcium 4.00% w/w Solvent cycoto exanaon 20.00 solvent. (Solvent) 64.60% w/w. Manfactured by Syngenta India limited. 14.1. Tata Road Mumbai.

Animals

Male albino mice, 7-8 weeks old, weighing 130-140g were used for the study. The animals were obtained from National Institute of Nutrition, Hyderabad and maintained in Central animal house, Rajah Muthiah Institute of Health Science, Annamalai University, Annamalainagar, India. The rats were housed in olypropylene cages at room temperatures (27±2°C) with relative humidity 55±5%, in an experimental room. In Annamalainagar, the LD (light: dark) cycle is almost 12:12h. The local institutional animal ethics committee (Registration Number 160/1999/CPCSEA), Annamalai University, Annamalainagar, India, approved the experimental design (Proposal No.527, dated 25.05.2007). The animals were maintained as perthe principles and guidelines of the ethical committee for animal care

of Annamalai University in accordance with the Indian National Law on animal care and use. The animals were provided with standard pellet diet (Amrut Laboratory Animal Feed, Mysore Feeds Limited, Bangalore, India) and water *ad libitum*. The mice were divided into two groups. Each group having 6 mice. The group I was control and Group II was treated with Lufenuron (0.1520 mg/kg). After the treatment, the Liver tissues were isolated from mice. The liver tissue was used for various biochemical estimations.

Estimation of ABT (Aspartate aminotransferase)

To 100µl of serum, 1ml of given reagent mix is added. The Mixture is mixed thoroughly and contents are transferred into cuvette. The first reading is recorded at 60th second, and subsequently three more readings are taken with 30 seconds interval at 340 nm in UV spectroscopy.

Estimation of ABT (Alanine aminotransferase)

To 100µl of serum, 1ml of given reagent mix is added. The Mixture is mixed thoroughly and contents are transferred into cuvette. The first reading is recorded at 60th second, and subsequently three more readings are taken with 30 seconds interval at 340 nm.

Estimation of alkaline phosphatase

Twenty microlitres of serum is mixed with 1 ml of given buffered substrate, mixed well and absorbance is read at 30, 60, 90 and 120 seconds at 405nm.The mean change in absorbance per minute is determined and test results.

Estimation of lipid peroxidation

Lipid per oxidation was estimated (Yagi, 1987) as evidenced by the formation of thiobarbituric acid reactive substances (TBARS). To o.5ml of plasma 4.0ml of o.o83N sulphuric acid was added. To this mixture, o.5ml of 10% phosphotungstic acid was added and mixed. After standing at room temperature for 5 mins, the mixture was centrifuged at 3000g for 10 minutes, was mixed with 2.0ml of sulphuric acid and 0.3ml of 10% phosphotungstic acid. The mixture was shaken well and centrifuged at 3000g for 10 mins. The sediment was suspended in 4.0ml distilled water and 1.0ml of TBA reagent was added. The reaction mixture was heated at 95%c for 60mins. After cooling 5ml of n-butanol was added and the mixture was shaken vigourously and centrifuged at 3000g for 15 mines. The color extracted in the butanol layer was read at 530nm. Standard malondialdehyde solution (To 5 moles) in 4.0ml volumes and a blank containing 4ml distilled water were processed along with test samples.

Estimation of reduced glutathione

The activity of reduced glutathione was determined by the method of Beutler and Kelly, 1963). 0.2ml of sample (plasma) was mixed with 1.8ml of EDTA solution. To this 3.0ml of precipitating reagent was added, mixed thoroughly and kept for 15mins before centrifugation. To 2ml of the filtrate, 4ml of 0.3ml disodium hydrogen phosphate solution and 1ml of DTNB reagent were added and read at 412nm. A set of standard solutions containing 20-100µ g of reduced glutathione was treated similarly. Values are expressed as mg/dl for plasma.

Estimation of glutathione peroxidase

Glutathione activity was assayed be the method of Beutler and Kelley, 1997). The reaction mixture in a total volume of 1.0ml contained 0.2ml of phosphate buffer,0.2ml of the enzyme (plasma), 0.2ml of glutathione and o.1ml of Hydrogen peroxide were added to the mixture and and incubated at 37°C for 10 mins. The reaction was arrested by addition of 0.5ml of 10% TCA. After centrifugation, the supernatant was assayed for glutathione content using DTNB as described by Beutler and Kelley. A blank was treated similarly to which o.2ml of the enzyme was added after the incubation. The activity of glutathione peroxidise is expressed as U/L plasma.

Estimation of catalase

To 1ml of the phosphate buffer taken in each of four test tubes, 0.1ml of plasma was added. To this, 0.4ml of H_2O_2 was added, The reaction was stopped at 15, 30, 45 and 60 seconds by the

addition of 2ml of the dichromatic acetic acid reagent. The tubes were boiled for 10mins, cooled and read at 620nm. For standards, different amounts of hydrogen peroxide ranging from 20-100µ moles were processed similarly along with a blank. Activity of catalase is expressed as U/L plasma.

Estimation of superoxide dismutase

The active of superoxide dismutase was assayed by the method of Kakkar *et al., 1984* based on the formation of NADH Phenazine metho sulphate – nitroblue tetrazolium formayan. The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of phenazine methosulfate and o.3ml of nitroblue tetrazolium. O.2ml of plasma and water in a total volume of 3ml. The reaction was started by the addition of 0.2ml of NADH. After incubation at 30°C for 90 seconds, the reaction was arrested by the addition of 1ml of glacial acetic acid. The reaction mixture was stirred and shaken with 4ml of n-butanol. The mixture was allowed to stand for 10mins, centrifuged and the butanol layer was separated. The colour intensity of chromogen in butanol was measured at 560nm. A system devoid of enzyme served as control. Enzyme activity is expressed as U/L plasma.

Statistical analysis

The data are expressed as mean ± SD. Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT).The results were considered statistically significant if the P values were less 0.05.

RESULTS

Level of lipid peroxidation (TBARS)

In the normal untreated control mice, the level (µmole/ml) of TBARS content in the liver tissue was 1.59 ± 0.13. At sub lethal Lufenuron treated animal shows an increased level of TBARS content (1.74 ± 0.09, µmole/ml) P<.0.05) as compared to control mice.

Level of reduced glutathione (GSH)

In the normal untreated control mice, the level of reduced glutathione content in the serum was 24.65 ±3.10). At sub-lethal dose of Lufenuron treatment decreased level of glutathione content (21.92± 2.90; P< 0.05) was noticed.

Table. 1. The level of lipid peroxidation and antioxidants in control and Lufenuron treated mice

Parameters	Group I (Control)	Group II (Treated)
TBARS ((nmoles/ml))	1.59 ± 0.13	1.74 ± 0.09*
GSH (mg/dl)	24.65 ± 3.10	21.92± 2.90*
Glutathione peroxidise (U/L)	1.59 ± 0.13	72.1 ± 8.17*
Catalse (U/L)	1.82± 0.10	1.01 ± 0.18*
Superoxide dismutase (U/L)	2.98± 0.23	1.81 ± 0.29*

Values are expressed as mean ± SD (n = 06). Values that are not sharing a common superscript letter in the same column differ significantly differ at $p < 0.05$ (DMRT).

Table 2. The level of liver enzymes in serum of mice treated and control mice

Parameters	Group I (Control)	Group II (Treated)
AST(U/L)	82±1.3	133±1.9*
ALT(U/L)	67±1.3	114±1.7*
ALP(U/L)	92±1.3	286±1.1*

Values are expressed as mean ± SD (n = 06). Values that are not sharing a common superscript letter in the same column differ significantly differ at $p < 0.05$ (DMRT).

Level of glutathione peroxidase (GPx)

In the normal untreated control mice, the level of GPX activity in the In the normal untreated control mice, the level of GPX activity in the was 1.59 ± 0.13. At sub-lethal dose of Lufenuron treatment for animal shows the increased level of GPX and when compared to normal mice.

Level of catalase (CAT)

The level of catalase in the normal untreated mice was 1.82± 0010 & in treated animal showed sub-lethal dose of Lufenuron intoxicated animal liver tissue shows the significantly decreased level of catalase activity (1.01 ± 0.18, $P<0.05$ nmol /mg. of protein).

Level of superoxide dismutase (SOD)

The level of SOD activity in the normal untreated mice was 2.98± 0. At sub-zethal dose of Lufenuron treatment, the intoxicated liver tissue shows the significantly decreased level of SOD activity (1.81 ± 0.29, $P<0.05$).

DISCUSSION

Liver is the main site for all metabolic activities and also for all detoxification reactions. It is strongly bound to which is in enzymes by replacing the hydrogen atom to form covalent bond as mercaptides (Klassen *et al.,* 1986). It is also capable of biotransformation of foreign chemicals (Mehandale, 1985). Poisoning induced physiological and biochemical changes in the liver can be regarded as an index for the identification of pollutant stress (Rao *et al.,* 1995; Shambunath Bose *et al.,* 1994). Antioxidants such as GSH, SOD, CAT and GPX are the main defense against O_2 - and H_2O_2 mediated injury. Antioxidants both in enzymatic and non-enzymatic, together with the substance that are capable of either reducing or preventing their formation, form a powerful reducing buffer which affects the ability of the cell to counteract the action of oxygen metabolites forming the protective mechanism which maintains the lowest possible level of the inside the cell (Sies, 1993). Lipid peroxidation is a chemical mechanism capable of disrupting the structure and the function of the biological membranes that occurs as a result of free radical attack on lipids. The ability of lufenuron to produce Reactive Oxygen Species (ROS) was indicated in our study by the increased amount of lipid peroxides measured as TBARS.

Glutathione (GSH) is the significant component of the collective antioxidant and defense, and highly potent antioxidant. The-SH groups of GSH are important for many facets of cell function.GSH plays multiple regulatory role at the cellular level.

The GSH is essential for functional and structural integrity of the cell, tissues and organ system (Apple, 1978).Glutathione peroxidase (GPx) is the well-known antioxidant enzyme against oxidative stess, which in turn requires glutathione as cofactor. It catalyzes the oxidation of GSH to GSSG at the expense of H_2O_2. It contains selenium molecules at the active sites and transtens reducing equivalents from glutathione to H_2O_2 and producing water and GSSG. GPx is antiperoxidative enzyme present in the cell and mitochondrial matrix. Catalase (CAT) is an enzyme , which is present in most cell, and catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a heme containing protein, and is an efficient inhibitor when H_2O_2 accumulates in the tissue containing ferrous ions. It is mainly found in the peroxisomes, and removes H_2O_2 produced oxidation. SOD is an important defense enzyme, which converts superoxide radicals to hydrogen peroxide (Mccord *et al.,*1984). CAT is a heme protein, which decomposes hydrogen peroxidase and protects the tissue from highly reactive hydroxyl radicals (Chance *et al*, 1982).

In the present study, the level of glutathione and CAT, SOD significantly decreased but the level of LPO, GPX content increased in the mice when treated with sub-lethal dose of Lufenuron. The reduction of these enzymes may be due to oxidative stress of pesticide intoxication. The present study showed that the increased level of LPO content suggested that the excess production of ROS might be explained by its ability to produce alteration by blocking the permeability transition

pore (Nilcoli *et al.,* 1995) and alteration in mitochondrial electron transport chain. These events cause the oxidative phosphorylation uncoupling and subsequent increase in ROS production (Salducci *et al.,* 1999).

The decreased activities of antioxidant represents increased utilization due to oxidative stress. Chandravathy and Reddy (1999), catalase is an enzyme catalyses decomposition of H_2O_2 to water and O_2 and efficient inhibitor of LPO when hydrogen peroxide accumulates in a cell containing free ferrous ions. In the present study the decreased level of catalase activity in serum following lufenuron exposure may therefore be an important role in enhancing oxidative stress of cellular system. GPx is another antiperoxidative enzyme, which is present, both in cytosol and mitochondrial matrix and is found to increased during exposure to lufenuron. The pesticide may inhibit the GPx directly by impairing the functional groups, or indirected by rendering the supply of reduced glutathione and NADPH. Sharma, *et al.,* (2005) reported that the decreased level of antioxidant defense system mainly responsible for generating hydroxyl radicals leading to promote oxidative damage by Fenton reaction. . This inhibition of antioxidant defense may be coupled with lowered total sulfhydryl (TSH) contents (Ramanathan *et al.,* 2002) or depletion of glutathione (Yu, 1994). From this study, we conclude that the Lufenuron induces free radicals oxidative damage in hepatic tissue elevating the liver enzymes AST, ALT and ALP. Therefore we suggest further study of toxic effect of Lufenuron on the liver tissue of mice.

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