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The Study of Lipid Peroxidation, Liver Enzymes and Antioxidantstatus Inlufenuron 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.
Key words: Lufenuron, LPO, Antioxidants Liver, Mice.

Introduction

The effect of environmental contamination on human health is one of the most challenging problems that face the world today. The growing world economy and movement towards global marketing have driven competition in industrial and technological development at a high speed towards the betterment of mankind. However, in nearly all countries such developments have focused on increased production and economic gains before realizing their impact on the environment and human health[1].

High level demand and respiratory exposure to pesticides during on-farm and house use; and chronic exposure to low levels of pesticides residues in food and water represent a serious source for the induction of genetic lesions[2,3]. It is taken for granted that the degree of mutagenic potentiality of environmental pollutants evaluated in one test system may or may not be the same in another; therefore testing for the induction of DNA lesions and for mutagenicity using a variety of short-term assays, has become an essential part of the toxicological evaluation of contaminants (e.g. pesticides, cosmetics, drugs, food and feed additives etc)[4]

Evidences accumulated in the last two decades have indicated that a large number of pesticides are capable of inducing genetic damage to human as well as domestic animals and economical plants. In recent years, there has been increasing awareness of the genotoxic potential of a wide variety of chemicals to which

the human population is exposed either environmentally or occupationally. This awareness is paralleled by the recent development of appropriate, sensitive and practical methods for detecting and assessing the possible genetic and biological effects of these substances[5].

Since, from the mouse samples are easily obtained and morphology is rapidly quantified as observed in comparison to humans, these observations suggest that the mouse may be an applicable screen for biological and environmental effects of pesticides[6].

The mouse has been used in biomedical research since the early 20th century. Today, over 3000 genetically defined strains of lab order mice are used for research laboratories, although the latter practice is becoming more common with some genetically engineered mice. Most of mice used in laboratories are white albino house mice (*Musmusclus*). The house mouse, a member of the rodent family, originated in ancient Asia and later spread throughout Europe.

Several characteristics have made the mouse an appealing research subject. These induce the mouse genetic similarity to human (at least 80% of DNA in mice is identical to that of humans), small size, short lifespan and reproductive cycle, low maintenance in captivity, and mild manner. For these reasons, house mice constitute the majority of mammals used in research, testing and education. The mouse, although the smallest of the common laboratory animals is in great demand in terms of numbers as an experimental animal. Up to 80% of all animals used in laboratories are mice. The small size, rapid reproduction, and relatively high position on the evolutionary scale provide numerous characteristics useful in all areas of research. The mouse is used in a wide variety of studies including drug toxicity, microbiology, radiobiology, cancer research, behaviour research, nutrition, and genetic studies. Test mice are subjected to the “classical” LD50 test [7].

Pesticides are widely used throughout the world in agriculture to protect crops and in public health to control diseases transmitted by vectors or intermediate hosts. Insect Growth Regulators (IGR’s) are third-generation insecticides less toxic and compatible with insect pest management that were developed to reduce the pollution of food and environment. These compounds have a specific mode of action on insects and have a lower toxicity against vertebrates than conventional insecticides. IGR’s include compounds that affect moulting and metamorphosis by mimicking Juvenile Hormone (JH agonists) or usually antagonizing JH activity (ecdysteroid agonists) or by interfering with cuticle formation (chitin synthesis inhibitors)[8,9,10].

During application of IGR’s on plants, part of the agent usually falls on the soil surface. Its subsequent penetration into the subsurface environment can cause pollution of soil, sediment and ground water. Evaluation of the corresponding ecotoxicity of IGR’s should be taken into consideration, in addition to the actual agent used, also its degradation products arising for the most part as metabolites of soil aerobic microorganisms[11,12]. IGR’s have a large potential for becoming an environmentally and economically important group of chemicals, however, very few toxicological studies have been carried out to evaluate the acute and chronic toxicity effects of Lufenuron on the laboratory animals. The researchers reported the obvious residues belonging to IGR group on fruit and vegetables during food processing, especially in acidic food becoming more persistent and less decayed even when used at high temperature.

Lufenuron is not broken down by the liver or kidneys. Lufenuron antifungal property may bedue to the fact that exoskeleton of insects is madeup of Chitin. The interesting fact about Chitin is that it is not just used by insectsand arthropods; it also makes up about half of the fungal cell wall. And fungi - also Candida alb cans- can't survive with half of their cell wall gone. The effect it has on Chitin production (Chitin is notfound in humans) makes this "off-label" use of Lufenuron an excellent broad-spectrum antifungalsuccessfully tested on a variety of animals in many countries around the world. It is not approved foruse as an antifungal medicine in humans. This is not because of side effects, but simply because themanufacturer is not interested in getting this drug certified for use as an antifungal in humans.

Materials and Methods

Chemical

Lufenuron 5.4% (w/w) (Cigna) Chemical composition of Lufenuron 540% w/w Emulsifyingagents caster of polyglcal, ether 36.40.6.00 w/w. Emulsifying agents linear alkylbenzone sulfonicacid. Calcium 4.00% w/w Solvent cycoto exanaon 20.00 solvent.(Solvent) 64.60% w/w.

Animals

Male albino mice, 7-8 weeks old, weighing 130- 140g were used for the study. The animals wereobtained from National Institute of Nutrition, Hyderabad and maintained in Central animalhouse, Rajah

Muthiah Institute of Health Science, Annamalai University, Annamalainagar, India.The rats were housed in polypropylene cages at room temperatures (27±2°C) with relative humidity55±5%, in an experimental room. In Annamalainagar, the LD (light: dark) cycle is almost 12:12h. The localinstitutional 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 careof Annamalai University in accordance with the Indian National Law on animal care and use. Theanimals were provided with standard pellet diet (Amrut Laboratory Animal Feed, Mysore FeedsLimited, Bangalore, India) and water ad libitum. The mice were divided into two groups. Eachgroup having 6 mice. The group I was control and Group II was treated with Lufenuron (0.1520mg/kg). After the treatment, the Liver tissues were isolated from mice. The liver tissue was used forvarious biochemical estimations.

Estimation of AST (Aspartate aminotransferase)

(Pentila*et al.*1983)[13]

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

Estimation of ABT (Alanine aminotransferase)

(Hafkenshield and Dijid 1979)[14]

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

Estimation of alkaline phosphatase(Hafkenshield and Dijid 1979) [14]

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

Estimation of lipid peroxidation (Yagi 1987)

To 0.5ml of plasma 4.0ml of 0.083N sulphuric acid was added. To this mixture, 0.5ml of 10% phosphotungstic acid was added and mixed. After standing at room temperature for 5 mins, the mixture was centrifuged at 3000 rpm 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 3000rpm 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 3000rpm for 15 minutes. The color extracted in the butanol layer was read at 530nm. Standard malondialdehyde solution (5 moles) in 4.0ml volumes and blank containing 4ml distilled water were processed along with test samples. Lipid peroxide levels are expressed as moles/ml plasma.

Estimation of reduced glutathione

(Beutler and Kelly 1963) [16]

0.2ml of sample (plasma) was mixed with 1.8ml of EDTA solution. To this 3.0ml of precipiating 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 20-100µg of reduced glutathione was treated similarly.Values are expressed as mg/dl for plasma.

Estimation of glutathione peroxidase(Beutler and Kelley 1963) [16]

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 0.1ml of hydrogen peroxide were added to the mixture and

incubated at 37^0C 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 (1963). A blank was treated similarly to which 0.2ml of the enzyme was added after the incubation.

The activity of glutathione peroxidase is expressed as U/L plasma.

Estimation of Catalase (Sinha 1972) [17]

To 1ml of the phosphate buffer taken in each of four test tubes, 0.1ml of plasma was added. To this,0.4ml of H2O2 was added, The reaction was stopped at 15, 30, 45 and 60 seconds by theaddition of 2ml of the dichromatic acetic acid reagent. The tubes were boiled for 10mins, cooledand 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/Lplasma.

Estimation of superoxide dismutase (Kakkaret al.1984) [18]

The active of superoxide dismutase was assayed by the method of Kakkar et al., 1984 based on theformation of NADH Phenazinemetho sulphate – nitrobluetetrazoliumformayan. The assay mixturecontained 1.2ml of sodium pyrophosphate buffer, 0.1ml of phenazinemethosulfate and o.3ml ofnitrobluetetrazolium. O.2ml of plasma and water in a total volume of 3ml. The reaction was startedby the addition of 0.2ml of NADH. After incubation at 30°C for 90 seconds, the reaction wasarrested by the addition of 1ml of glacial acetic acid. The reaction mixture was stirred and shakenwith 4ml of n-butanol. The mixture was allowed to stand for 10mins, centrifuged and the butanol layerwas separated. The colour intensity of chromogen in butanol was measured at 560nm. A systemdevoid 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 analysisof variance (ANOVA), followed by Duncan's multiple range test (DMRT).The results wereconsidered 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 tissuewas 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	Untreated Mean ± Sd	Lufenurontreated Mean ± Sd
TBARS(nmoles/ml)	1.59±0.13	1.74±0.09*
GSH(mg/dl)	24.65±3.10	21.92±2.90*
Glutathione peroxidase (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 thesame column differ significantly differ at p < 0.05 (DMRT).

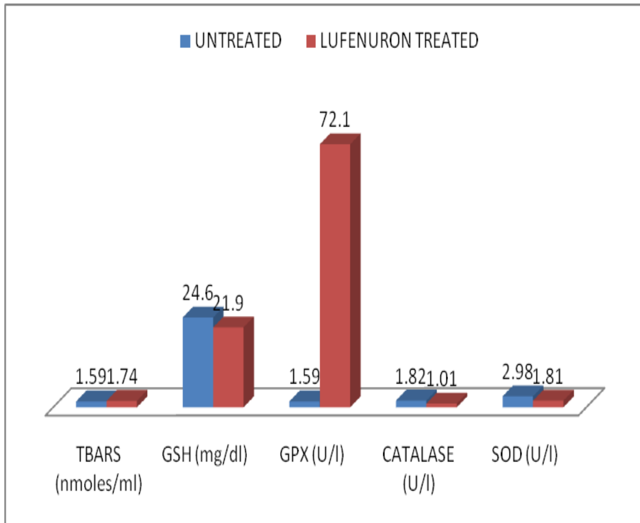

Fig.1.1 Mean Level of Lipid Peroxide and Antioxidative Stress Levels in *M.* musculus

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

Liver Function Parameters	Untreated Mean ± Sd	Lufenurontreated Mean ± Sd
SGOT (IU/L)	82±21.30	133±133.90
SGPT (IU/L)	67±17.30	114±29.70
ALP (IU/L)	92±18.30	286±39.10
TOTAL PROTEIN (g/dl)	5 ± 4.10	4.4 ± 3.10
ALBUMIN (g/dl)	3 ±1.70	2.1±1.30
SERUM GLOBULIN (g/dl)	2 ± 0.87	1.3 ± 0.58

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).

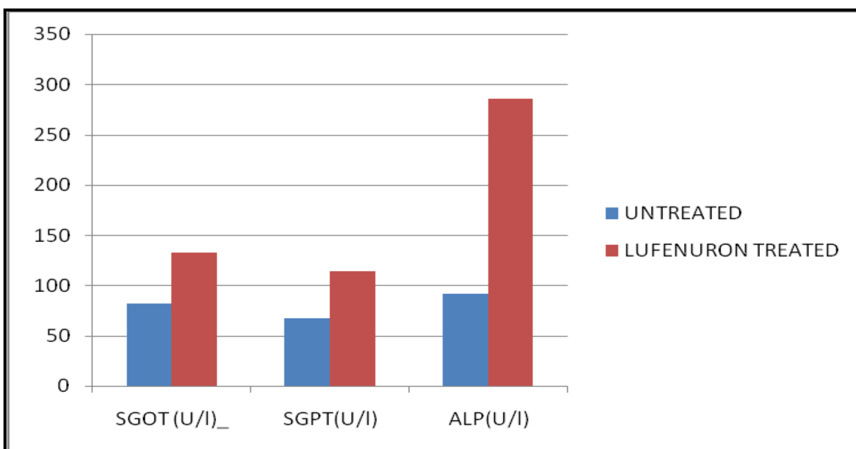

Fig.1.2 Variations in the Mean Level of Liver Enzymes of Control and Lufenuron Treated *M.* musculus

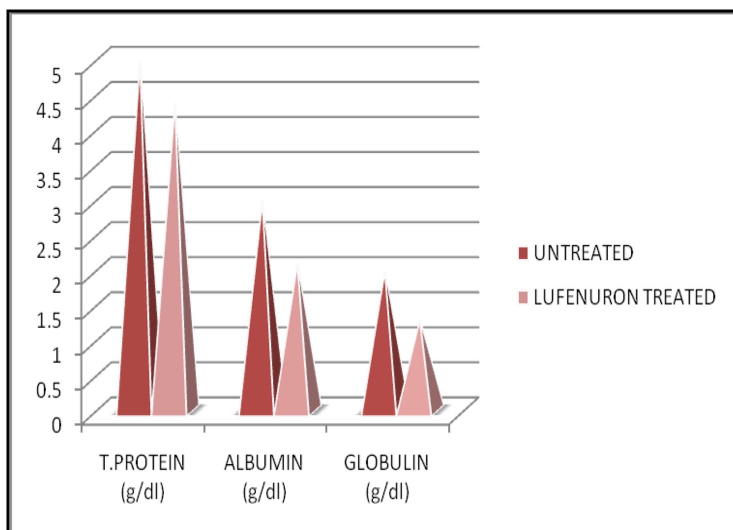

Fig. 1.3: Mean Variations of Serum, Total Protein, Albumin and Globulin Level in *M.* musculus

Level of glutathione peroxidase (GPx)

In the normal untreated control mice, the level of GPX activity in the In the normal untreated controlmice, the level of GPX activity in the was 1.59 ± 0.13. At sub-lethal dose of Lufenuron treatment foranimal 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 ofcatalase 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 Lufenurontreatment, 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 isstrongly bound to which is in enzymes by replacing the hydrogen atom to form covalent bond asmercaptides[19]. It is also capable of biotransformation of foreign chemicals[20]. Poisoning induced physiological and biochemical changes in the livercan be regarded as an index for the identification of pollutant stress [21]. Antioxidants such as GSH, SOD, CAT and GPX are the main defense againstO2 - and H2O2 mediated injury. Antioxidants both in enzymatic and non-enzymatic, together with thesubstance that are capable of either reducing or preventing their formation, form a powerfulreducing buffer which affects the ability of the cell to counteract the action of oxygen metabolitesforming the protective mechanism which maintains the lowest possible level of the inside the cell [22]. Lipid peroxidation is a chemical mechanism capable of disrupting the structure and the functionof the biological membranes that occurs as a result of free radical attack on lipids. The ability oflufenuron to produce Reactive Oxygen Species (ROS) was indicated in our study by the increasedamount of lipid peroxides measured as TBARS.

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

The GSH is essential for functional and structural integrity of the cell, tissues and organsystem [23].Glutathione peroxidase (GPx) is the well-known antioxidant enzymeagainst oxidative stess, which in turn requires glutathione as cofactor. It catalyzes the oxidationof GSH to GSSG at the expense of H2O2. It contains selenium molecules at the active sites andtranstens reducing equivalents from glutathione to H_2O_2 and

producing water and GSSG. GPx isantiperoxidative enzyme present in the cell and mitochondrial matrix. Catalase (CAT) is anenzyme, 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 H2O2accumulates in the tissue containing ferrous ions. It is mainly found in the peroxisomes, and removesH_2O_2 produced oxidation. SOD is an important defense enzyme, which converts superoxideradicals to hydrogen peroxide [24]. CAT is a heme protein, which decomposeshydrogen peroxidase and protects the tissue from highly reactive hydroxyl radicals [25].

In the present study, the level of glutathione and CAT, SOD significantly decreased but the level ofLPO, GPX content increased in the mice when treated with sub-lethal dose of Lufenuron. Thereduction of these enzymes may be due to oxidative stress of pesticide intoxication. Thepresent study showed that the increased level of LPO content suggested that the excess productionof ROS might be explained by its ability to produce alteration by blocking the permeability transitionpore [26] and alteration in mitochondrial electron transport chain. Theseevents cause the oxidative phosphorylation uncoupling and subsequent increase in ROSproduction [27].

The decreased activities of antioxidant represents increased utilization due to oxidativestress. [28], catalase is an enzyme catalyses decomposition of H2O2 towater and O2 and efficient inhibitor of LPO when hydrogen peroxide accumulates in a cell containingfree ferrous ions. In the present study the decreased level of catalase activity in serum followinglufenuron exposure may therefore be an important role in enhancing oxidative stress of cellularsystem. GPx is another antiperoxidative enzyme, which is present, both in cytosol and mitochondrialmatrix and is found to increased during exposure to lufenuron. The pesticide may inhibit the GPxdirectly by impairing the functional groups, or in directed by rendering the supply of reducedglutathione and NADPH. [29]reported that the decreased level of antioxidantdefence system mainly responsible for generating hydroxyl radicals leading to promote oxidativedamage by Fenton reaction. . This inhibition of antioxidant defence may be coupled with loweredtotal sulfhydryl (TSH) contents [30] or depletion of glutathione [31]. Fromthis study, we conclude that the Lufenuron induces freeradicals oxidative damage in hepatic tissueelevating the liver enzymes AST, ALT and ALP. Therefore we suggest further study of toxic effectof Lufenuron on the liver tissue of mice.

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