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### Histological assessment for the effect of Cypermethrin in the skin of local breed rabbits

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#### ABSTRACT

Research has been designed to focusing and evaluation the effect of cypermethrin as insecticide material in the skin of the rabbits. Cypermethrin was applied on the skin after shaving the dorso-lateral aspect of rabbits. The animals (12) were classified into four groups (A, B, C and D), three animals per group. Groups (B, C and D) were exposed to cypermethrin at doses (0.001, 0.005, 0.010)mg/kg respectively, while (A) was a control group. After ten days of drug's application, animals of group (D) were died at ending of the study because they received heavy dose. The groups (B and C) shown excitation signs, followed by inhibition and depression of their activity. The animals of groups (A, B and C) were scarified and a specimens (0.5) cm<sup>3</sup> of skin from the dorso- lateral aspect were obtained, washed with water and put in formalin (10)% then processed after (24) hours by using histological technique, sectioned at (5)µm thickness, stained with Hematoxylin and Eosin a then examined under light microscopic at different powers.

#### 1. Introduction

Cypermethrin insecticide has been used in agricultural, veterinary and home formulations for more than (40) years and account for approximately one-fourth of the worldwide insecticide market [1]. The cypermethrin one most important pesticides belonging to the group of Pyrethroids manufactured and the most toxic, used in a wide range against the formation of a wide range of insect in agriculture; vegetables, fruits as well as animal, made and market as an industrial industry active for wetting and sometimes in the form of liquid, it has the ability to mix with other pesticides, which is classified by the world health organization[2], and by the environmental protection agency as a moderately toxic and a contaminant of the toxic environment, and part of the third category of hazardous substances, in addition classified by the European bureau of chemicals in the hazardous substances[3]. As a result, a wide range of disorder related to the use of cypermethrin has been reported on insect, animal, birds, and fishes even human. Acute toxicity including abnormal facial sensations, dizziness, headache, nausea, anorexia and fatigue, vomiting, increased stomach secretion, irritant to skin and eye, persistently muscular twitching and convulsive

attacks. Its effects on various systems in the body including hematological, biochemical, dermatological, muscular, urinary, central nervous system and digestive system [4].

The pesticides of acute toxicity should not be used in developing countries Palestine 1984, However, in Pakistan pyrethroids insecticides including cypermethrin are extensively used pesticide comprises; (88) % insecticides, (11) % herbicides and one percent fungicides[5]. This cypermethrin is photo stable with a half-life of (8-16) days in direct sunlight, in soil and water, half-life is as long as (56) and (100) days, respectively. In spite of wide range of effectiveness, cypermethrin is not free from side effects signs like muscular tremors, ataxia, weakness of limbs, convulsions, coma and death from respiratory depression have been reported after ingesting high doses of it, while its dermal contact in the skin area may cause a subjective sensation of tingling or numbness [6]. Hematological values are widely used to determine the systemic relationship and physiological adaptations including the assessment of general health condition for animals, most studies on the effects of pyrethroids on the skin changes, and little attention has been paid to the

hematological modulations induced by these pesticides rabbit [7]. A few information is available about cypermethrin toxicity from different regions of the world, yet little work has been accomplished on cypermethrin toxicity especially in relation to clinical signs symptoms[8], and hematological changes in experimental animals under local conditions, this study mentioned as affected by cypermethrin treatment in rabbits[9], cypermethrin is primarily absorbed by gastrointestinal tract, may also be absorbed via inhalation of spray mist and only simply through the intact skin. Due to its lipophilic nature, cypermethrin has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries and brain [10].

The aim of study was to show the effect of cypermethrin in the skin, after application of different concentrations of it in the skin of rabbits.

## 2. Materials and methods.

The dorsum skin of all rabbits (local breeds) were shaved and sterilized by iodine solution (1) %, and then the shaved skin was dipping in cypermethrin with different concentrations and at the end of the experiment whole rabbits were housed separately.

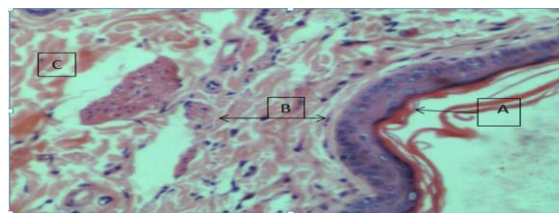
The material used in experiment for one animal was cypermethrin. The rabbits were of (1-1.5) kg weight were used the study and put in steel cages of volume (100 x 60 x 20) cm. Mature rabbits were obtained from Animal House from College of Veterinary Medicine in Tikrit University, they divided into four groups (4 animals for each one), group (A) as a control while groups (B), (C) and (D) treated by cypermethrin of different concentrations, the skin of all animals were shaved and then dipping in cypermethrin mixed with water as for experimental group (B), (C) and (D), the study prolonged for (10) days. The group (A) was dipping in tap water daily for ten days. Sampling from the area was taken (0.5) cm<sup>3</sup> of skin, each segments of skin was immersed in (10) % formalin for (24) hours, followed by immersion in graded series of alcohol from (70, 80, 90 and 100) %, then clearing with xylene and embedded in paraffin wax at (60) °C. Blocking of the samples were done, and the sectioning were performed with (5) µm thickness by using a rotary microtome. After staining with haematoxylin and Eosin, tissues sections were mounted on the slides using D.P.X and covered by cover slides [11]. The slides were examined by using light microscope, and photographed by manipulated camera prepared for this purpose.

## 3. Results

### 3.1 Control Group

The epidermis was formed by stratum basal (cuboidal-columnar cells) and stratum spinosum (scale-like cells) and the granular layer, which was covered by keratin. The hair follicles were present in the dermis invading the epidermis, as well as, dermis was containing groups of the hair follicles formed by external sheath, internal sheath and the core in

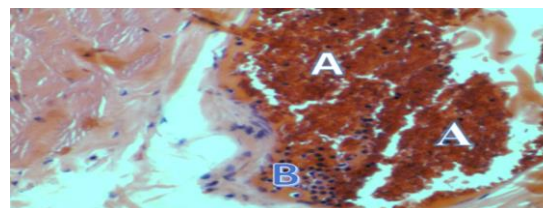
between these groups were present, a few number of lymphocyte and collagen bundles were also present around it (Figure 1). The dermis and hypodermis were containing multiple blood vessels and bundle of collagen fibers with its fibroblasts.



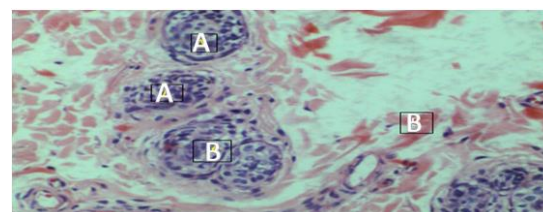
**Fig. 1: Control group (A), demonstrating the epidermis with keratin (A), dermis (B), hypodermis (C). (H&E X 20).**

### 3.2 Effect of cypermethrin in skin of rabbit with concentration (0.001)

The epidermis was thin and its cells were difficult to be recognized, the keratin on its surface was present as thread like, the granular cell layer nearby keratin was very darkened and appeared as a black zone. As for dermis, was containing lymphocyte and macrophage with loosening of the collagen bundle. The blood clot was seen in the dermis due to hemorrhage of certain blood vessels, also there was multiple aggregations of white blood cell in the form of corpuscles in the deepest layer of dermis nearby hypodermis with breaking down of collagen fibers. (Figures 2 and 3).



**Fig. (2): Effect of cypermethrin in skin of the rabbit with concentration (0.001), appeared the blood clot in dermis of skin (A), with lymphocytic aggregation (B). (H&E X 40).**



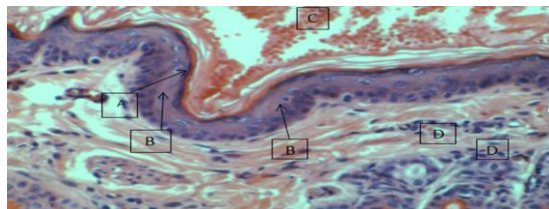
**Fig. 3: Effect of cypermethrin in skin of the rabbit with concentration (0.001), appeared the multiple aggregation of WBC in the deepest layers of dermis (A), with breaking down of collagen fibers (B). (H&E X20) .**

### 3.3 Effect of cypermethrin in skin of rabbit with concentration (0.005)

The epidermis was very narrow, and most of the cells of stratum spinosum were disappeared and the cells of the stratum basale were discontinued, other the keratin on the surface of epidermis was appeared of thin thread, intermingled by blood mass from outside. As for the dermis, was containing white blood cells infiltration, and most of the hair follicles were darkened in staining due to degeneration of the sheath cell of



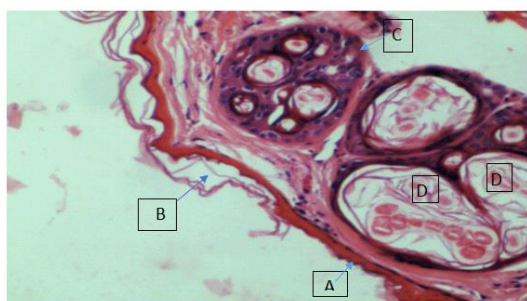
the hair follicle, which were surrounded by a few number of the lymphocyte and few bundle of collagen fibers. The deepest layer of dermis was containing thickened walls of blood vessels, and a few collagenous fibers, separated it from hypodermis which was containing skeletal muscles fibers which were present loosening from each other (Figure 4).



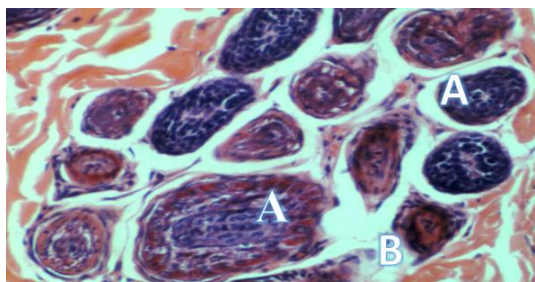
**Fig.4 :** Effect of cypermethrin in skin of the rabbit with concentration (0.005), appeared the epidermis showing its narrow layers (A), with disappearance of stratum spinosum remnant of keratin (B), mass of blood clot (C), inflammatory WBC in dermis around sweat gland (D). (H&E X 20).

### 3.4 Effect of cypermethrin in rabbit skin with concentration (0.010)

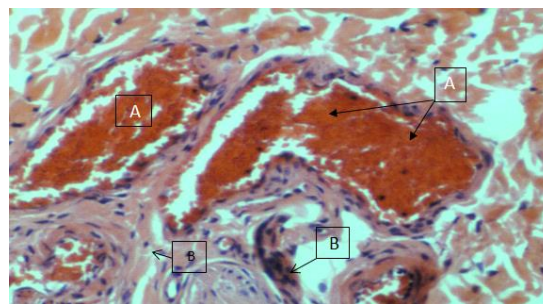
The epidermis was completely destroyed and its remnants were transformed into a zone of keratin, resemble to that of separated threads of keratin, the dermis was containing degeneration pattern of hair follicles and sweat gland, surrounded by dense connective tissue. The external and internal sheath of hair follicles were darken in its color due to its degeneration. The dermis of skin injury was containing sever blood congestion in its blood vessels, and the hypodermis was containing loosening skeletal muscle fibers (Figures 5, 6, 7 and 8).



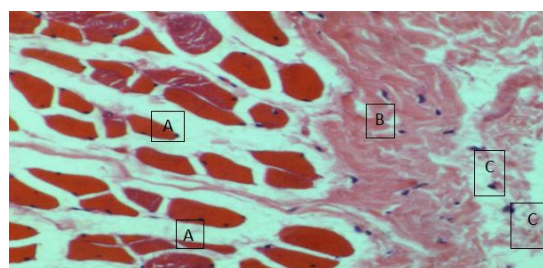
**Fig. 5:** Effect of cypermethrin in skin of the rabbit with concentration (0.010), appeared the destruction of epidermis (A), threads of keratin (B), degeneration of hair follicle (C), and sweat glands (D). (H&E X 40).



**Fig. 6:** Group given cypermethrin (0.010) demonstrating degeneration of external and internal sheath of hair follicle (A), of the macrophages (B), (H&E X 40).



**Fig. 7:** Effect of cypermethrin in skin of the rabbit with concentration (0.010), appeared the severe congestion of B.V (A), WBC and macrophage aggregation (B). (H&E X40).



**Fig. 8:** Effect of cypermethrin in skin of the rabbit with concentration (0.010), appeared the hypodermis with loosening of the skeletal muscle fibers (A), dense C.T of dermis (B), macrophages (C). (H&E X 20).

## 4. Discussion

The present study was designed to investigate the effect of cypermethrin after application on the skin of rabbit for (10) continuous days, so the cypermethrin was put on the shaved skin for three different doses. Application of this material demonstrated that the shape of the epidermis and dermis at the site of application was affected at different degrees, which means that increasing the concentration of dose leads to severely effect.

The present results indicated that the cypermethrin as a toxic chemical insecticide has powerful effect in the skin due to its active ingredients which lead to destructive effect on the skin if applied for un limited time and intensive doses, these were referred by [6], and in another study, was confirmed this concept for same cypermethrin in nervous system[12]. However the most studies were mentioned the effect of this material histological and histopathologically like in rat[5].The application of this material was indicated the insult of cypermethrin at any concentration even mild dose, so our suggestion that this drug must not be used by owner, just used by supervision of the veterinarian. Most of the studies were focused at its effect as concentration for the animal[1] and the reflection of this application be followed to the involved individuals which are consumption the product of these animals.

In conclusion, cypermethrin in high dose may be fatal and toxic for animals, and be result of good to treatment of ectoparasitic, as well as weakness, anorexia, histopathological lesion as; degeneration of epithelial cells, blood composition at dermal region

associated with degeneration of follicular sheath of hair follicle, and lymphocyte infiltration.

The cypermethrin has toxic effect on public health especially on skin tissues, but intensity of degree damage depend on exposure time, dose concentration. As a results, strict law need to be formulated to

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prevent use of cypermethrin during applying in the agricultural sector, and it should to be reflected in our health policy, in addition, should not be use high dose of cypermethrin which cause dead animals, and appearance of excitation signs[13] .

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## التقييم النسيجي لتأثير السايبرمثرين في الجلد لسلالة الارانب المحلية

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### الملخص

صمم البحث الحالي لتسليط الضوء وتقييم تأثير السايبرمثرين كمبيد حشري على الجلد في الأرانب. وضع السايبرمثرين على جلد الارانب بعد حلاقة الناحية الظهرانية الجانبية منه.

استعمل (12) من الارانب المحلية، وزعت اربع مجاميع (A,B,C,D) بواقع ثلاث ارناب في كل مجموعة. تم تعريض مجاميع (B,C, D) للسايبرمثرين بجرع (0.001, 0.005 و 0.010) ملغم / كغم على التوالي، بينما مجموعة (A) عدت سيطرة.

بعد عشرة ايام من استعمال العقار نفقت مجموعة (D) عند نهاية الدراسة لتلقيها جرعة عالية منه، اما المجموعتان (B,C) فقد اظهرتا تهيجاً، خمولاً وانخفاضاً في النشاط. تم قتل الحيوانات في المجاميع (A,B,C) واخذ عينات من الجلد من المنطقة الظهرية الوحشية بنسبة 0.5 سم<sup>2</sup> غسلت بالماء ثم وضعت في مثبت الفورمالين 10 % وبعد (24) ساعة من التثبيت تم اعتماد التقنية النسيجية معها وقطعت بسمك 5 مايكرومتر ثم لونتباستعمال الهيماتوكسليين والايوسين و فحصت تحت المجهر الضوئي باستعمال قوى تكبير مختلفة.