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Combined Effects of Cypermethrin and Lead on Biochemical and Molecular Parameters in Albino Mice

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Abstract

In this study, the combined toxicity of cypermethrin and lead were investigated using molecular and biochemical parameters in albino mice. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP),blood urea nitrogen (BUN) and creatinine levels were studied and used as indicators of toxicity. Comet test was also utilized to evaluate the mechanism of genotoxicity of cypermethrin, lead and the (cypermethrin and lead) group. Albino mice were divided into eight groups and given intraperitoneal cypermethrin injections at 1.75 mg/kg/day and lead nitrate at 1.75 mg/kg/day, and combined doses of each one respectively. Blood samples were collected after two and four weeks. Cypermethrin raised AST, AL, BUN and creatinine levels as treatment time and doses rose, as did lead nitrate which elevated liver and kidney parameters in response to the toxicant. As evaluated by comet test molecular characteristics, the impact of cypermethrin and lead nitrate was an increase in DNA damage.

Keywords: Lead, Cypermethrin, Biochemical parameters, Comet assay, DNA damage

التأثير المشترك للسايبرمثرين والرصاص على المؤشرات الكيموحيوية والجزيئية في الفئران البيضاء

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الخلاصة

في هذه الدراسة ، تم فحص السمية المشتركه للسايبرمثرين والرصاص باستخدام المؤشرات الجزيئية والكيموحيوية في الفئران البيضاء. تم استخدام ناقلة أمين الأسبارتات ((AST) ، انزيم الفوسفتاز القلوي (ALP)، ونيتروجين اليوريا في الدم (BUN) ، ومستويات الكرياتينين كمؤشرات على السمية. كما تم استخدام اختبار المذنب لتقييم آلية السمية الجينية للسايبرمثرين والرصاص ومجموعة (للسايبرمثرين والرصاص). قسمت الفئران البيضاء إلى ثماني مجموعات وأعطيت الجرع داخل الصفاق من السايبرمثرين عند 1.75 ملغم/كغم / يوم ونترات الرصاص عند 1.75 ملغرام/ كغم / يوم ، ومجموع الجرعتين لكل مجموعه معامله على التوالي. تم جمع عينات الدم بعد أسبوعين وبعد أربعة أسابيع. رفع السايبرمثرين مستويات BUN،ALT،AST والكرياتينين مع زيادةالجرع والفترة الزمنية، وكذلك نترات الرصاص ، مما أدى إلى ارتفاع مؤشرات الكبد والكلى استجابةً للمادة السامة. اما فيما يتعلق بالمؤشرات الجزيئية لاختبار المذنب ، كان تأثير سايبرمثرين ونترات الرصاص هو زيادة في تلف الحامض النووي.

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1. Introduction

Pesticides are progressively polluting the living environment, causing harmful biological impacts on target species as well as non-target species. Pyrethroids belong to natural compounds-derived insecticides and are generally thought to be quite harmless [1].

Due to its lipophilic nature,cypermethrin are accumulative in different parts of the body leading to neurotoxicity by either altering the gamma-aminobutyric acid level or stimulating the generation of free radicals[2]. Previous research has shown that cypermethrin causes toxicity by inhibiting acetylcholinesterase function, in addition to its impact *via* oxidative stress [3].

Several metals, like lead and cadmium, are categorized hazardous as they are very dangerous even at low doses and over extended periods of time [4]. Lead is characterized as a persistent metal which is assigned within the high-risk materials [5,6]. It causes changes in blood elements leading to an acute damage to red blood cells (RBCs) and white blood cells (WBCs) that generate negative consequences in biochemical parameters [6]. Biochemical and physiological descriptions of species under stress from pollution serve as essential indicators in the ecosystem [7]. Arsenic, cadmium, chromium, copper, lead, nickel and zinc are among the most often detected heavy metals, all of which pose health and environmental hazards [8]. The toxicity of heavy metals is owed to the formation of complexes with nitrogen-, oxygen-, or sulphur-containing biological molecules [9-11]. Such complexes may inhibit or alter the essential enzyme systems alongside the protein structure, resulting in cellular malfunction and eventually necrosis [12]. The functional status of homoeostasis is represented by serum marker enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and biochemical indicators such as blood nitrogen urea (BUN), creatinine. Thus, when animals are exposed to external modulators such as pesticides, the serum concentration of such marker enzymes and other biochemical factors represent their general health condition [13]. Metal pesticide combinations may produce unanticipated toxicity by chemical interaction with the surrounding environment and each other. Metal-pesticide combinations may produce unanticipated toxicity by chemically reacting with the environment as well as each other. Since humans do not expose to a sole xenobiotic consecutively, researching the toxicity of combinations has never been more important [14].
The absence of fundamental understanding about the harmful consequences of pesticides and metals, either individually or in combinations, has resulted in uncertainty over study results, data interpretation, and eventually, identifying a correct categorization for the effects of pesticide-metal combination. Hence, the current investigation was conducted to assess the time-dependent effects of cypermethrin, lead nitrate and their combinations utilizing the serum of male albino mice as a reporter system.

2. Materials and Method

2.1 Ethical Statement

All participants agreed to provide the investigator with the specimens. The ethics committee of College of science, University of Baghdad approved this work (Ref: CSEC/1122/0149).An informed consent according to the declaration of Helsinki was obtained from all participants.

2.2 Animals and Experimental Design

Swiss albino adult male mice, weighing 25-35 g, were maintained in plastic cages in the animal house at Biotechnology Research Center/Al-Nahrain University, Baghdad, Iraq, at 22-

23°C with 50-55% humidity, and a light/dark cycle of 14:10 h. All animals were fed a regular diet and given free access to water.

After one week of acclimatization, experimental protocols were carried out on eight groups of ten animals each. The negative control was called group A. Lead nitrate and cypermethrin were given intraperitoneally to groups B and C at single doses of 1.75 and 3.5 mg/kg/day respectively. In addition, animals in group D were given a solution containing lead nitrate and cypermethrin (0.875/0.875mg/kg/day). There was no fatality throughout the experiments. Blood samples (3ml) were taken and tested for biochemical markers and DNA damage after 2 and 4 weeks.

2.3 Biochemical Variables

The levels of biochemical indices were studied in blood serum that was taken byafter whole blood collection whichwas then allowed to clot by leaving it undisturbed at room temperature. This usually takes 30 minutes, no more than one hour. The clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes. Biochemical variables were determined using a spectrophotometer at certain wavelengths for example AST, ALT, ALP at 340 nm , urea at 405nm, and creatinine at 505nm in accordance with the protocol of kits given by Cypress Diagnostics, Belgium. The levels of AST and ALT were measured by a semiautomatic analyser BTS-350 as specified in a previous study[15].

2.4 Comet Analysis

The comet experiment was carried out exactly as laid down by Zhang *et al.*[16]. The DNA damage was quantified for each cell as comet tail length m = maximal total length - head diameter. Comet Assay Software Project (CASP) was used to examine the images[17].

2.5 Statistical Analysis

All tests were carried out in triplicate. The statistical analyses were carried out by SPSS version 26 using two-way ANOVA and Tukey's post hoc test [18].

3. Results

3.1 Biochemical Variables

Table 1 shows that treatment with cypermethrin alone resulted in a negligible (P>0.05) rise in blood BUN in the treated groups after 2 and 4 weeks, compared to other groups. However, following 4 weeks of treatment with the combined solution (group D), the BUN level increased significantly (P< 0.05) to 77.33 mg/dl. However, neither the period nor the therapy with cypermethrin, lead nitrate or the combination had any effect on creatinine levels.

Table 1: Changes in mean ±SD in Urea, Creatinine, ALT and AST of albino mice exposed to cypermethrin and lead after (2) and (4)weeks.

Parameter	Duration	Treatment					
		Cypermethrin	SD	Lead Nitrate	SD	Cypermethrin+ Lead Nitrate	SD
Urea (mg/dl)	Control	43.100Aa ±2.252		43.100Aa ±2.252		43.100Aa ±2.251	
	2 Weeks	60.033Ba ±10.649		52.600Bb ±8.544		66.667Ba ±8.386	
	4 Weeks	62.233Ba ±10.399		61.633Bb ±8.695		77.333Ba ±4.163	
Creatinine (mg/dl)	Control	1.007Aa ±0.621		1.007Aa ±0.621		1.007Aa ±0.621	
	2 Weeks	0.900Aa ±0.870		1.833Aa ±0.503		1.633Aa ±0.737	
	4 Weeks	1.467Aa ±0.929		1.623Aa ±0.908		2.267Aa ±0.569	
ALT (U/l)	Control	91.233Aa ±10.727		91.233Aa ±10.726		91.233Aa ±10.727	
	2 Weeks	106.433Bab ±16.720		134.967Ba ±19.123		140.667Bb ±13.796	
	4 Weeks	113.967ABab ±27.054		134.967Aba ±19.123		169.067ABb ±15.799	
AST (U/l)	Control	132.7Aa ±15.0		132.7Aa ±15.0		132.7Aa ±15.0	
	2 Weeks	230.7Bab ±74.6		130.0Ba ±33.0		334.7Bb ±106.5	
	4 Weeks	183.8ABab ±27.7		134.0Aba ±35.6		218.0ABb ±35.4	
ALP (U/l)	Control	110.000Aa ±43.093		110.000Aa ±43.093		110.000Aa ±43.093	
	2 Weeks	181.900Ba ±31.795		208.667Ba ±49.238		237.333Ba ±43.879	
	4 Weeks	182.767Ba ±31.687		198.667Ba ±44.658		214.000Ba ±17.692	

Different lower-case letters in the same row indicated significant differences.
Different upper-case letters in the same column indicated significant differences. SD(**Standard deviation)**

After 2 weeks of cypermethrin administrating, there was a significant rise ($P< 0.05$) in AST and ALT levels versus the control group (Table 1). Intriguingly, the 4-week therapy had no effects on the levels of these enzymes ($P > 0.05$). Treatment with a cypermethrin and lead nitrate combination significantly ($P< 0.05$) increased AST and ALT levels more than cypermethrin and lead nitrate individually. In terms of ALP, 4 weeks of therapy resulted in the greatest rise above the control and 2 weeks ($P< 0.05$) (P 0.05). Despite this, none of the three therapies had any effect on its level ($P> 0.05$).

3.2 The Alkaline Comet Analysis

The results described in Table 2 demonstrate that cypermethrin, lead nitrate and the combination therapy produced considerable harm ($P< 0.05$) after 2 weeks of administration (Figure 1). Given that no significant changes ($P> 0.05$) were seen between 2 and 4-week durations (Figure 2), it is reasonable to conclude that time had no influence on DNA damage. Nonetheless, lead nitrate and the combination induced much more DNA damage ($P< 0.05$) than the control.

Table 2: Mean ± standard deviation of comet tail length (µm) of mice white blood cells.

Duration	Cypermethrin (1.75 mg/kg)	Lead Nitrate (1.75 mg/kg)	Mixture of Lead and Cypermethrin (0.875/ 0.875 mg/kg)
Control	65.66 ± 0.57 Aa	65.66 ± 0.57 Aa	65.66 ± 0.57 Aa
2-weeks	63.00 ± 2.64 Ab	92.00 ± 1.73 Bb	83.66 ± 2.5 Bb
4-weeks	80.66 ± 6.11 Ab	83.00 ± 1.00 Bb	85.00 ± 1.73 Bb

Different lower-case letters in the same column denote significant differences. Different upper-case letters in the same row denote significant differences.

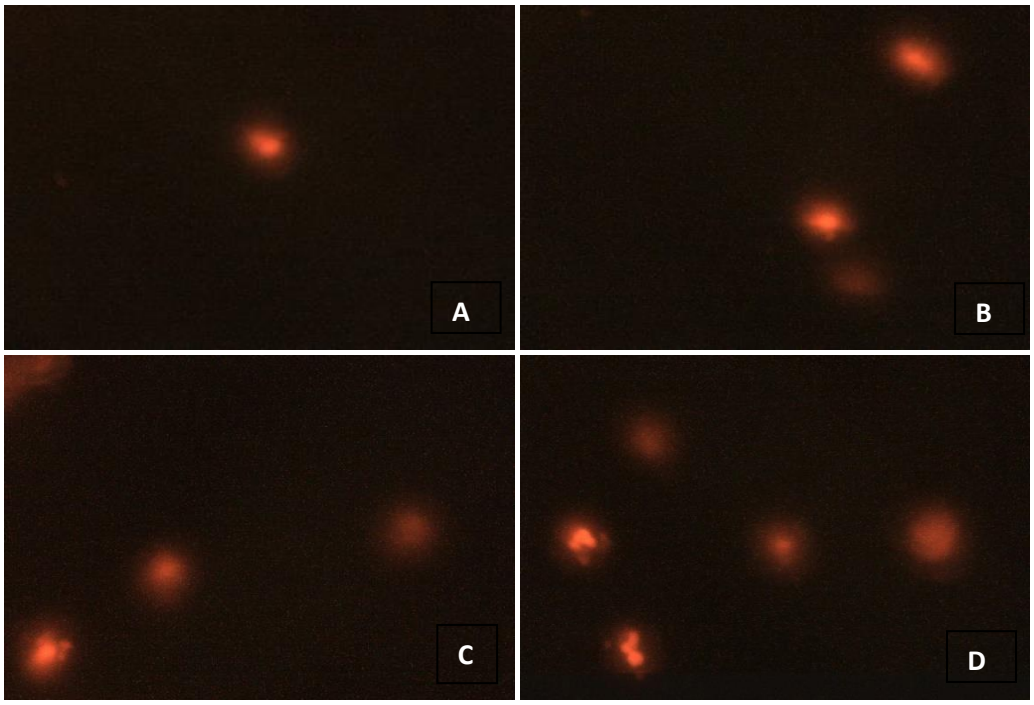

Figure 1: Images of single field of alkaline comet assay revealing different levels of DNA damage in white blood cells of mice after 2 weeks treatment with (A) Negative control (B) cypermethrin at 1.75 mg/kg/day (C) lead nitrate at 1.75 mg/kg/day (D) mixture of lead and cypermethrin at 1.75/1.75 mg/kg/day. The images were acquired at a magnification of 200× and analysed using Comet Assay Software Project (CASP) [17].

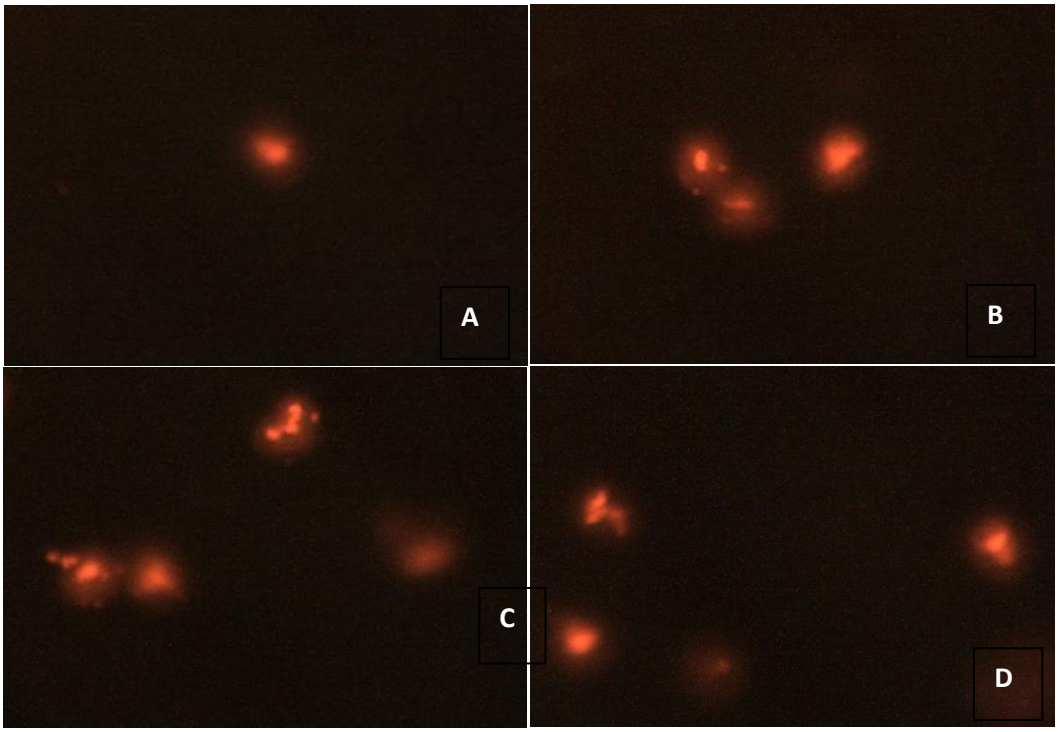

Figure 2: Images of single field of alkaline comet assay revealing different levels of DNA damage in white blood cells of mice after 2 weeks treatment with (A) Negative control (B) cypermethrin at 1.75 mg/kg/day (C) lead nitrate at 1.75 mg/kg/day (D) mixture of lead and cypermethrin at 1.75/1.75 mg/kg/day. The images were acquired at a magnification of 200× and analysed using Comet Assay Software Project (CASP) [17].

4. Discussion

An increase in blood urea is regarded as a strong indicator of renal impairment. Such increase is associated with enhanced protein breakdown leading to ammonia formation urea due to enzymatic hydrolysis of ammonia [19]. According to a current research, serum AST, ALT and ALP values are indicators for hepatic disorders. Increased activity of these enzymes in serum indicates liver injury and, as a result, changes in liver function. As a result, several enzyme activities such as AST and ALT were estimated since they indicate the functional condition of the liver. Enzyme activity in blood serum can also be employed as a meaningful stress indicator [20].

Cypermethrin treatment has been proven to cause oxidative stress by producing ROS and decreasing antioxidant defence mechanisms. Thus, it is possible that cypermethrin-induced oxidative stress is to blame for degenerative alterations in organs such as the liver, lungs, heart and kidneys. In a nutshell, elevated blood concentrations of AST, ALT and ALP are thought to be an indicator of organ damage. Thus, the elevated activity of these enzymes found in this study most likely owes to cypermethrin-induced degenerative changes in the liver and kidney tissues [21]. Recently, individuals with acute lead poisoning symptoms and abnormal liver chemistry tests among lead mine workers the AST and ALT ranges for liver enzymes were 153.08 U/L and 28.53 U/L respectively [22].

In their environment, all organisms are regularly exposed to a cocktail of xenobiotics (pesticides, heavy metals, poisonous gases, and so on). It has been shown that xenobiotic chemicals induce toxicity in animals as well as important organs in humans. As a result, the mutual interactions of these compounds in addition to their interaction with various mammalian systems are life-threatening [23]. In comparison to rats exposed just to Cd, animals exposed to Cd plus ethanol had higher levels of norepinephrine in their hypothalamus and midbrain [24].

Experiment results have demonstrated that many agrochemical chemicals exhibit mutagenic capabilities, causing mutations, chromosomal changes, or DNA damage, resulting in negative consequences on exposed animals and people. A large body of research has established that agrochemicals are a significant contributor of DNA damage [25].

Numerous studies have shown that the comet test was effective for assessing DNA damage induced by agrochemicals. And hence, it has been widely utilized for environmental biomonitoring. The comet test had also been widely used as it could detect modest amounts of DNA damage in individual cells in a very short period of time [26-29].

Cypermethrin induces significant DNA damage and a state of disequilibrium in the lymphocytes pro-oxidant and antioxidant. Ojha and Gupta [30] reported genotoxic effects of cypermethrin in human peripheral lymphocytes. Another possible cause of DNA damage is the suppression of enzymes responsible for the DNA replication or repairing. The DNA integrity is important component of normal biological function. A vital component of healthy biological activity is the organism's DNA integrity. DNA changes might be caused by cell structure loss, cell proliferation, tissue development and tissue breakdown, resulting in a whole lack of cellular regulatory system [31]. Plenty of pesticides cause DNA alterations and structural modifications at the chromosomal level. The physiochemical activity of pesticides with DNA results in a range of main alterations, including single- and double-strand breaks, DNA protein crosslinking, and purine and pyrimidine base damage [32].

The triphosphate in DNA as well as proteins are believed to covalently bond with Pb which inhibits the synthesis of nucleic acids and proteins. Metals have been proven to influence molecule conformation of DNA. Thus, it is probable that Pb's attraction for DNA phosphate groups may lead to the deformation and destabilising of DNA structure and eventually a mutation. Such interactions might be related to the process through which $Pb(NO_3)_2$ can induce breaks in DNA single strand. Nevertheless, Pb may block the repair of enzymes responsible [33].

Pb has been shown to block the enzymatic repair mechanism resulting in alterations to replication and repair pathways of DNA. Pb has a high affinity for sulfhydryl groups that may lead to a decrease in the cellular glutathione concentrations, promote peroxidation of lipids and intensify lipofuscin genesis, resulting in DNA damage[34].

As a result of our findings, we may infer that Pb $(NO_3)_2$ is a possible genotoxic agent capable of causing DNA damage, as demonstrated by the comet experiment. As a result, given the extensive usage of Pb and its derivatives, this metal should be treated with greater caution.

5. Conclusion

This study found that low dose cypermethrin, lead and their combination had an effect on BUN in male albino mice. Serum marker enzymes such as ALT, AST and ALP, on the other hand, remained changed after the administration of the pesticide, either as individual or in combination. The findings of the experiments revealed that even low-dose administration of these pesticides, both alone and in combination, had a deleterious impact on the cell physiology. It can, therefore, be concluded that further research is required into the mechanisms underlying the interaction between lead nitrate toxicity and cypermethrin.

Conflict of Interest Declaration

The authors state that they do not have any known competing financial interests or personal relationships that may seem to have influenced the work in this study.

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