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The Effect of Lufenuron on Biochemical Parameters in Serum of Mice,Musmusculus species

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Abstract : The present study examined the impact of Lufenuron on biochemical parameters of albino mice.The biochemical parameters such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) and Total protein in serum were observed. The mice exposed with Lufenuron 0.1520 mg/kg) for 30 days. The present study suggests that the level of AST, ALT and ALP were increased and Total protein content was decreased in Lufenuron treated mice. The present study concludes that the Lufenuron damages the liver tissue. Due to the damage of liver tissue these enzyme increased in the serum.
Key words: Lufenuron, AST, ALT, ALP, Total protein, 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]

Lufenuron is the active ingredient in the veterinaryflea control medication, program, and the veterinary co-formulation flea control, heartworm prevention, and anti-helminthic medicine. Lufenuron is stored in the animal's body fat and 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 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, eriophidmites, and Western flowerthripsit has approval in a number of countries for use on a variety of crops, including soyabeans 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 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 reported to the toxic to fresh water zooplankton.

Bioavailability is a major factor in toxicity of environmental samples. According to the United States Environmental Protection Agency (US EPA), bioavailability measures the physicochemical access that a

toxicant has to the biological processes of an organism. The less the bioavailability of a toxicant, the less its toxic effect on an organism. Bioavailability refers to the difference between the amounts of a substance, such as a drug, herb, or chemical, to which a person is exposed and the actual dose of the substance the body receives. Bioavailability accounts for the difference between exposure and dose. A chemical toxicity is determined by the dose received at the target site in the body. The dose at the target site is determined by the amount of the substance absorbed by the body, which depends on its bioavailability. When a substance is ingested, its bioavailability is determined by the amount that is absorbed by the intestinal tract. If a substance is inhaled, its bioavailability is determined by the amount that is absorbed by the lungs. In dermal contact, its bioavailability is determined by the amount that is absorbed by the skin. Bioavailability started to be studied in drug and micronutrient field such as minerals and antioxidants in food[2,3]. Today questions of bioavailability of toxicants are sometimes at the root of disagreements about what are the appropriate actions to be taken to protect public health and the environment. Understanding bioavailability is critical to determine the level of chemical exposure that is likely to produce toxicity. The bioavailability of chemical contaminants in the environment depends on the nature of the medium in which they are found within other factors.

Previous studies have confirmed that soil matrix is an important factor in determining a pesticides' bioavailability [4]Some authors have reported pesticides bioavailability rates in dermal absorption from water. Also, there are publications about lung atmosphere Nevertheless; knowledge about the bioavailability of pesticides from foods is still limited. Several food components are able to form soluble or insoluble complexes with trace elements under gastrointestinal conditions. These food components thereby increase or decrease the availability for absorption in the small intestine, and thus the bioavailability of pesticides. Due to the complexity of food products, the relative contributions of food components to the bioavailability of pesticides are not clear. Although in vivo experiments would be the best way to study the intestinal bioavailability, in vitro methods also have been done . Therefore, there is a great need in human nutrition for an in vivo method which predicts the intestinal bioavailability. In vivo methods in which gastrointestinal conditions are simulated and the amount of the dialysed elements through tubing is measured, have obtained satisfying results to predict the intestinal bioavailability in vivo from foods. The in vitro method used in this work is expected to give good correlation with the intestinal bioavailability observed in vivo[3]

Due to the controversy generated about the abusive use of chemical pesticides in previous years, more biological pesticides such as IGR's have been designed to control pests in crops[5]. It is important to determine the bioavailability of these new pesticides from foods so that it is possible to assess the risk of these compounds for human. Lufenuron and organophosphates have been studied in samples of citrus fruits. The analytical methodology consisted of a Solid Phase Extraction (SPE) and determination by liquid chromatography coupled to diode array detection (LC-DAD) used The in vivo bioavailability of the compounds was studied not only in fresh fruit but also in standards and canned food in order to establish possible differences related to the matrix and to know the intestinal bioavailability in all possible situations[6].

Materials and Methods

Chemical

Lufenuron 5.4% (w/w) (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 cycotoexanaon 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 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 polypropylene 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 per the 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 blood samples were collected from venipuncture of mice.

The centrifuged blood samples were stored and serum were separated and used for various biochemical estimations.

Estimation of serum aspartate aminotransferase

(AST) and alanine aminotransferase (ALT)(Hafkenshield and Dijid 1979)[7]

The conversion of NADH to NAD+ is proportional to the concentration of AST in serum, and ismeasured at 340nm as rate of decrease in absorbance. To 100µl of serum, 1ml of givenreagent mix is added. The Mixture is mixed thoroughly and contents are transferred intocuvette. The first reading is recorded at 60^{th} second, and subsequently three more readings aretaken with 30 seconds interval at 340 nm.

Assay of alkaline phosphatase (ALP) in serum(Hafkenshield and Dijid 1979)[7]

Alkaline Phosphatase in a sample hydrolyses para – nitro phenyl phosphate into Paranitro phenol and phosphate, in the presence of magnesium ions. The rate of increase in absorbance of thereaction mixture at 405nm due to liberation of paranitrophenol is proportional to the alkaline Phosphatase activity. Twenty micro litres 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 are calculated.

Estimation of total protein in serum (Goodwin 1970)[8]

The peptide bonds of protein react with copper ions in alkaline solution to form blue violet coloured complex (biuret reaction). Each copperion complexes with 5 or 6 peptide bonds. The colour formed is proportional to the protein concentration and is measured at 546nm. To 20µl of serum and 20µl of standard, 1 ml of reagent is added in two different test tubes, mixed well and incubated for 10 minutes at 37C. The absorbance of test and standard are measured at 546nm.

Estimation of serum Albumin (Keyser 1962)[9]

Albumin binds with the dye Bromocresol Green in a buffered medium to form a green coloured complex. The intensity of the colour formed is directly proportional to the amount of albumin present in the sample. To 10µl of serum and 10µl of standard, 1 ml of reagent is added in two different test tubes, mixed well and incubated for 5 minutes at room temperature. The absorbance of test and standard are measured at 630nm against reagent blank.

Estimation of urea (Dumas 1971)[10]

To 0.1 ml of serum 1.0 ml of enzyme solution was added, mixed well and incubated for three Minutes at 37 0 C (Bumas*et al*., 1971). Then 1 ml of chromogen solution was added. This was mixed well and incubated for five minutes at 37 0 C. The standard and blank were treated similarly. The absorbances of the test and standard were measured against blank at 578 nm.

Estimation of serum Creatinine (Henry 1974)[11]

Fifty microlitres of serum was mixed with 1.0 ml of working solution (Henry *et al.*, 1974). The absorbance of assay mixture was read at exactly 30 seconds after the addition of serum and then again at 90 seconds. The standard was treated in a similar manner.

Estimation of serum glucose (Barham*et al*., 1972)[12]

To 10 µl of serum, 1 ml of enzyme reagent was added. Mixed well and incubated for ten minutes at 37 C. Similarly 10 µl of standard and 10 µl of deionized water as blank were treated. The absorbance of standard and test was measured against reagent blank at 505 nm.

Results

The level of AST was 82±1.3 U/L. in the serum of control mice. During the Lufenuron exposure the activity of AST was significantly increased in the serum (133±1.9) U/L. In the control mice, the level of ALT

was 67±1.3 U/L. At sublethal dose of Lufenuron the ALT increased upto114±1.7 U/L. in serum of Lufenuron treated mice. Serum Glucose, Urea, Creatinine, was observed to be in normal limit in both the groups.

The level of ALP was 92±1.3 U/L in control mice and 286±1.1 in treated serum of mice. The evel of total protein was 5 ± 1.1 g/dl in normal mice. During the Lufenurontreatment the level of total protein was decreased (4.4 ± 1.5) in serum of mice.

Table 1.1: Liver Function Test Levels in *M. musculus*

Liver Function Parameters	Untreated Mean ± Sd	Lufenuron Treated 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	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)

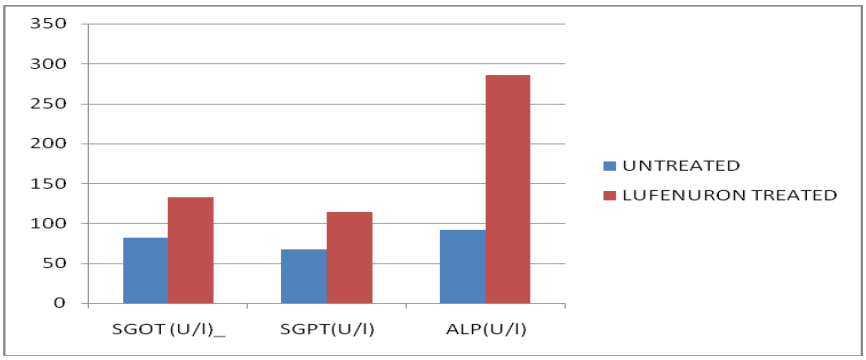

Fig.1.1: Variations in The Mean Level of Liver Enzymes of Control and Lufenuron Treated *M. musculus*

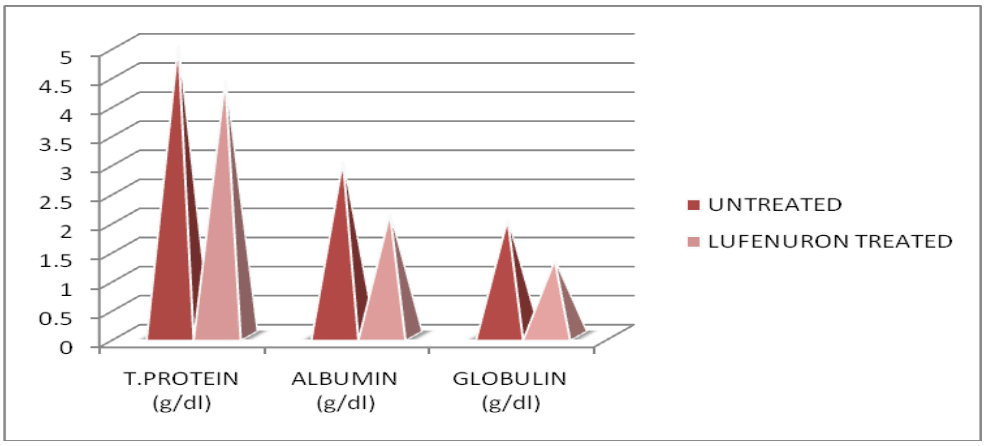

Fig. 1.2: Mean Variations of Serum, Total Protein, Albumin and Globulin Level in *M. musculus*

Table 1.2:The level of General Biochemical Parameters in serum of Lufenuron treated & control mice

Biochemical Parameters (Mg/Dl)	Untreated Mean ± Sd	Lufenuron Treated Mean ± Sd
GLUCOSE (mg/dl)	106 ± 6.70	161± 7.20
TOTAL CHOLESTEROL (mg/dl)	139 ± 22.10	154 ± 26.50
TRIGLYCERIDE	84 ± 12.40	181 ± 28.50
HDL- CHOLESTEROL (mg/dl)	30 ±3.10	43 ±4.30
LDL- CHOLESTEROL (mg/dl)	75 ±7.90	92 ± 8.90

Values are expressed as mean ± SD (n = 06).

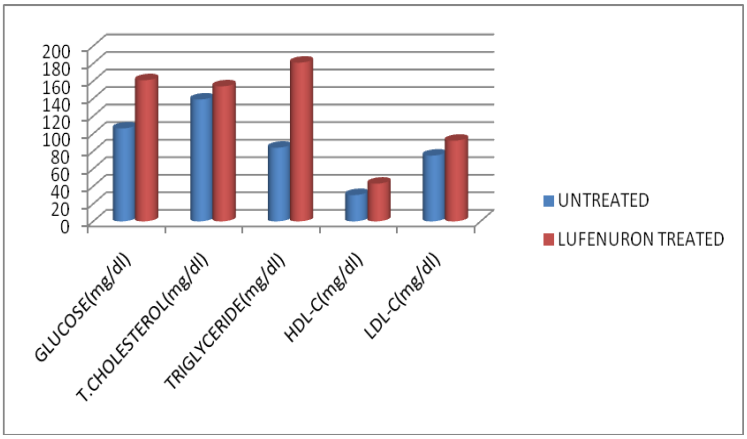

Fig. 1.3: A Comparison of Mean Blood Glucose and Lipid Profile Parameters in Control and Lufenuron Treated Mice

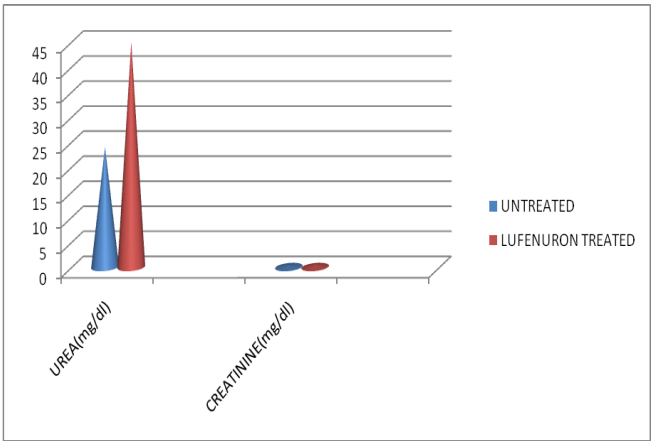

Fig. 1.4: Mean Serum Urea and Creatinine in Untreated and Lufenuron Reated Mice

Table 1.3: Blood Urea And Creatinine Levels In The Study Groups

Renal Function Parameters	Untreated Mean ± Sd	Lufenuron Treated Mean ± Sd
UREA (mg/dl)	24 ± 2.50	45 ± 3.10
CREATININE (mg/dl)	0.38 ± 0.18	0.58 ± .24

Values are expressed as mean ± SD (n = 06);

Discussion

The determination of the patho-physiological enzymes like AST and ALT is a common mean ofdetecting the liver status. Alterations in AST and ALT values are reported in hepatic disease or damage. AST, ALT and ALP are considered the bio-markers for liver functions[13,14]. AST is responsible for transferring amino group from aspartate to α- βglutaric acid forming glutamate and oxaloacetate. The rise in AST level is virtually responsible forall types of hepatic disease. Its peak concentration and ratio to other enzymes reflect the type of hepatic damage[15].

ALT is responsible for transferring an amino group from alanine to α-ketaglutaric acid forming glutamate and pyruvate. It is well known that AST is very specific enzyme for hepatic tissue. It is more sensitive to hepatic damage and its level rises faster and higher in most types of hepato cellular damage [15]. The present study shows significant increase in the level of AST and ALT in the serum of mice treated with sub lethal dose Lufenuron. This result indicates that the increase in AST and ALT in serum may be due to hepato cellular necrosis, which causes increase in the permeability of thecell membrane resulting in the release of Transaminase in the blood stream. Similar results were found by[16]. They reported that the serum AST and ALT are elevated due to heavy metals in chronic hepatic disease indicating toxic liver damage and correlating with the development of fibrosis[17,18,19] reported disturbances in the liver functions after exposure reported the increase in the level of AST and ALT activities in the serum of mice when treated with.[20]have also observed similar results in mice serum when treated with lufenuron. The increased AST and ALT activity in rats exposed to may reveal possible leakage of enzymes across damaged plasma membranes and or the increased synthesis of enzyme in the liver. Meanwhile, the elevation of ALP also correlates with exposure level and time of animals. The Increasedserum ALP has been explained by pathological processes such as liver impairment and kidney dysfunction [21,22]

Alkaline phosphatase is a brush border enzymes, splits various phosphate esters at an alkaline pH and mediates membrane transport[23]. Alkaline phosphatase is a membrane bound enzyme and its inactivation leads to membrane damage of hepatic cells[24]. Increased Alkaline phosphatase is responsible for intra-and extra-hepatic disease. These enzymes are indicative of various aspects of metabolism and they have been used to evaluate the physiological, biochemical and metabolic defects in the brain, liver and kidney tissues. Alkaline phosphatase is involved in the synthesis of nuclear proteins, nucleic acids and phospholipids as well as in the cleavage of phosphate esters. These enzymes are associated with transport mechanism, ion transport, maintenance of ionic strength and cell growth in the organ[36]. The significant increase in the activities of acid and alkaline phosphatises may be attributed to the destruction of all membranes and lysosomes which in turn might cause tissue damage [25,26]. Similar observations were made by various authors [27,28,29].

Proteins are important organic constituents of the animal cells. It plays a vital role in the process of interactions between intra and extra-cellular media being a part of cell membrane and an enzyme. It participates the intricately balanced sub cellarfraction[31,30]. Proteins are important organic substances required by an organism in the tissue building, the cellular organelles repair and alsocellular metabolism[32]. Its synthesis is considered as premier biochemical parameters since it is the most sensitive and earlier indicator of stress. It can be reflected by a large number of exogenous substances, mainly through reduction of the endoplasmic reticulum in the cells[33].

The present study showed that the level of total protein content significantly decreased in serum of mice when treated with sub-lethal dose of Lufenuron. This result suggests that the decreased level of total protein might be due to their catabolism to liberate energy during the stress of Lufenuron toxicity similar type of results was observed by [34,35]. in rat and mice when they treated with lead and cadmium respectively. The liver enzymes in the present work (AST, ALT and ALP) was increased and total protein in serum were significantly increased were observed in Lufenuron intoxicated animals (Table 1). These results suggested that the Lufenuron has induced treated mice hepatotoxicity Serum glucose, urea, Creatinine to be is normal limit in both the groups.

These results may be due to hepatic cellular necrosis which causes increase in the permeability of cell membrane resulting in the release of these enzymes in the blood stream. As the liver is the centre for detoxifying many foreign compounds entering the body.Lufenuron which is to mice is neither not properly detoxified in body or have caused its direct toxic effect on hepatic tissue. So in the present study, It is concluded that treated albino mice with Lufenuron showed significant changes in the level of ALT,AST,&

ALP without returning to normal levels even at the end of 30 days of recovery period. So further toxic effects of Lufenuron on hepatic tissue is to be considered.

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