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Editorial

Corresponding author
Bashir M. Rezk, PhD
Associate Professor
Department of Natural Sciences
Southern University at New Orleans
6400 Press Drive
New Orleans, LA 70126, USA
Tel. +1(504)284-5405; +1(504)957-8806
E-mail: batteia@suno.edu

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Electronic Cigarettes: Toxicity and Addiction

Bashir M. Rezk, PhD[1,2*]; Merhan E. Y. Khedr, MD[2]; Suresh C. Sikka, PhD, HCLD[2]; Wayne J. G. Hellstrom, MD, FACS[2]; Asim B. Abdel-Mageed, DVM, PhD[2]

[1]Department of Natural Sciences, Southern University at New Orleans, New Orleans, LA 70126, USA

[2]Department of Urology, Tulane University School of Medicine, New Orleans, LA 70112, USA

Cigarette smoking remains a leading cause of preventable disease and premature death in the United States and other countries. More than 20 million Americans have died as a result of smoking since the 1st Surgeon General's report on smoking and health was released in 1964.[1] Approximately, 2.5 million were non-smokers who died from heart disease or lung cancer caused by exposure to secondhand smoke (SHS).[1] Generally, smoking causes 20% of deaths in the United States each year.[1] Premature death from complications of smoking is approximately 50%.[1] Electronic cigarettes [also known as e-cigarettes and electronic nicotine delivery systems (ENDS)] are battery-operated devices designed to deliver flavored nicotine to users in a vapor, as a substitute to conventional cigarettes, cigars, and pipes. E-cigarettes were invented and patented by the Chinese engineer Hon Lik in 2003. E-cigarettes are now the most commonly used tobacco products among youth and the rising popularity of e-cigarettes among teens. The Centers for Disease Control and Prevention (CDC) reported that more than 3 million middle and high school students were users of e-cigarettes in 2015, compared to 2.46 million in 2014.[2,3] In 2013-2014, 81% of youth e-cigarette users cited the availability of appealing flavors as the primary reason for use.[4] Candy-flavored, fruit-flavored and menthol-flavored e-cigarettes appeal to adolescents more than tobacco-flavored or alcohol-flavored e-cigarettes, as well as common beliefs that e-cigarettes offer reduced harm.[5] The online availability of e-cigarettes allows this age group to easily order them. In May 2016, the National Institute of Drug Abuse (NIDA) reported that, the Food and Drug Administration (FDA) has finalized new regulations to prohibit minors from buying e-cigarettes in person or online.[6] The FDA rule extended the regulatory authority to cover all tobacco products, including vaporizers, vape pens, hookah pens, electronic cigarettes, e-pipes, and all other ENDS. FDA now regulates the manufacturing, import, packaging, labeling, advertising, promotion, sale, and distribution of ENDS.

E-cigarettes usually contain major ingredients such as propylene glycol, glycerol, ethylene glycol and polyethylene glycol mixed with concentrated flavors, and optionally, a variable percentage of nicotine.[7-9] Other organic compounds can be found in liquid formulated products and/or the vapor phase produced by an e-cigarette unit. These include tobacco specific nitrosoamines such as N-nitrosonicotine, N-nitrosoanabasine, N-nitrosoanabatine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, or tobacco-specific impurities such as cotinine, anabasine, and myosmine.[7-9] Data on the daily exposure from liquid-phase aerosol particulate matter in e-cigarettes showed significant formation of formaldehyde and formaldehyde hemiacetals when high voltage (5 V) was applied.[10] Geiss et al[11] reported that, when considering concentrations in each inhaled puff, the short-term indoor air guideline value for formaldehyde was already exceeded at the lowest wattage of 5 W, which is the wattage applied in most second-generation e-cigarettes. The e-cigarette aerosol also contained metals such as silver, iron, nickel, aluminum, silicate, chromium, lead, tin, and cadmium.[12] The concentrations of these elements in e-cigarettes aerosol were higher than or equal to the corresponding concentrations in conventional cigarette smoke.[12] Evaluating the potential harm associated with e-cigarette use requires detailed analysis of various aspects of these products and their metabolites, including their toxicological profiles.[13]

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Sellers of e-cigarettes have made a variety of claims indicating that e-cigarettes are safer than conventional cigarettes and that their use facilitates smoking cessation.[14] However, e-cigarette manufacturers do not provide complete information on the chemicals used in the manufacturing process, or the chemicals that may be released or synthesized during the aerosol generation process that occurs during use.[14] Generally, e-cigarettes required stronger vacuums to smoke than conventional brands, and the effects of this on human health could be adverse.[15,16] Health risks associated with the intended use of electronic cigarettes cannot be excluded at present.[14]

Nearly 50 mg of nicotine are lethal for adults.[17] However, for children; a dose of only 6 mg is life-threatening.[17] Liquids used in e-cigarettes usually contain up to 36 mg/ml of nicotine. Study of Morean et al[18] reported that e-cigarettes smokers, males, and those who purchased their own e-cigarettes reported using the highest nicotine levels. Smoking is a highly efficient form of drug administration.[19] Inhaled nicotine enters the circulation rapidly through the lungs and moves into the brain within seconds and reinforces the effects of the drug.[19] However, nicotine addiction is more threatening than its toxic effects. This addiction consists of a physical and a psychological component, making cessation particularly hard.[19,20] It is possible that e-cigarettes smokers who were more experienced in using the products pulled harder at the devices leading to increased nicotine delivery.[16] Since users of electronic cigarettes absorb nicotine when vaping, it is possible that smokers who have switched to e-cigarettes or are using them in addition to conventional cigarettes may maintain nicotine dependence because of the smoking ritual being maintained.[16] It is particularly important to protect young people and children from toxicity and addition of e-cigarettes.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Research

*Corresponding author
Sujata Maiti Choudhury, PhD
Department of Human Physiology with Community Health
Vidyasagar University
Midnapore, West Bengal, India
Tel. + 9474444646
Fax: + 3222 275 329
E-mail: smaitichoudhury@yahoo.com; sujata.vu2009@gmail.com

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Lambda Cyhalothrin Elicited Dose Response Toxicity on Haematological, Hepatic, Gonadal and Lipid Metabolic Biomarkers in Rat and Possible Modulatory Role of Taurine

Rini Ghosh, MSc; Tuhina Das, MSc; Anurag Paramanik, MSc; Sujata Maiti Choudhury, PhD*

Department of Human Physiology with Community Health, Vidyasagar University, Midnapore 721102, West Bengal, India

ABSTRACT

Extensive application of pesticides is usually accompanied with serious problems of pollution and health hazards. Lambda-cyhalothrin (LCT), a type II synthetic pyrethroid, is widely used in agriculture, home pest control and protection of foodstuff. This study designed to evaluate the dose dependent haematological, hepatic and gonadal toxicity of LCT at different dose levels in Wistar rat. Investigations were also done to find out the toxic effect of lambda cyhalothrin on lipid metabolism in female rat and its amelioration by taurine. Rats were exposed to different doses of lambda cyhalothrin over a period of 14 consecutive days. Exposure to LCT produced ataxia, agitation, rolling and also tremors which were considered as the signs of toxicity. Significant decrease in erythrocyte count, haemoglobin percentage, seminal fructose concentration, hepatic and testicular reduced glutathione (GSH) content was observed. Increase in leukocyte count, serum aspartic and alanine transaminase, hepatic and testicular malondialdehyde (MDA), testicular and ovarian cholesterol after LCT treatment were seen in male rats at the dose level of 10.83 mg/kg body wt. ($1/7^{th}$ LD_{50}). Elevated ovarian cholesterol and MDA and reduced 3β hydroxy steroid dehydrogenase (HSD) and GSH level were also observed in lambda cyhalothrin exposed female rat at the dose level of 6.29 mg/kg body wt. ($1/9^{th}$ LD_{50}). LCT caused increase in serum triglyceride, cholesterol, low density lipoproteins (LDL), very low density lipoproteins (VLDL) and bilirubin and decrease in serum high density lipoproteins (HDL) in female rat. Taurine pretreatment ameliorated LCT induced altered lipid metabolic biomarkers in female rat.

KEYWORDS: Lambda cyhalothrin; Taurine; Wistar rat; Hepatic and gonadal toxicity; Lipid metabolism.

ABBREVIATIONS: LCT: Lambda-cyhalothrin; GSH: Glutathione; MDA: Malondialdehyde; HSD: Hydroxy Steroid Dehydrogenase; LDL: Low Density Lipoproteins; VLDL: Very Low Density Lipoproteins; HDL: High Density Lipoproteins; DNA: Deoxyribonucleic acid; EC: Emulsifiable Concentrate; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals.

INTRODUCTION

The interaction of xenobiotics with the biological system is a multifaceted phenomenon, which comprises interplay between the environment, the host and the chemical substance. Chemical contagion as a result of pesticide introduction has been assumed as one of the factors for the deterioration of natural fauna. Indiscriminate application of pesticides is usually accompanied with serious problems of pollution and health hazards.[1]

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Pyrethroids, derivatives of natural pyrethrins have been well known for their high effectiveness against insects and can be easily biodegraded than other types of pesticides.[2] In spite of claims of low mammalian toxicity of pyrethroid, several investigations reported the toxicological evidence of pyrethroid among various species of animals.[3] Pyrethroids are easily absorbed through gastrointestinal and respiratory tract due to their lipophilic nature and also make them easier to be stored in the lipid rich internal tissues like body fat, skin, liver, kidney, central and peripheral nervous systems. Hemato-biochemical studies are important for the analysis of the functional status of animals to suspected toxic agents. It may act as a strong evidence against toxicity of contaminated pyrethroid insecticides. Recent reports have clarified that exposure to pyrethroid leads to a significant modifications in hematological findings.[4] On the other hand the liver is the first organ encountered when toxicants enter into the body. The liver was found to accumulate a huge pyrethroid residues as it is the primary site for pyrethroid metabolism. A large number of man-made chemicals such as pesticides stated to produce liver damage.[5] Several investigations also reported that pesticides adversely affect the testicular functions in experimental animals[6-9] as well as they are potent endocrine disrupters.[10,11] Pesticides are responsible for oxidative stress that causes free radicals generation, leading to deoxyribonucleic acid (DNA) fragmentation.[12,13]

Lambda-cyhalothrin (LCT), a type II pyrethroid pesticide, is used worldwide to control pests in a variety of agricultural crops. LCT is chemically alpha-cyano-3phenoxybenzy3-(2-chloro-3,3,3 trifluoropropenyl)-2,2,dimethylcyclo- propane carboxylate. It revealed that lambda-cyhalothrin is moderately toxic for mammals[12,13] and highly toxic for fish, aquatic invertebrates and bees. LCT at low concentrations can cause death in these species.[14,15] The degree of concentration and nature of solvent are important for the toxicity of lambda cyhalothrin.[16]

Taurine (2-aminoethane sulphonic acid), a free intracellular sulfonated beta amino acid, is present in many animal tissues especially muscle, brain, liver, heart, etc.[17] From the metabolism of methionine and cysteine, taurine is derived and is much more concentrated in pro-inflammatory cells such as polymorphonuclear phagocytes and in the retina.[18] Taurine takes part in bile acid conjugation, detoxification, osmoregulation and modulation of cellular calcium level.[19-21] Taurine acts as major antioxidant in most living organism which attributes its ability to stabilize biomembranes[17] and to scavenge reactive oxygen species.[22]

For any pesticide, the exposure concentration is important in the alteration of its toxicity. So in our present study, LCT were administered at different concentrations to find out the exact toxic dose levels of LCT which can produce hematological, hepatic and gonadal toxicity in male and female Wistar rat. At the same time, investigation was also carried out to evaluate the protective role of taurine against LCT induced alterations in lipid metabolism of female rat.

MATERIALS AND METHODS

Chemicals and Reagents

Lambda cyhalothrin 5% emulsifiable concentrate (EC) was procured from RPC Agro Industries, Kolkata. Taurine was purchased Sigma Aldrich Inc., USA. All other chemicals used were of analytical grade and were purchased from Merck India Ltd, Himedia India Ltd, etc.

Animals and Care

For the present study mature Wistar male and female albino rats (weighing 130-150 g) were taken and the animals were housed in polypropylene cages at an ambient temperature of 25 °C ±2 °C with 12 hrs light-dark cycle. The rats were acclimatized for one week prior to different treatments. The standard laboratory feed and water were supplied throughout the period of experimentation. The present study was approved by the Institutional Animal Ethical Committee (IAEC), registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India and performed in accordance to the relevant laws and guidelines of the CPCSEA.

Experimental Design

A commercial formulation of lambda cyhalothrin 5% EC 'Karate' was used for the study. Dilution of lambda cyhalothrin was done in distilled water to acquire the test concentrations. The test concentrations of lambda cyhalothrin were measured from the percentage of the active ingredient present in above mentioned commercial formulation of lambda cyhalothrin.

Healthy mature Wistar rats (n=6, for control and each dose group) of either sex selected by random sampling, were used for the study. The rats were kept fasting for overnight providing only sufficient water, after which LCT were administered orally for 14 consecutive days at the dose level of 6.89 mg/kg body wt. (i.e. 1/11th LD_{50} dose) for male and 5.15 mg/kg body wt. (i.e. 1/11th LD_{50} dose) for female. Sharma et al[23] reported that oral LD_{50} dose of LCT for mature male and female rats were 75.85 and 56.69 mg/kg body wt. respectively. The procedure was repeated for the dose levels of 7.58(1/10th LD_{50}), 8.42(1/9th LD_{50}), 10.83(1/7th LD_{50}), 15.17(1/5th LD_{50}), 18.96(1/4th LD_{50}), 25.28(1/3rd LD_{50}) mg/kg body wt.[24] for male rats. For female rats, lambda-cyhalothrin were administered orally at the dose levels of 5.66(1/10th LD_{50}), 6.29(1/9th LD_{50}), 8.09(1/7th LD_{50}), 11.33(1/5th LD_{50}), 14.17(1/4th LD_{50}), 18.89(1/3rd LD_{50}) mg/kg body weight. Dose solutions were freshly prepared immediately before usage.

In another separate set of experiment, female rats were divided into the following six groups each containing six to as-

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sess LCT induced toxicity to lipid metabolism and to find out the protective effect of taurine against this toxicity. Taurine was administered at the dose level of 50 mg/kg body wt., which was effectively used to protect the toxicity induced by various xenobiotics.[25,26]

1. Group A: Control (no treatment).
2. Group B: Taurine control (50 mg/kg body wt.).
3. Group C: LCT low dose (1/9 of LD_{50} value i.e., 6.3 mg/ kg body wt.).
4. Group D: Taurine (50 mg/kg body wt.)+lambda-cyhalothrin low dose (6.3 mg/kg body wt.).
5. Group E: Lambda-cyhalothrin high dose (1/5 of LD_{50} value i.e., 11.33 mg/kg body wt.).
6. Group F: Taurine (50 mg/kg body wt.)+lambda-cyhalothrin high dose (11.33 mg/kg body wt.).

At the end of the doses, the animals were fasted overnight. On 15th day, the rats were anesthetized with pentobarbital sodium (35 mg/kg) and sacrificed by cervical dislocation. Samples were collected and stored at -80 ºC until analysis.

Study on Body Weight

Body weight of individual overnight fasting male and female animals was taken regularly before the administration of LCT. After 24 hrs of the treatment of the last dose, all animals were weighed and then sacrificed.

Measurement of Haematological Parameters in Male Rat

Erythrocyte count: Erythrocyte count was done by the dilution of blood with red blood cell (RBC) dilution fluid (1:200) and the total erythrocyte counts[27] were expressed as $\times 10^6/mm^3$.

Estimation of haemoglobin percentage: The haemoglobin percentage was measured by cyanmethemoglobin method.[28] Using Drabkin's diluent as a blank, the optical density was measured at 540 nm.

Total leukocyte count: Blood was diluted (1:20) with white blood cell (WBC) dilution fluid and total leukocyte were counted using Neubaur haemocytometer.[27]

Study of Hepatic Biomarkers in Male Rat

Assay of serum glutamate-oxaloacetate transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT): For SGOT sample, 1 ml of buffer substrate (2.66 gm aspartic acid, 60 mg α-ketoglutaric acid and, 20.5 ml of 1(N) NaOH and 100 ml of volume was made by 0.1 M phosphate buffer, pH-7.4) and for SGPT sample, 1 ml of buffer substrate (1.78 gm DL-alanine, 30 mg α-ketoglutaric acid, 20 ml of 0.1M phosphate buffer and 1.25 ml of 0.4(N) NaOH) were taken and waited for 5 min at 37 ºC. Then 0.2 ml of serum sample was mixed and incubated at 37 ºC for 60 min. To prepare standard, 0.2 ml of working standard (200 µM/100 ml) was taken in a test tube and 0.8 ml of buffer substrate was added. For blank, 1.0 ml of buffer substrate was taken. In each of sample, standard and blank test tubes, 1 ml of 2,4-dinitrophenylhydrazine hydrochloride (DNPH) solution were added and waited for another 20 min. Then 10 ml of 0.4(N) NaOH was mixed and waited for 10 minutes. Readings were taken at 520 nm in spectrophotometer[29] (UV-245 Shimadzu, Japan).

Assay of hepatic malondialdehyde (MDA): Malondialdehyde (MDA) was determined by the mixing of 1 ml of sample with 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of acetate buffer (20%, pH-3.5) and 1.5 ml of aqueous solution of thiobarbituric acid (0.8%) and the mixtures were boiled for 60 min at 95 ºC. After heating when the red pigment was produced, that was extracted with 5 ml of n-butanol-pyridine (15:1) and centrifuged at 5000 rpm for 10 min at room temperature. The optical density of supernatants was measured at 535 nm.[30]

Estimation of hepatic reduced glutathione (GSH) content: 100 µl of sulfosalicylic acid was mixed with 200 µl of sample. Then the mixture was allowed for centrifugation at 3000 rpm for 10 min. With the supernatant, 1.8 ml of DTNB was included and shaken well.[31] Final reading was noted at 412 nm.

Study of Male Reproductive Parameters

Measurement of seminal fructose concentration: In a centrifuge tube, 1 ml of diluted seminal plasma (five times dilution was done by mixing 0.1 ml of seminal plasma with 4.9 ml of distilled water) was added with 0.3 ml of 1.8 gm% $ZnSO_4$ and 2 ml of 0.1 M NaOH. After 15 min, the mixture was centrifuged at 2000 g to obtain the supernatant. Then seminal fructose concentration was measured by taking 0.5 ml of supernatant as sample, 0.5 ml of 0.14 mM and 0.28 mM fructose solutions as two standards and 0.5 ml of distilled water as blank. Then, 0.5 ml of indole reagent and 5 ml of concentrated HCl were added to each test tube. The test tubes were then incubated at 50 ºC for 20 min and were cooled in ice water and then in room temperature.[32] The reading was taken at 470 nm in spectrophotometer (UV-245 Shimadzu, Japan).

Estimation of testicular cholesterol: Testicular tissue was homogenized with 0.5% $FeCl_3$ solution at a conc. of 20 mg/ml. Supernatant was collected after centrifugation of the homogenized tissue at 2000 rpm for 10 min. Then 0.1 ml of supernatant was added with 6 ml of glacial acetic acid to prepare sample. Simultaneously 5.9 ml of glacial acetic acid was added with 0.1 ml of working standard and 0.1 ml of distilled water to prepare standard. Blank was prepared by mixing 6 ml of glacial acetic acid and 0.1 ml of distilled water. Then 4 ml of colour reagents were added to each, mixed vigorously and stand for 20 minutes for spectrophotometric reading at 570 nm against blank.[33]

Assay of testicular malondialdehyde (MDA) and reduced glutathione (GSH) content: Testicular malondialdehyde (MDA) and

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reduced glutathione (GSH) content were measured by the respective above mentioned methods.[30,31]

Study of Female Reproductive Parameters

Estimation of ovarian Δ⁵, 3β-hydroxysteroid dehydrogenase (Δ⁵, 3β-HSD) activity: Homogenizing media was prepared by 20 ml of glycerol, 0.01 M EDTA in 0.05 M phosphate buffer in 100 ml with redistilled water. Tissue homogenate (20 mg/ml homogenizing media) was centrifuged at 10,000 rpm for 30 min at 4 ºC in a cold centrifuge. One ml of supernatant was mixed with 1 ml of sodium pyrophosphate buffer and 40 µl of dehydroepiandrosterone (DHEA). After addition of 0.1 ml of NAD, the activity of Δ^5,3β-HSD was measured at 340 nm against a blank (without NAD).[34]

Assay of ovarian cholesterol, malondialdehyde (MDA) and reduced glutathione (GSH) content: Ovarian cholesterol, malondialdehyde (MDA) and reduced glutathione (GSH) content were measured by the respective above mentioned methods.[33,30,31]

Study of the protective effect of taurine on serum bilirubin and lipid profile in female rat in selected dose levels

Serum bilirubin was determined by the method of Jendrassik and Grof using commercial diagnostic reagent kit.[35] Serum cholesterol (CHO), triglyceride (TG) and high density lipoprotein cholesterol (HDL) were estimated by the commercial diagnostic reagent kit.[36] VLDL was calculated using the formula (TG/5).[37] LDL concentration (mg/dL) was estimated indirectly from the concentrations of CHO, TG and HDL using the equation[37] LDL=CHO-(VLDL+HDL).

Statistical Analysis

The data was expressed as Mean±SEM. The differences between the means of each group were tested using a one way ANOVA test (using a statistical package, Origin 6.1, Northampton, MA, USA). $p<0.05$ was considered to indicate a statistically significant difference.

RESULTS

General Observations

After each treatment of lambda cyhalothrin, the animals were kept under observation at least once in 30 min interval. Special attention was given up to 4 hrs from the time of treatment. Rats those were exposed to different doses of lambda cyhalothrin over a period of 14 days produce ataxia, agitation, rolling and also tremors which were considered as the signs of toxicity up to eleventh day of treatment after which the symptoms were reduced.

Changes in Body Weight, Food and Water Intake

Significant changes in final body weight were found at the dose level of 10.83 mg/kg body wt. ($1/7^{th}$ LD_{50}) in male rats and at the dose level of 5.15 mg/kg body wt. ($1/11^{th}$ LD_{50}) in female rat (Figure 1). No differences in food and water consumption were seen in last consecutive four days of treatment in the experimental schedule but there was little non-significant alterations observed in food consumption at the exposure dose levels of $1/4^{th}$ and $1/3^{rd}$ LD_{50}.

Effect on Haematological Parameters in Male Rat

To find out the dose dependent effect of LCT, some clinically significant biochemical parameters from each system were studied. From the study it was seen that total erythrocyte count was decreased significantly ($p<0.05$) from the dose level of 10.83 mg/kg body wt. ($1/7^{th}$ LD_{50}) to 25.28 mg/kg body wt. ($1/3^{rd}$ LD_{50}) in male rat (Table 1). No significant changes were found below 10.83 mg/kg body wt. ($1/7^{th}$ LD_{50} dose). Maximum toxic effect

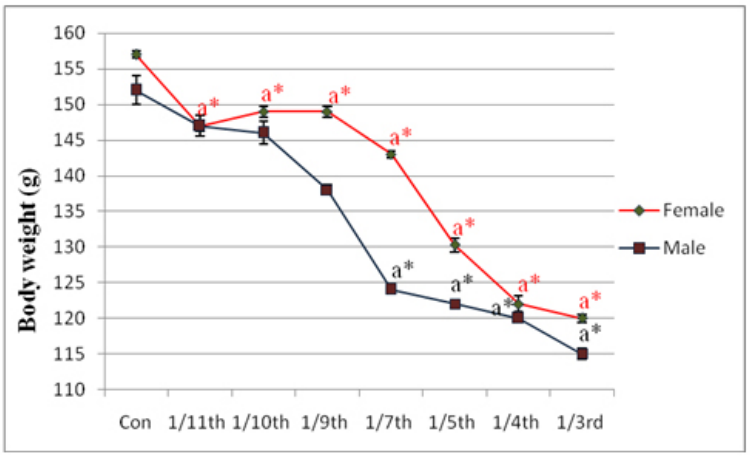

Figure 1: Effect of lambda cyhalothrin on body weight of male and female rat. Results are expressed as Mean±SEM. Analysis is done by one way ANOVA. Superscript a, control group *versus* all other groups (*indicates $p<0.001$). (For male and female the LD_{50} values are different which are mentioned in materials and methods section).

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Group	Erythrocyte count ($\times 10^6$ / mm^3)	Hb percentage (gm/dL)	Leukocyte count ($\times 10^6$/µl)
Control	7±0.3	14±0.5	6±0.17
1/11th LD_{50} dose (6.89 mg /kg body wt.)	7.2±0.2	14±1.1	6±0.11
1/10th LD_{50} dose (7.58 mg /kg body wt.)	7±0.1	13.3±1.4	6.03±0.14
1/9th LD_{50} dose (8.42 mg /kg body wt.)	7±0.06	13.6±0.6	6.03±0.15
1/7th LD_{50} dose (10.83 mg /kg body wt.)	6±0.2a*	10±0.6a**	8±0.11a***
1/5th LD_{50} dose (15.17 mg /kg body wt.)	5.9±0.1a*	8±0.6a**	9.2±0.12a***
1/4th LD_{50} dose (18.96 mg /kg body wt.)	5.9±0.2a*	8±0.6a**	9.1±0.17a***
1/3rd LD_{50} dose (25.28 mg /kg body wt.)	5.9±0.1a*	8±1.1a**	9.2±0.12a***

Table 1: The effect of lambda cyhalothrin on haematological parameters in male rat. Results are expressed as Mean±SEM. Analysis is done by one way ANOVA. Superscript a, Control group *versus* all other groups (*indicates p<0.05,**indicates p<0.01, ***indicates p<0.001).

of LCT was exhibited at the 15.17 mg/kg body wt. (1/5th LD_{50} dose) without any mortality. Interestingly, the rate of mortality was increased from the dose level of 18.96 mg/kg body wt. (1/4th LD_{50}). Similar results were found in case of haemoglobin percentage (p<0.01). Table 1 displays the significant (p<0.001) increase in leukocyte count in lambda cyhalothrin treated male rats from the dose level of 1/7th LD_{50} to 1/3rd LD_{50}.

Effect on Hepatic Biomarkers in Male Rat

Activities of serum glutamate-oxaloacetate transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT): The effects of LCT on SGOT, SGPT are shown in Figures 2A, and 2B respectively. The activity of these two important hepatic transaminase enzymes were increased significantly (p<0.001) with increase in the concentration of LCT as compared to the control rats. No significant alterations were noted below 10.83 mg/kg body wt. (1/7th LD_{50} dose) in male rat.

Hepatic lipid peroxidation and glutathione content: Hepatic malon-di-aldehyde (MDA) and reduced glutathione content in the control and experimental groups of male rats are shown in Figures 2C and 2D. MDA was found to be significantly increased (p<0.01) whereas reduced glutathione content was decreased significantly (p<0.001) in a dose-dependent manner from the dose level of 10.83 mg/kg body wt. (1/7th LD_{50} dose) onwards.

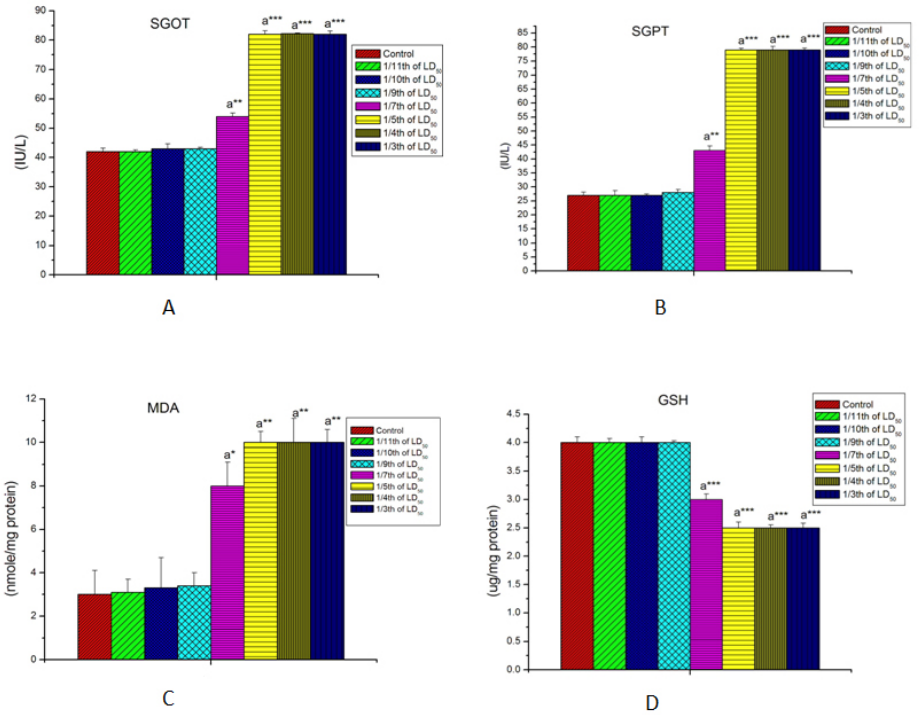

Figure 2: Effect of lambda cyhalothrin on some hepatic biomarkers in male rat.
A= Effect on SGOT. B= Effect on SGPT. C=Effect on liver MDA. D=Effect on liver GSH. Results are expressed as Mean±SEM. Analysis is done by one way ANOVA. Superscript a, Control group *versus* all other groups (*indicates p<0.05, **indicates p<0.01, ***indicates p<0.001).

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Effect on Male Reproductive System

Effect of LCT on seminal fructose concentration and testicular cholesterol: As shown in Table 2, the LCT induced reproductive toxicity by reducing seminal fructose concentration ($p<0.001$) and elevating testicular cholesterol level ($p<0.05$).

Impact of LCT on testicular oxidative stress and antioxidant status: Table 2, also shows the effect of LCT on MDA and GSH. Testicular MDA was found to be increased significantly ($p<0.01$) in response to LCT treatment whereas a significant decrease ($p<0.001$) in testicular GSH level was also noted. Significant changes were detected from the dose level of 10.83 mg/kg body wt. ($1/7^{th}$ LD_{50} dose) onwards.

Effect on Female Reproductive Function

Ovarian steroidogenic key enzyme activities and cholesterol content: LCT induced ovarian toxicity was exhibited by significant diminution in ovarian steroidogenic enzyme activities and elevation in ovarian cholesterol content at the dose level of 6.29 mg/kg body wt. ($1/9^{th}$ LD_{50} dose) and were continued towards increased dose levels of LCT (Figures 3A and 3B).

Effect of LCT in malondialdehyde (MDA) and reduced glutathione (GSH) level: LCT intoxicated rats shows a marked dose-dependent increase ($p<0.001$) in the lipid peroxidation, specified in term of MDA (Figure 1). Decline in GSH levels in LCT treated rat may also an indication of oxidative stress as GSH is used

Group	Seminal fructose concentration (nmole of fructose/lit of seminal plasma)	Testicular Cholesterol (mg/gm)	Testicular MDA (nmole/mg protein)	Testicular GSH (µg/mg protein)
Control	20±0.3	1.4±0.1	2.5±0.2	3.5±0.03
$1/11^{th}LD_{50}$	20±0.6	1.4±0.2	2.6±0.2	3.4±0.12
$1/10^{th}$ LD_{50}	20±0.5	1.4±0.05	2.6±0.1	3.4±0.03
$1/9^{th}$ LD_{50}	19.5±0.2	1.4±0.1	2.6±0.3	3.4±0.09
$1/7^{th}$ LD_{50}	18±0.1a**	1.9±0.1a*	4±0.2a*	2.9±0.12a**
$1/5^{th}$ LD_{50}	15±0.3a***	2.4±0.3a*	5±0.3a**	2.36±0.22a***
$1/4^{th}$ LD_{50}	15±0.2a***	2.4±0.1a*	5±0.2a**	2.36±0.25a***
$1/3^{rd}$ D_{50}	15±0.4a***	2.4±0.05a*	5±0.5a**	2.36±0.24a***

Table 2: Shows the effect of lambda cyhalothrin on some male reproductive parameters.
Results are expressed as Mean±SEM. Analysis is done by one way ANOVA. Superscript a, control group *versus* all other groups (*indicates $p<0.05$, **indicates $p<0.01$, ***indicates $p<0.001$).

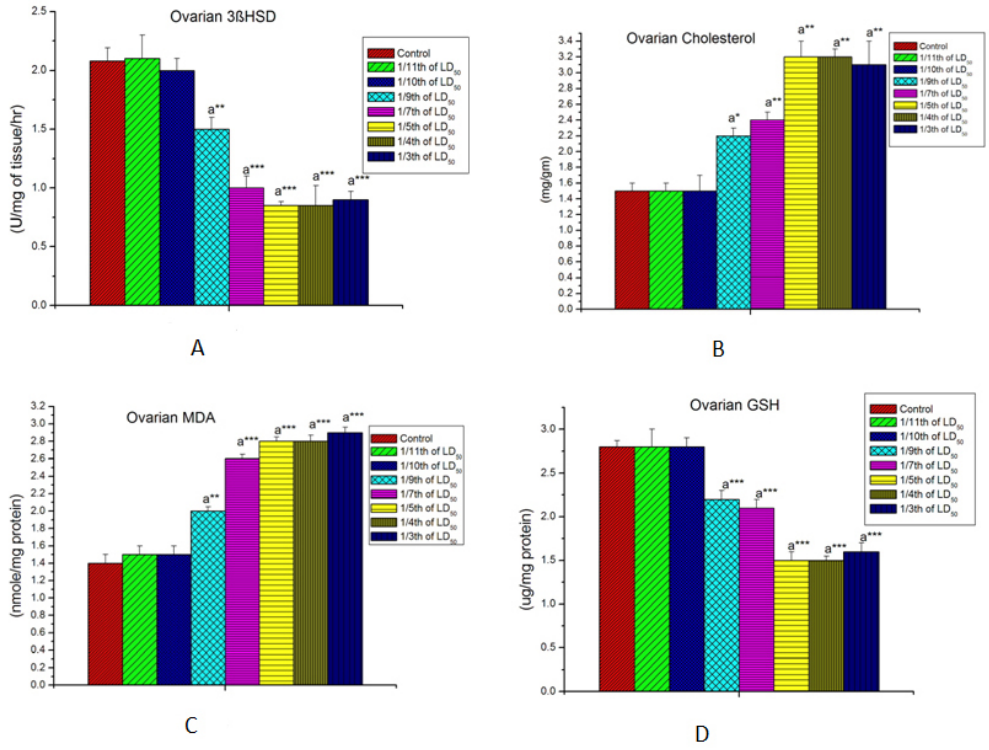

Figure 3: Effect of lambda cyhalothrin on female reproductive biomarkers.
A=Effect on ovarian 3β HSD. B= Effect on ovarian cholesterol. C= Effect on ovarian MDA. D=Effect on ovarian GSH. Results are expressed as Mean±SEM. Analysis is done by one way ANOVA. Superscript a, control group *versus* all other groups (*indicates $p<0.05$, **indicates $p<0.01$, ***indicates $p<0.001$).

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Parameters	Group-A	Group-B	Group-C	Group-D	Group-E	Group-F
Serum total Cholesterol (mg/dL)	120±0.7	112±1.5a***	138±0.8a***	127±1.3a***b***	149±1.5a***	137±1.2a***c***
Serum triglyceride (TG) (mg/dL)	98±1.0	92±1.0a**	110±1.0 a***	98±0.5b*	121±1.0a***	104±1.5a*c***
High density lipoprotein (HDL) (mg/dL)	45±0.7	51±1.0a***	38±0.2 a***	47±1.2b***	29±1.0a***	37±0.5 a***c***
Very low density lipoprotein (VLDL) (mg/dL)	19.6±0.2	18.4±0.2	21.6±0.1a***	20±0.3b*	24.2±0.2a***	20.8±0.4a**c***
Low density lipoproteins (LDL) (mg/dL)	55.3±1.8	42.6±2.7a**	78.4±0.8a***	60±0.2a*b***	95.8±0.7a***	79.2±1.4a**c***
Serum bilirubin (mg/dL)	0.3±0.05	0.4±0.06	1±0.07a***	0.5±0.02a**b***	1.7±0.1a***	1.2±0.1a***c**

Table 3: Effect of LCT and taurine on some biomarkers of lipid metabolism in female rats.
Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail *t*-tests. Superscript a, Group A (control group) *versus* all other groups, superscript b, Group C *versus* Group D and superscript c, Group E *versus* Group F (*indicates $p<0.05$, **indicates $p<0.01$, ***indicates $p<0.001$).

for the detoxification of reactive toxic substances resulted from LCT exposure at the dose level of 6.29 mg/kg body wt. ($1/9^{th}$ LD_{50} dose) (Figures 3C and 3D).

Selection of Experimental Dose of Lambda Cyhalothrin

From these above findings for male rats, 10.83($1/7^{th}$ LD_{50} dose) and 15.17($1/5^{th}$ LD_{50} dose) mg/kg body wt. and for female rats, 6.29 mg/kg body wt. ($1/9^{th}$ LD_{50} dose) and 11.33($1/5^{th}$ LD_{50} dose) mg/kg body wt. were selected as effective doses for our further studies. At the $1/7^{th}$ LD_{50} dose, significant alterations were seen in different systemic biochemical parameters of male rat. In female rat first significant toxic response was found at the dose level of 6.29 mg/kg body wt. ($1/9^{th}$ LD_{50} dose). So in case of male and female rat, $1/7^{th}$ LD_{50} dose and $1/9^{th}$ LD_{50} dose were considered as effective low dose. Better toxic response was found at $1/5^{th}$ LD_{50} dose for both male and female rat after which animal mortality was increased. So this dose was considered as effective high dose for future studies.

Effect on Lipid Profiles in Female Rat

The data from Table 3 reflected marked alterations in the levels of serum total cholesterol, lipoproteins and bilirubin. The total cholesterol concentration was found to be significantly increased ($p<0.001$) in the LCT treated group compared to control. Serum triglyceride, VLDL-cholesterol and LDL-cholesterol in the LCT treated rats were also found to be elevated when compared to normal rats where as HDL was markedly ($p<0.001$) reduced in LCT treated female rats. Our results also showed a significant increase of serum bilirubin level in LCT intoxicated rats. Pre treatment with taurine has caused the significant alleviation in serum total cholesterol, LDL cholesterol and HDL cholesterol in LCT treated rats. Pretreatment with taurine also improved the altered serum triglyceride and bilirubin level (Table 3).

DISCUSSION

The present study was carried out to assess the toxic effects of LCT on the different system of male, female Wistar rats, and also to search out the alleviating role of taurine under this toxic condition. In toxicological studies, body weight is a basic benchmark for evaluation of organ toxicity. In the present study, oral administration of LCT brought about a significant reduction in body weight of both male female rats.

A significant change in erythrocyte counts, haemoglobin percentage and leukocyte were detected in lambda cyhalothrin exposed rats and these pointed out the physiological disruption in the rat haemopoietic system. Haemolysis of blood cells[38] may cause the decrease in erythrocyte counts in LCT intoxicated rats which in turn tends to be responsible for the reduction in haemoglobin percentage. In the present study, decreased biosynthesis of haem in bone marrow may also lead to the significant reduction in haemoglobin percentage. Increased leukocyte in lambda cyhalothrin treated group may arise due to the immediate activation of the immune system of the body[39] against lambda cyhalothrin.

SGOT, SGPT are two important hepatic enzyme biomarkers of hepatotoxicity. In the present study a significant increase in SGOT, SGPT level after LCT treatment at different concentration point out towards active utilization of amino acids in energy-yielding metabolic processes like gluconeogenesis. Pyrethroids induced oxidative stress by the elevation of lipid peroxidation products.[40,41] Elevated MDA level in LCT intoxicated rat liver was in an agreement with the above statement. This type of result also suggested that LCT produces hepatic injury and pathogenesis through the generation of free radicals and by the alteration of antioxidant system. Decrease in cellular GSH concentrations may be through low production or non-enzymatic oxidation of GSH to glutathione disulfide (GSSG) due to oxidative stress in LCT treated rat liver at different dose levels.

Here the results also reflect the male reproductive dysfunction after LCT exposure at different dose levels. The reduction in fructose content in seminal fluid collected from LCT intoxicated rats were drawn attention towards the secretory ability of seminal vesicles and the nutritive potential for the semen. Previous studies[42-44] reported that ROS were involved in the toxicity of various pesticides. ROS inhibits steroidogenesis by disrupting cholesterol transport to mitochondria.[45] LCT elevated testicular cholesterol at the dose level of 10.83 mg/kg body wt. ($1/7^{th}$ LD_{50} dose) and also on above dose levels. An increase in MDA, the mostly used biomarker of lipid peroxidation, indicates serious

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cellular damage, inhibition of several enzymes and cellular functions.[46] GSH, one of the most potent biological molecules, play a key role in the detoxification of the reactive toxic metabolites. A considerable decline in GSH levels in liver LCT treated rat may be due to its utilization to challenge the common oxidative stress.

According to the results, increase in ovarian cholesterol and significant decrease in ovarian steroidogenic enzyme activity pointed out towards the LCT induced ovarian toxicity through diminishing steroidogenesis. The maintenance of high redox potential is a prerequisite for assuring the reproductive system functions in a healthy organism.[47,48] Increased ovarian MDA and decreased ovarian GSH level in LCT intoxicated rats compared to control may be an indication of oxidative stress due to LCT exposure at the dose level of 6.29 mg/kg body wt. ($1/9^{th}$ LD_{50} dose) and also on above dose levels.

The elevation in serum total cholesterol level in female rats was observed in the LCT treated groups indicating the hypercholesterolemic action of LCT due to blockage of liver bile ducts causing reduction or cessation of its secretion to the duodenum.[49] Pretreatment of taurine by its hypocholesterolemic action caused reduction in serum total cholesterol. Elevated serum triglyceride, VLDL-cholesterol and LDL-cholesterol with reduction of HDL in LCT treated rats may suggest that LCT has strong toxic potential to alter normal body physiology. These may be attributed to the increased fat catabolism in response to LCT.[50] Our results also showed a significant increase of serum bilirubin level in LCT intoxicated rats. The increased level of total bilirubin in treated female rats is an indicator of hyper-bilirubinemia, a useful indicator of hepatocellular dysfunction.[51,52] Pretreatment with taurine improved the altered lipid profile and bilirubin level in female rats. Taurine play a role in conjugation reaction with bile acids in the liver. Consequently, bile acid synthesis is increased[53] by simultaneous rise in the messenger RNA (mRNA) expression and activity of cholesterol 7α-hydroxylase, a rate-limiting enzyme in hepatic bile acid synthesis.[54] The primary mechanisms responsible for the hypocholesterolemic action of taurine may be due to the increased conversion of cholesterol into bile acids through the stimulation of cholesterol 7α-hydroxylase enzyme activity.

CONCLUSION

The present study showed that LCT exposure produced hematological, hepatic and gonadal toxicity at 10.83 mg/kg body wt. ($1/7^{th}$ LD_{50} dose) in male and at 6.29 mg/kg body wt. ($1/9^{th}$ LD_{50} dose) in female rat and also on above dose levels. Taurine has the potential to mitigate LCT induced altered lipid metabolic biomarkers in female rat.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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TOXICOLOGY AND FORENSIC MEDICINE

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Case Series

*Corresponding author
Wilson I. B. Onuigbo, MD, PhD
Department of Pathology
Medical Foundation and Clinic
8 Nsukka Lane
Enugu 400001, Nigeria
E-mail: wilson.onuigbo@gmail.com

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The Morals of the Ibo Ethnic Group and the Abortion Deaths Among their Maidens in Nigeria

Wilson I. B. Onuigbo, MD, PhD*

Department of Pathology, Medical Foundation and Clinic, 8 Nsukka Lane, Enugu 400001, Nigeria

ABSTRACT

Among the Ibo or Igbo ethnic group in Nigeria, a "daughter's chastity" is highly valued. Little wonder that maidens could be tempted to commit abortion to conceal the illegitimate pregnancy. These abortions may have led to the sudden death in 3 cases presented here, in which microscopy revealed chorionic villi in the womb. This is comparable to other published cases of death in pregnancy. The future is important with reference to prevention practices.

KEYWORDS: Pregnancy; Concealment; Abortion; Death; Prevention; Ethnic group; Nigeria.

INTRODUCTION

The intimate work of a British anthropologist, Basden,[1] carried out among the Ibo or Igbo ethnic group in Nigeria is rich in customs. Basden's work includes the pride in a "daughter's chastity." Undoubtedly, on this account, maidens are tempted to conceal illegitimate pregnancy. Thus, this could lead to abortion practices which might end with sudden death. The author's experience of this tragedy emanated from his headship of a Reference Pathology Laboratory which facilitated the establishment of a histopathology data pool whose utility was promoted by a Birmingham (UK) group[2] regarding epidemiological analysis. Elsewhere, I mentioned in passing in my book on Expert Evidence,[3] that 3 cases occurred locally to my knowledge. Here, I propose to use the experiences gained by local Police Doctors. One of them, Lashari,[4] reported that he had Tuesdays for police cases including post-mortems and Wednesdays for going to court to testify to his findings although made "some months ago". What did 3 other Police Doctors see?

CASE HISTORIES

1. OR, a 22-year-old girl, suddenly collapsed and died in a Patent Medicine Store. The owner denied giving any drug to the patient. An autopsy was carried out at the Parklane General Hospital, Enugu, by Dr. Ezeanyagu at the insistence of the Police. PM findings–collapsed left lung and flabby heart. Several pieces of tissues, the largest 4 cm across, were sent to me. There was massive edema of the lungs as well as autolysed looking endometrium.

2. UC, a 19-year-old girl, not married, disappeared from the father's house. Three days later, she was brought dead to the Queen Elizabeth Specialist Hospital, Umuahia, by the Police. At autopsy by Dr. Akanwa, a bulky uterus was found with retained products.

3. CH, a 17-year-old girl was brought in dead from home by the Police. She was said to be procuring an abortion by taking some indigenous drugs. At autopsy by Dr. Okafor, there was marked hepatomegaly as well as congestion. Marked areola tissue of the breasts was noted with some blood. Clots in the uterus were also present.

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REGIONAL PATHOLOGY LABORATORY SERVICE

From 1963, I headed the Reference Pathology Laboratory established by the Government of the Eastern Region of Nigeria. It was on this account that the local histopathology data pool became available for epidemiological analysis, including forensic analysis. Indeed, all 3 above cases were bolstered with the specimens sent to me for an opinion. In all of them, chorionic villi were identified from the wombs (Figure 1).

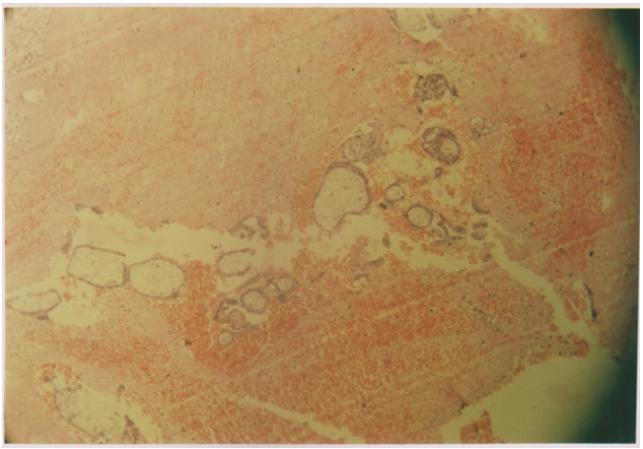

Figure 1: Chorionic villi in the midst of blood.

DISCUSSION

The above mundane cases probably rested to some extent on the administration of unknown drugs including injections which may result in anaphylactic shock. They may be especially contrasted with the sophisticated dimensions found in the world literature.

Thus, one case was in the series from 1972-1975. It was reported by Grimes and Gates and in it, the case involved fatal amniotic *fluid* embolism that resulted from induced abortion.[5] Another case was due to *air* embolism which resulted in a 27-year-old female when the husband had intercourse in the knee-chest position.[6] The patient was dead on arrival at the hospital. At autopsy, the wall of the uterus crackled with air; air was also present in the uterine veins, vena cava, and jugular veins, while a large amount of air was expelled from the right side when the heart was opened under water.

Unsafe abortion leading to unnecessary maternal mortality was discussed fully by Haddad and Nour,[7] who highlighted the ominous fact that The World Health Organization (WHO) stated that "*every 8 minutes a woman in a developing nation will die of complications arising from an unsafe abortion*". Little wonder that the Lancet[8] highlighted unsafe abortion as a preventable pandemic.

In this context, a Nigerian report, fortunately, came from the South-western Region.[9] The report concluded that "*The rate of women seeking repeat abortions is high in Nigeria, the rate of contraceptive use is low while contraceptive failure rate is high*". Wide literature searches were undertaken from 1970-2006 concerning the 11-24 years age group by Williamson and her associates.[10] As they concluded, "*young women often relied on traditional methods of abortion*". Perhaps, in the final analysis, let us listen to one Sedgh[11]: "*Despite recent declines, teen pregnancy rates remain high in many countries. Research on the planning status of these pregnancies and on factors that determine how teens resolve their pregnancies could further inform programs and policies*".

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Research

*Corresponding author
Sujata Maiti Choudhury, PhD
Department of Human Physiology with Community Health
Vidyasagar University
Midnapore, WB 721102, India
Tel. + 9474444646
E-mail: smaitichoudhury@yahoo.com; sujata.vu2009@gmail.com

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Sub-Acute Toxicity Study of *Calotropis gigantea* Latex Extracts in Male Swiss Albino Mice

Saswata Sanyal, MSc; Pralay Maity, MSc; Ananya Pradhan, MSc; Madhubanti Bepari, MSc; Surya Kanta Dey, MSc; Tamanna Roy, MSc; Sujata Maiti Choudhury, PhD*

Department of Human Physiology with Community Health, Vidyasagar University, Midnapore, WB 721102, India

ABSTRACT

Background: *Calotropis gigantea* of family Asclepiadaceae, is traditionally used in ayurveda for its anti-helminthic, anti-pyretic, and anti-malarial activities. The present study was designed to investigate the sub-acute toxic effects of ethanol and water extract of *Calotropis gigantea* latex in mouse model using haematology, serum biochemistry and histopathological changes as toxicity indices.
Methods: In the sub-acute 28-day toxicity study, ethanol and water extract of *Calotropis gigantea* latex were administered intraperitoneally at the dose levels of 50, 100, 200, 500, 1000 and 2000 mg/kg body wt./day.
Results: Significant ($p<0.05$) difference were not observed in relative organ weights and haematological, hepatic and renal biomarkers up to the dose level of 500 mg/kg body wt./day for 28 days except blood glucose and serum glutamate pyruvate transaminase (SGPT) in comparison to the control group. No significant toxicity was seen up to the dose level of 1000 mg/kg body wt./day for 28 days in case of blood glucose and SGPT.
Conclusion: The findings suggest that *Calotropis gigantea* latex extracts do not cause sub-acute toxicity up to the level of 1000 mg/kg body wt./day for 28 days and may be considered as phytomedicinal therapeutic agents.

KEYWORDS: *Calotropis gigantea*; Sub-acute toxicity; Relative organ weight; Haematological hepatic and renal biomarkers; Histopathological changes.

ABBREVIATIONS: EECGL: Ethanol extract of *Calotropis gigantea* latex; WECGL: Water extract of *Calotropis gigantea* latex; EDTA: Ethylenediaminetetraacetic acid; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; RBC: Red Blood Cell; WBC: White Blood Cell; SGOT: Serum Glutamate Oxaloacetate Transaminase; SGPT: Serum Glutamate Pyruvate Transaminase; ALP: Alkaline phosphatase.

INTRODUCTION

Presently, 25% of prescribed drugs worldwide are derived from plant sources in spite of the great progress and advancement of organic synthesis.[1] Medicinal plants offer unlimited opportunities for the discovery of new drugs in biomedical field. Most of the natural products used in folk remedy have scientific evidences with regard to their biological activities. However, there is little evidence available concerning the possible toxicity those drugs or products from medicinal plants may cause to the consumers.[2] The traditional use of any plant for medicinal purposes requires guarantees for the safety of such plant. Herbal medicines are usually complex mixtures of many bioactive compounds. Herbal medicines may differ from single-agent pharmaceuticals, phytomedicines due to the different mechanisms of action of their bioactive constituents.[3] This is also reflected in their dose-response relationships and in synergistic/combinatorial effects. Pharmacological investigations have established their rising relevance in search of more reliable herbal drugs free of any side effects.[4] Different types of interactions can arise

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whenever a chemical substance or drug administered to a biological system and a series of dose-related responses can occur. Thus, to identify potential health hazards before the drugs are administered to man, toxicity testing in animals is usually done on new drugs with doses well above the expected therapeutic range. Toxicity studies include a wide range of tests in different species with regular monitoring for biochemical or physiological anomalies seen in long-term administration of the drug.[5]

The types of toxicity studies which are routinely executed by pharmaceutical manufacturers in the search of a new drug include acute, sub-acute and chronic toxicity. Administration of a single dose or multiple doses in a period not beyond 24 hours, up to a limit of 2000 mg/kg produces acute toxicity. Objective of acute toxicity tests is to detect a dose producing major adverse effects and an assessment of the minimum dose causing lethality.[6]

Calotropis gigantea (Linn.) R. Br. of family Asclepiadaceae is a perennial shrub and widely distributed in tropical and subtropical region and most abundant in Bangladesh, India, Burma and Pakistan.[7-10] The root, stem, leaves, flower and latex of *C. gigantea* are reported to be utilized in traditional medicine for the treatment of toothache, ear ache, eczema, syphilis, elephantiasis, injury, pain, ulceration, epilepsy, anxiety and mental disorders.[11,12]

Traditionally, the root of *Calotropis gigantea* is used in treatment of leprosy, asthma, bronchitis, and as an expectorant.[13,14] *Calotropis gigantea* reported to exhibit free radical scavenging[15,16] and pro-coagulation activity,[17] pregnancy interceptive,[18] anticancer,[19] immunomodulatory,[20] wound healing,[21] anti-inflammatory[22-25] and hepatoprotective[16,26] activity.

The major phytochemicals of *Calotropis gigantea* are flavonol, glycoside, uscharidin, calotropin, frugoside, calotroposides A to G. Other constituents are α-amyrin, β-amyrin, taraxasterol, β-sitosterol, α- and β-amyrinmethylbutazone, gigantursenylacetate A and B. It's latex rich in lupeol, calotropin, calotoxin and uscharchin.[27] In current times there is a growing awareness and interest in medicinal plants and their preparations.[28] Lack of scientific and clinical data in support of better understanding of the efficacy and safety of the drugs, is the major difficulty to the use of traditional herbal preparations.

The sub-acute toxicity data may be required to envisage the safety and effects of long term exposure to a specific medicinal plant. This study therefore was carried out to evaluate the sub-acute toxic effects of the ethanol and water extract of *Calotropis gigantea* latex in mice.

MATERIALS AND METHODS

Collection and Extraction of Plant Material

C. gigantea plant was collected from the out fields of Vidyasagar University, Midnapore, West Bengal, India and authenticated from Botanical Survey of India (BSI), Ministry of Environment and Forest (MoEF), Government of India, Howrah (Identification No. CNH/2014/Tech.II/55).

The fresh latex of *C. gigantea* was collected from the aerial parts of mature plants and was then spread into petri dishes for air dry in room temperature under shade up to 4 to 5 days. Then 250 g of dried latex was subjected for size reduction to coarse powder. Then in 450 ml of ethanol and water, powdered latex was dissolved separately and was incubated for 48 hours in room temperature and was filtered using Whatmann filter paper. The filtrates were dried in EYELA CCA 1110 rotary evaporator to produce ethanol extracts of *Calotropis gigantea* latex (EECGL) and water extracts of *Calotropis gigantea* latex (WECGL) extracts (yields 4.5% and 2.8% respectively).[29] Dried extracts were stored in air tight containers at 4°C till further use.

Animals

Healthy male Swiss albino mice (20-25 g) were selected for toxicity test. The mice were grouped and housed in poly acrylic cage (38×23×10 cm) with 6 animals per cage. The animals were kept on a 12 h light: 12 h dark regime at 25°C for 7 days before commencement of the experiment. The animals had free access to standard diet and water.[30] Mice were deprived of food but not water prior to administration of the test extracts. The study was approved by the Institutional Animal Ethical Committee (IAEC), registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forests and Climate Change (MoEFCC), Government of India and performed in compliance with the relevant laws and guidelines of the CPCSEA.

Treatment Schedule

The sub-acute toxicity study on the above mentioned plant extracts was performed as per the Organisation for Economic Co-operation and Development (OECD) guidelines 407[31] with slight modifications, where 2000 mg/kg was used as the limit test dose. The ethanol and water extracts of *Calotropis gigantea* latex were administered intraperitoneally prior up to the bladder[32] at the dose levels of 50, 100, 200, 500, 1000 and 2000 mg/kg body weight to the group II to VI animals respectively and distilled water to the control group (Group I) animals by sterile syringe daily for 28 days.[33,34] Toxicity studies were conducted as per internationally accepted protocol in Swiss albino mice. The animals were divided into 6 groups containing 6 animals each. The experimental design was given below:

Group I : Control
Group II : EECGL /WECGL (50 mg/kg body weight)
Group III : EECGL/WECGL (100 mg/kg body weight)
Group IV : EECGL/WECGL (200 mg/kg body weight)
Group V : EECGL/WECGL (500 mg/kg body weight)
Group VI : EECGL/WECGL (1000 mg/kg body weight)

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Group VII : EECGL/WECGL (2000 mg/kg body weight)

The feed and volume of water consumed by mice in each group were monitored daily. Weights of the mice in all the groups were recorded daily during the treatment schedule and on the last day of study. Doses of the extract administered were adjusted accordingly. On the 29th day of the experiment, the mice were anesthetized with pentobarbital sodium (50 mg/kg, i/p) and sacrificed by cervical dislocation. The test organs of the animals were opened up surgically and blood samples were collected by cardiac puncture.

Blood Sample Collection

On the last day of the experiment, 2 sets of blood samples were collected from all the animals *via* cardiac puncture using a 5 ml sterile syringe. Two ml of blood sample was collected into sterile container containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant for the determination of haematological parameters. For serum analysis blood samples were collected into another anticoagulant free sterile container, allowed to stand at room temperature and centrifuged at 2000 rpm for 10 minutes. The supernatants were then collected and stored at -20ºC for biochemical analysis.

Organ Weight

On 29th day, all the animals were sacrificed. Heart, liver, lungs, spleen, and kidneys were carefully dissected out and weighed.

Haematological Analysis

Red blood cell (RBC) count: Blood was diluted with RBC dilution fluid (1:200). Total erythrocytes were counted in the Neubaur haemocytometer chamber.[35]

White blood cell (WBC) count: Blood was diluted with white blood cell (WBC) dilution fluid (1:20) and loaded in Neubaur haemocytometer chamber. Four large (1 sq mm) corner squares of the haemocytometer chamber were counted under the microscope.[35]

Determination of haemoglobin: At first 20 µl of blood was transferred into a test tube containing 5 ml of Drabkin's solution. After adjusting the photoelectric colorimeter at 540 nm with a blank the OD of sample was read.[36]

Estimation of Urea

Urea was determined by the modified method of Natelson et al.[37,38] To 0.1 ml of serum, 3.3 ml of water, 0.3 ml each of 10% sodium tungstate and 0.67 N sulphuric acid were added. The suspensions were centrifuged and to 1.0 ml of the supernatant, 1.0 ml of water, 0.4 ml of diacetylmonoxime and 2.6 ml of 0.67 N sulphuric acid-phosphoric acid reagents were added. Standard urea (20 to 50 µg/ml) were also treated in a similar manner and all the tubes were heated in a boiling water bath for 30 minutes, cooled and the color developed was measured at 480 nm in a Shimadzu spectrophotometer (UV-Shimadzu-245, Japan). The values were expressed as mg of urea/dl of blood.

Estimation of Creatinine

Creatinine was described according to the modified method of Brod and Sirota.[39,40] At first, protein free filtrate was prepared. One ml of serum was precipitated with 8.0 ml of water, 0.5 ml of 2/3 N sulphuric acid and 0.5 ml of 40% sodium tunsgstate. After that, 5.0 ml clear filtrate was taken and 1.5 ml of saturated picric acid and 1.5 ml of 0.75 N sodium hydroxide were added to it. Standard and blank were also prepared similarly. The color intensity was measured at 530 nm in a Shimadzu spectrophotometer (UV-Shimadzu-245, Japan). The values were expressed as mg of creatinine/dl of blood.

Measurement of Serum Alkaline Phosphate (ALP)

Activity of serum alkaline phosphatase were estimated by taking 0.25 mL of serum in a centrifuge tube containing 1 ml buffer (1 mM of p-nitrophenol phosphate in 1 M Tris buffer, pH 8.0); the mixture was then subjected to be incubated at 37ºC for 30 minutes in a water bath. The activity was measured spectrophtometrically at 420 nm.[41]

Measurement of Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT)

For the estimation of SGOT, to 1.0 ml of the buffered substrate (200 mM/L of DL-aspartate and 2 mM/L of α-ketoglutarate, pH=7.4), 0.1 ml of serum was added and incubated at 37°C for one hour. Then 1 ml of dinitrophenyl hydrazine (DNPH) was added and kept for 20 minutes at room temperature. After 20 minutes, 10 ml of 0.4 N sodium hydroxide was added and the color intensity was measured at 505-540 nm in a Shimadzu spectrophotometer (UV-Shimadzu-245, Japan) after 10 minutes against the reagent blank. The standard and blank were also prepared by the same process. The enzyme activity in serum was expressed as IU/lit.

For the estimation of SGPT, to 1.0 ml of the buffered substrate (200 mM/L of DL-alanine and 2 mM/L of α-ketoglutarate pH=7.4), 0.1 ml of serum was added and incubated at 37°C for on hour. The reaction was arrested by the addition of 1.0 ml of dinitrophenyl hydrazine and left aside for 20 minutes at room temperature. The color developed by the addition of 10 ml of 0.4 N sodium hydroxide was read at 505-540 nm in a Shimadzu spectrophotometer (UV-Shimadzu-245, Japan) against the reagent blank. The enzyme activity in serum was expressed as IU/lit.[42]

Estimation of Blood Glucose

Blood glucose was measured according to the method of Nelson-Somogyi.[43,44] Somogyi's copper reagent was prepared by

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dissolving 24 g anhydrous sodium carbonate and 12 g of sodium potassium tartrate in about 250 ml of distilled water. To this 4 g copper sulphate as a 10% (w/v) solution was added and mixed followed by the addition of 16 g of sodium bicarbonate. Then 180 g of sodium sulphate was dissolved in about 500 ml of distilled water and boiled to expel air. After cooling, the two solutions were mixed and the volume was made up to 1000 ml. Nelson's arsenomolybdate reagent was prepared by dissolving 25 g ammonium heptamolybdate in 450 ml of water. Then 21 ml of sulphuric acid was added and mixed well. To the mixture 3.0 g disodium hydrogen arsenate dissolved in 25 ml of distilled water was added. The solution was mixed well and incubated for 24 hours at 37 °C. From the sample, one ml of aliquot was pipetted out. To this 1.0 ml of Somogyi's copper reagent was added. The mixture was then placed in a bath of boiling water and heated for 20 minutes. After cooling under tap water 1.0 ml of Nelson's arsenomolybdate reagent was added with immediate mixing till the effervescence ceased. The intensity of color was measured spectrophtometrically at 540 nm.

Measurement of Serum Cholesterol

At first 0.1 ml of serum was mixed with 6 ml of glacial acetic acid. After addition of 4 ml of color reagent (1 ml of 10% $FeCl_3$, 6 H_2O, 15 ml of conc. H_2SO_4) it was mixed vigorously and allowed to stand for 20 min. The reading was taken at 570 nm.[45] The amount of cholesterol present is calculated by plotting the standard curve.

Measurement of Serum Total Protein

Different dilutions of BSA solutions are prepared by mixing stock BSA solution (1 mg/ml) and water. The final volume in each of the test tubes is 6 ml. The BSA range is 0.05 to 1 mg/ml. From these different dilutions, protein solutions were pipetted out to different test tubes and 5 ml of alkaline copper sulphate reagent were added. The solutions were mixed well. Then 0.5 ml of reagent Folin-Ciocalteau solution (reagent solutions) was added to each tube and incubated at 37°C for 30 min. The optical density was measured at 660 nm.[46] The absorbance was plotted against protein concentration to get a standard calibration curve.

Histopathology Analysis

All the animals from each group were sacrificed for histopathological examinations of major internal organs. Organs such as liver, kidney were collected from all the animals. The collected organs were weighed and preserved in 10% neutral buffered formalin, then dehydrated in alcohols and embedded in paraffin. Five micron thickness of tissue sections were stained with haematoxylin and eosin (H and E) for histopathological study.

Statistical Analysis

All the parameters were performed in triplicate manner. The data was expressed as Mean±SEM, comparisons between control and treated groups were analyzed by using the one-way analysis of variance (ANOVA) considering $p<0.05$ as a limit of significance.

RESULTS

Relative Organ Weights (ROW) of Male Mice

Relative organ weights of 28-days treated mice are shown in Tables 1A and 1B. The relative organ weights recorded in the treatment groups did not show any significant difference ($p<0.05$)

Weight	Control	50 mg/kg body wt.	100 mg/kg body wt.	200 mg/kg body wt.	500 mg/kg body wt.	1000 mg/kg body wt.	2000 mg/kg body wt.
Initial body weight (gm)	27.66±0.33	27±0.57	27.7±0.33	27.88±0.57	28±0.57	28±0.57	28±0.57
Final body weight (gm)	31±0.57	31±0.57	31±0.57	31±0.57	32±0.57	32±0.57	35±0.57*
Weight of heart (gm)	0.22±.005	0.22±.005	0.22±.005	0.21±.005	0.21±.003	0.2±.003	0.19±.005*
Weight of spleen (gm)	0.24±.008	0.24±.008	0.26±.005	0.27±.003	0.29±.005	0.30±.008	0.32±.011*
Weight of liver (gm)	0.33±.008	0.33±.008	0.34±.008	0.36±.005	0.36±.008	0.41±.008	0.41±.011*
Weight of kidney(gm)	0.38±.003	0.36±.005	0.36±.005	0.35±.005	0.35±.005	0.32±.003	0.3±.005*

Results are expressed as Mean ± SEM, Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$).
Table 1A: Effect of water extract of *Calotropis gigantea* latex (WECGL) administration for 28 days on body and different organ weight in Swiss albino mice.

Weight	Control	50 mg/kg body wt.	100 mg/kg body wt.	200 mg/kg body wt.	500 mg/kg body wt.	1000 mg/kg body wt.	2000 mg/kg body wt.
Initial body weight (gm)	27±0.57	27±0.57	27.33±0.33	28±0.57	28±0.57	28.66±0.88	29±0.57
Final body weight (gm)	31.33±0.66	31±0.57	31±.57	33±0.57	34±0.57	34.66±0.88	35±0.57
Weight of heart (gm)	0.23±.005	0.22±.005	0.22±.005	0.22±.008	0.2±.005	0.2±.008	0.19±.005*
Weight of spleen (gm)	0.27±.005	0.27±.003	0.27±.005	0.28±.005	0.29±.005	0.3±.005	0.32±.008*
Weight of liver (gm)	0.31±.005	0.32±.005	0.33±.005	0.32±.005	0.32±.011	0.33±.005	0.35±.015*
Weight of kidney(gm)	0.39±.005	0.36±.008	0.36±.005	0.35±.005	0.35±.005	0.33±.003	0.31±.005*

Results are expressed as Mean±SEM; n=6. Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$).
Table 1B: Effect of ethanolic extract of *Calotropis gigantea* latex (EECGL) administration for 28 days on body and different organ weight in Swiss albino mice.

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Haematological parameters	Control	50 mg/kg body wt.	100 mg/kg body wt.	200 mg/kg body wt.	500 mg/kg body wt.	1000 mg/kg body wt.	2000 mg/kg body wt.
Haemoglobin (%)	15±0.577	15±0.55	14.8±0.25	14.5±0.4	14.5±0.54	14±0.55	13.5±0.54*
Total RBC count (cu.mm)	6±0.145	6±0.012	5.8±0.104	5.8±0.05	5.5±0.057	5.1±0.04	5.1±0.02
Total WBC count (cu.mm)	5100±58	5100±50	5200±62	5300±60	5300±78	5400±65	5560±70*

Results are expressed as Mean ± SEM; n=6. Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$).

Table 2A: Haematological parameters after WECGL administration for 28 days in Swiss albino mice.

Haematological parameters	Control	50 mg/kg body wt.	100 mg/kg body wt.	200 mg/kg body wt.	500 mg/kg body wt.	1000 mg/kg body wt.	2000 mg/kg body wt.
Haemoglobin (%)	15±0.577	15±0.52	14.54±0.25	14.5±0.43	14.5±0.56	14±0.55	13.8±0.64*
Total RBC count (cu.mm)	6±0.145	6±0.109	5.5±0.124	5.5±0.057	5.6±0.057	5.2±0.042	5.2±0.022
Total WBC count (cu.mm)	5100±58	5100±52	5300±82	5345±80	5400±88	5545±85	5550±80*

Results are expressed as Mean±SEM; n=6. Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$).

Table 2B: Haematological parameters after EECGL administration for 28 days in Swiss albino mice.

up to the dose level of 1000 mg/kg body wt. compared to the control.

Haematological Parameters Analysis

The effects of *C. gigantea* latex extracts at sub-acute doses on haematological parameters are presented in Tables 2A and 2B. Most haematological parameters (haemoglobin, total RBC count, total WBC count) in treated mice were not significantly different from the control group animals, with the exception of marginal variations in the parameters. No significant toxicity seen up to the dose level of 1000 mg/kg body wt. in haemoglobin percentage and total WBC count in latex extracts treated mice than that of the control animals. There was no trace of toxicity in total RBC counts in latex extracts treated mice up to the dose level of 2000 mg/kg body wt.

Effect of EECGL and WECGL on Hepatic and Renal Biomarkers of Mice

The results of hepatic and renal biomarkers following the treatment of *C. gigantea* latex extracts in mice are shown on Figures 1 to 8.

No significant sub-acute toxicity was seen in the level of urea (Figure 1), creatinine (Figure 2), alkaline phosphotase (Figure 3), and serum cholesterol (Figure 7) in mice treated with EECGL and WECGL up to the dose level of 500 mg/kg body wt./day for 28 days when compared with the control animals.

In case of blood glucose (Figure 6), SGPT (Figure 5) and serum total protein (Figure 8), significant sub-acute toxicity was not observed up to the dose level of 1000 mg/kg body wt./

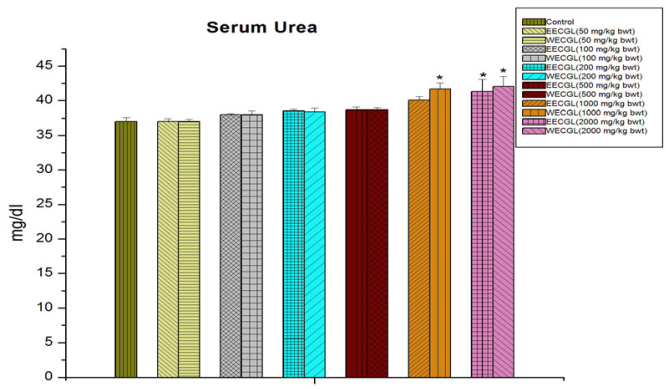

Figure 1: Shows serum urea level after treatment of EECGL and WECGL for 28 days in Swiss albino mice. Results are expressed as Mean±SEM; n=6. Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$).

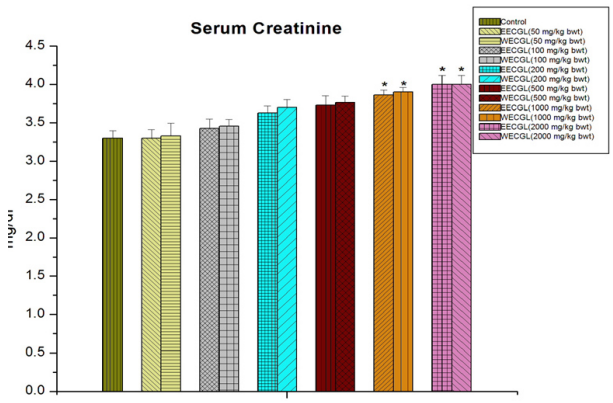

Figure 2: Serum creatinine after treatment of EECGL and WECGL for 28 days in Swiss albino mice. Results are expressed as Mean±SEM; n=6. Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$).

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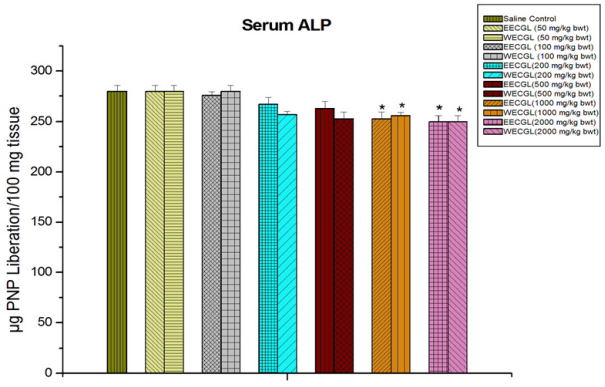

Figure 3: Shows serum ALP after treatment of EECGL and WECGL for 28 days in Swiss albino mice. Results are expressed as Mean±SEM; n=6. Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$).

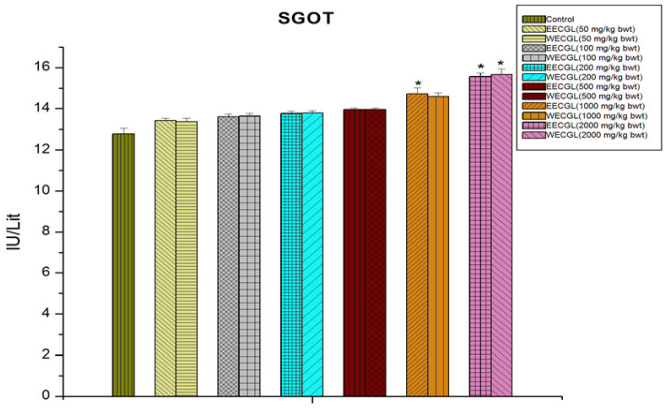

Figure 4: The effect of SGOT after treatment of EECGL and WECGL for 28 days in Swiss albino mice. Results are expressed as Mean±SEM; n=6. Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$).

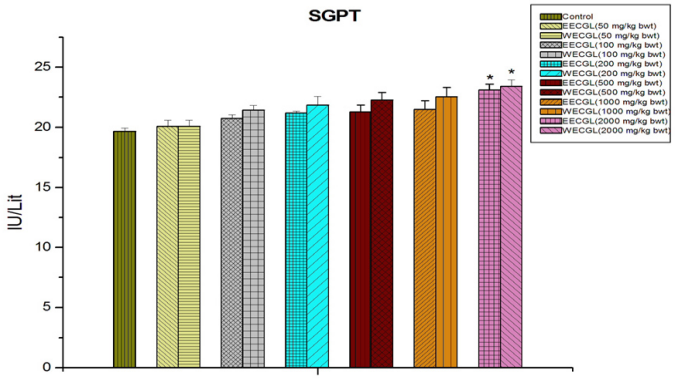

Figure 5: Level of SGPT after treatment of EECGL and WECGL for 28 days in Swiss albino mice. Results are expressed as Mean±SEM; n=6. Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$).

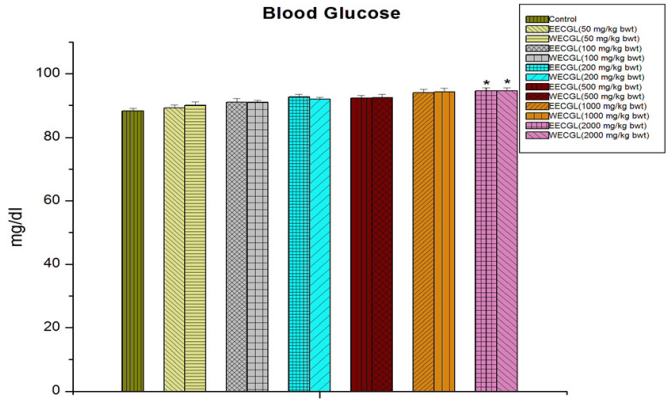

Figure 6: Shows the blood glucose level after treatment of EECGL and WECGL for 28 days in Swiss albino mice. Results are expressed as Mean±SEM; n=6. Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$).

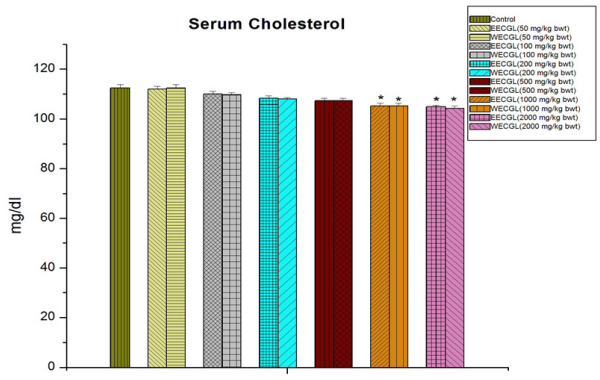

Figure 7: The diagram shows the measurement of serum cholesterol after treatment of EECGL and WECGL for 28 days in Swiss albino mice. Results are expressed as Mean±SEM; n=6. Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$).

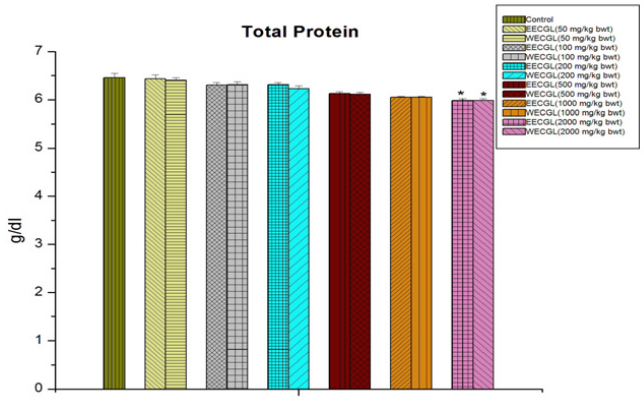

Figure 8: Shows serum total protein after treatment of EECGL and WECGL for 28 days in Swiss albino mice. Results are expressed as Mean±SEM; n=6. Analysis is done by one way ANOVA. Comparison was done between control group *versus* all other groups (*indicates $p<0.05$)

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day.

WECGL produces no toxicity in case of SGOT (Figure 4) up to 1000 mg/kg body wt./day but EECGL causes significant (p<0.05) toxicity at this dose level.

Histopathological Study

Histopathological studies of the liver and kidney tissues in the control and the *C. gigantea* extract treated groups showed no differences, indicating that treatment of EECGL and WECGL did not result in any adverse or abnormal toxicological effect on these vital organs (Figures 9, 10, 11 and 12).

DISCUSSION

For centuries, natural products especially medicinal plants are used for the treatment of different diseases.[47] In the screening of

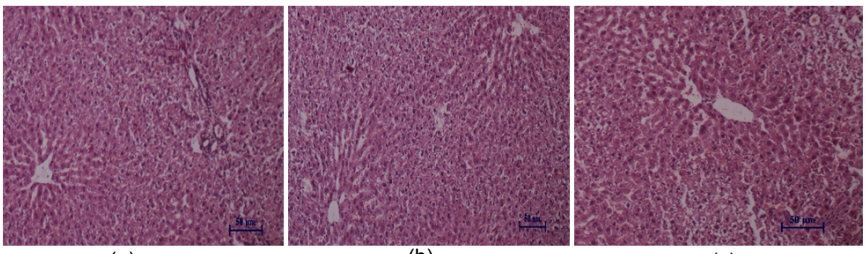

Figure 9: Histopathological studies of liver tissue: (a) Control mice, (b) EECGL treated (50 mg/kg body wt.), (c) EECGL treated (2000 mg/kg body wt.).

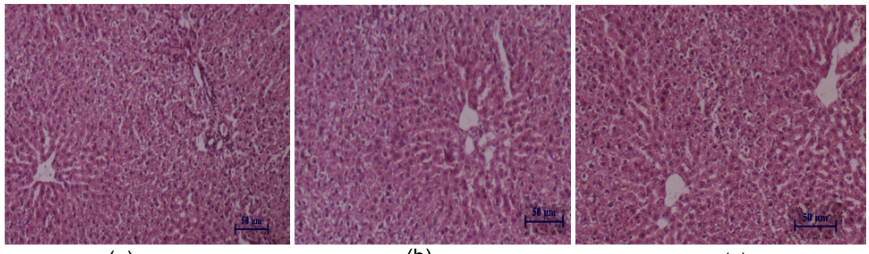

Figure 10: Histopathological studies of liver tissue: (a) Control mice, (b) WECGL treated (50 mg/kg body wt.), (c) WECGL treated (2000 mg/kg body wt.).

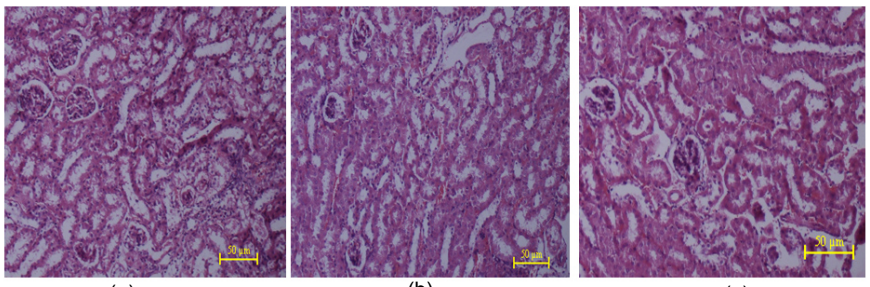

Figure 11: Histopathological studies of kidney tissue: (a) Control mice, (b) EECGL treated (50 mg/kg body wt.), (c) EECGL treated (2000 mg/kg body wt.).

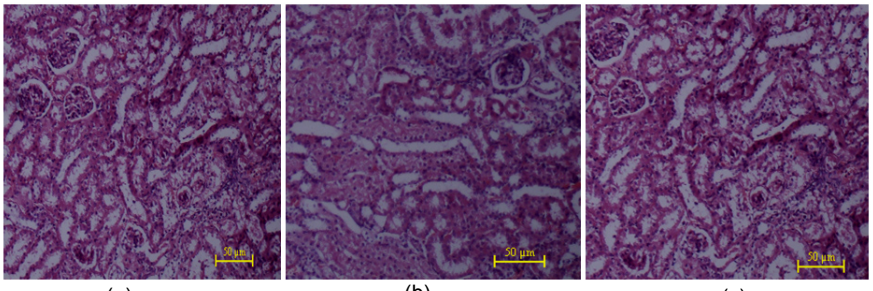

Figure 12: Histopathological studies of kidney tissue: (a) Control mice, (b) WECGL treated (50 mg/kg body wt.), (c) WECGL treated (2000 mg/kg body wt.).

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pharmacological efficacy of a medicinal plant, the initial step is the assessment and evaluation of its toxic characteristics and the present study was undertaken to investigate the sub-acute toxicity of *C. gigantea* in a mammalian model.

No significant changes was observed in the weights of the heart, liver, spleen, kidneys suggesting that the administration of *C. gigantea* extract at the sub-acute doses had no effect on the normal growth. In toxicity studies, the worth of weighing organs takes account of their sensitivity to envisage toxicity, enzyme stimulation, physiologic perturbations, and acute injury.[48] The relative organs weights are also relatively sensitive indicators for particular organs in toxicity studies.[49] The findings of this study exposed that the vital organs, such as liver, kidneys, were not adversely affected for toxicity throughout the treatment. Since there was no reduction in body and relative organ weights of the treated animals at any of the tested dose level, we concluded that the extracts are nontoxic to the analyzed organs.

To evaluate the probable changes in hepatic and renal functions influenced by the extracts the serum haematology and clinical biochemistry studies were done liver and kidney function analysis is very important in the toxicity evaluation of plant extracts as they are both necessary for the survival of an organism.[50] The assessment of activities of serum marker enzymes plays important role in the evaluation of plant extract for its toxicity risk. The enzymes considered in this study, are valuable marker enzymes of liver cytolysis and liver cell membrane damage. SGOT and SGPT, the transaminases are well known good indicators of liver function and used as biomarkers to conclude the probable toxicity of drugs. Normally, destruction to the liver parenchymal cells will result in an increase of both these enzymes in the blood.[51] Alkaline phosphatase is a marker enzyme for the plasma membrane and endoplasmic reticulum of the tissue studied. High levels of alkaline phosphatase are reported in liver diseases or hepatotoxicity.[52] The insignificant changes in alkaline phosphatase in male mice at all dose level suggest that acute administration of *C. gigantea* extract does not affect the hepatocyte function in mice. Renal dysfunction can be measured by simultaneous measurements of urea, creatinine and their normal levels observed at reduced renal problems.[53] Higher than normal levels of serum creatinine and urea are good indicators of renal function abnormality.[54] Thus, the decrease in serum creatinine concentrations with concomitant decrease in the serum urea concentration in the treated rats suggests that functioning of the kidney is normal. In the present study, changes in serum urea, creatinine levels in *C. gigantea* extract treated groups showed non-significant differences indicating a normal renal function up to the dose level of 500 mg/kg body wt.

Evaluation of haematological parameters can be used to estimate the extent of the harmful effect of *C. gigantea* extract on the blood of an animal. It can also be used to explain blood related functions of a plant extract or its products.[55] Moreover, for risk evaluation such analysis is important as haematological changes have higher prognostic value for human toxicity when the data are obtained from mammalian studies.[56] A haemogram was undertaken for all the *C. gigantea* extract treated and control groups and the results show no significant effects up to the dose level of 1000 mg/kg bw. The non-significant effect of the extract on total red blood cells, Hb percentage indicates that the *C. gigantea* extract does not affect the RBC morphology or formation, or its osmotic fragility.[57] Leukocytes are the first line of cellular defence that counter tissue injury, infectious agents or inflammatory process. Furthermore, *C. gigantea* extracts produced no significant changes in WBC count, which further confirmed the above findings. A normal haematological profile of *C. gigantea* extract treated groups also further justified the non-toxic nature of *C. gigantea* extract. In histopathological studies, the liver of treated animals showed normal histological feature at 50, 2000 mg/kg. No degeneration of hepatocyte, focal steatosis, congestion of central vein and inflammation of portal tract when compared with control animals. The kidney of treated rats showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney. Gross histological examination of liver and kidney on did not reveal any abnormalities. Thus, it was concluded that *C. gigantea* did not produce any toxic effect in male albino mice.

From these findings, we may conclude that EECGL and WECGL are not toxic up to the dose level of 500 mg/kg body wt./day for 28 days and did not produce any noteworthy significant ($p<0.05$) alterations in relative organ weights, haematological, hepatic and renal biomarkers except blood glucose, serum protein and serum glutamate pyruvate transaminase (SGPT). No significant toxicity was seen up to the dose level of 1000 mg/kg body wt./day for 28 days in case of blood glucose, serum protein and SGPT, which are important toxicity biomarkers. As there were no significant adverse effects on the liver and kidney histology, haematological and serum biomarkers up to the dose level of 500 mg/kg body wt., it may be concluded that the ethanolic and water extract of *C .gigantea* latex did not induce any damage to the vital organs and may be quite safe for mammals.

CONCLUSION

In conclusion, the present investigation establishes that the ethanolic extract and water extract of *C. gigantea* latex may be considered as absolutely safe up to 500 mg/kg body wt. dose level, as it did not create any toxicological effects on selected body organs, haematological and serum biomarkers of mice during the sub-acute toxicity study. So, the findings suggest that ethanolic and water extracts of *Calotropis gigantea* latex do not produce any effective sub-acute toxicity in mice and may be considered as phytomedicinal therapeutic agents.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Case Report

*Corresponding author
Irena Çeko Marko, MSc, MD
Addictology & Clinical Toxicology Service
UHC "Mother Theresa"
Rruga e Dibres, 372, Tirane, Albania
Tel. 00355 684722796
Fax: 00355 4 2363644/2362627
E-mail: irenaceko@yahoo.com

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Anaphylactic Reactions due to Snakebite Vipera and Administration of Antivenom

Irena Çeko Marko, MSc, MD[1*]; Sonil Marko, MSc, MD[2]; Ilir Alimehmeti, MSc, MD, PhD[3]; Zihni Sulaj, MD, PhD[4]

[1]Addictology & Clinical Toxicology Service, UHC "Mother Theresa", Rruga e Dibres, 372, Tirane, Albania
[2]Institute of Health Insurance, Tirana, Albania
[3]Department of Family and Occupational Health, Faculty of Medicine, University of Medicine, Tirana, Albania
[4]Department of Biomedical and Experimental, Faculty of Medicine, University of Medicine, Tirana, Albania

INTRODUCTION

A venomous snakebite is the single most important global cause of human injury from venomous and poisonous animals of all types.[1] The viper family (*Viperaberus, Viperaammodites, Viperaursine*)[2] is the 2nd most important venomous snake family of major medical importance globally and they are found in Albania. All have mobile fangs in the front of the mouth on hinged maxillae, allowing the fangs to fold away against the roof of the mouth when not in use. These long fangs are often coupled with large venom glands, allowing large quantities of venom to be injected, but "dry bites" can also occur.[2] Snake venom contains toxins designed to kill or immobilize the snake's prey. There are four main types of snake venom: Hemotoxins, neurotoxins, cytotoxins, and myotoxins.

Bites from Vipera lead to local tissue damage and systemic signs such as generalized edema, hypotension, gastrointestinal symptoms, hemolysis, and renal dysfunction. Rarely a patient bitten by vipers may experience anaphylaxis from the vemon itself. This can complicate the evaluation or mimic a severe systemic reaction to the venom. The presence of pruritis and urticaria, wheezing, edema of oropharix which is uncommon with the envenomation, suggests anaphylaxis. These signs respond to standard treatment for anaphylaxis (epinephrine, antihistamines and corticosteroids).[3]

A fifty-eight-year old female patient, accompanied by a health center nurse presented to the Emergency Department (ED), Tertiary University Hospital with pain of her right leg with a history of snakebite at the level of talocrural articulation two hours prior. She was working in the garden and was bitten by a viper snake which she saw and recognized. She was admitted within the first hour to the village health center. The nurse who accompanied her stated that the patient lost consciousness after the bite. On presentation to the ED, the patient was conscious and orientated. The vital signs were blood pressure=90/50 mmHg, SpO_2=95%. The area around the bite was swollen, painful and local edema occurred. Hypotension and tremor wheezing were also reported. The patient noted this was the first time that she had been bitten by a viper; however, she was allergic to bee stings.

Saline perfusion, prednisolone 50 mg and 1 ampoule of adrenaline 0.1%-1 ml were given subcutaneously and local treatment of the wound with potassium permanganate occurred at the village health center.

She was admitted to a Monitored Unit, (cardiac, hemodynamic, respiratory and body temperature monitoring, blood analysis). Two intravenous line were opened with a saline intravenous perfusion (500 ml) and 50 mg of prendisolone in one of the lines. The patient was conscious. Vital signs were stabilized and she was monitored for blood preasure, cardiac rate,

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and blood oxygen (SpO_2). The patient was administered intramuscularly 1 ml of Viper Venom Antiserum (equine). The patient developed severe hypotension, tremors, wheezing edema of the tongue, cyanosis of the extremities but she did not lose consciousness. In pulmonary auscultation there were rhonchi. In the other intravenous line the following were administered: saline perfusion 500 ml, calcium chloride 10%-10 ml, potassium chloride 8.4%-10 ml, magnesium sulfate 25%-5 ml, and epinephrine 0.1%-1 ml ½ of ampule sub cutis.

The first blood analysis revealed White Blood Cell of 14.6×10^3/ mm^3 (Lymphocytes 3.7%, Monocytes 1.4%, Granulocytes' 94.9%), Red Blood Cell 4.30×10^6 /mm^3, and Hemoglobin 10.5 g/dL, International Normalized Ratio (INR) 1.403.

After four hours in the Monitored Unit her blood preasure was 100/70 mmHg, SpO_2=95%, heart rate 100', body temperature 36.7 °C, sweating and local sign were pain, edema and ecchymosis up to her right knee. After 48 hours she developed edema and ecchymosis of the right leg up to sacroiliac joint, a painful and immobilized right leg, bilateral ptosis, blurred vision, sweating and vomiting. Blood analysis revealed a White Blood Cell of 21.2×10^3/ mm^3, Red Blood Cell 3.5×10^6/mm^3, and Hemoglobin 10.5 g/dl. After 10 days of hospitalization, systematic and local treatment, the blood analyses were within the normal level.[4] The patient had only local signs, edema and ecchymosis. She was discharged from the hospital.

During the hospitalization period she did not have another anaphylactic reaction. The rehabilitation period took more than six months.

CONCLUSION

The literature contains case reports of allergic reactions as a result of snakebites, which are life-threatening for the patient if they have serious and massive edema of the oropharynx. Antivenom is also an immunoglobulin and during its application, the patient can have allergic reaction. Before antivenom is administered, the patient should be asked about their history of asthma, atopy, and previous antivenom exposure. Not always all the anaphylaxis symptoms from snakebites are present but antivenom should be administered in a monitored unit where resuscitation can be performed and airways supplies can be quickly accessed.[5]

There are no studies in our country about the percentage of patients who develop anaphylactic reactions due to snake bite.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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