Role of Two Biocides in The Wounds Healing and side Effect in Rabbits

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Summary
This study was aimed to show the influence of two bioformulation on wounds healing, side effect in liver and kidneys in rabbits. The results indicated that no effect on wound healing were observed in bioformulation treated group compared with the controls. There were toxic effects on the liver which was shown on histopathological examination as centrilobular vacuolation, necrosis of hepatocytes around congested central vein, pyogranulomatous lesion around dilated central vein with marked vacuolation of hepatocytes. On kidneys, histopathological results revealed severe hemorrhage in the cortical area between glomeruli and renal tubules, severe atrophy of glomeruli in the cortical area with some congested blood vessels in the renal parenchyma, severe atrophy of glomeruli in the cortical area with some congested blood vessels in the renal parenchyma and some degree of dilation of renal tubules.

Keyword: histopathological, Wounds healing, Biological formulation, Rabbits.

دور مبيدات احيائيين في التئام الجروح واثارها الجانبية في الارانب

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الخلاصة:
هدفت الدراسة الحالية إلى اظهار تأثير مستحضري احيائيين في التئام الجروح والآثار الجانبية على الكبد والكليه في الارانب . أظهرت النتائج عدم وجود تأثير على التئام الجروح بين المجموعة المعالمة ومجموعة السيطرة ولم يحدث تأخر في الأنتام ، كما أظهرت النتائج النسيجية المرضية للكبد وجود فجوات في مركز الصيغات وتتخر الخلايا الكبدية التي تحيط الوريد المركزي المحتمل وقرحات حبيبية قيحية حول الوريد المركزي الموسع مع وجود فجوات بشكل ملحوظ في الخلايا الكبدية. أظهرت النتائج النسيجية المرضية للكليه وجود التهاب الكليه النوعي الشديد في منطقة القشرة بين الكبيبات والأدبيات الكلوية وضمور شديد للكبيبة في منطقة القشرة مع وجود بعض الأوعية الدموية المحتشتة في نسيج الكليه وتوزع نواعا في الأدبيات الكلوية.
Introduction

Bioformulations (biocides) are widely used as an alternative to chemical pesticides for controlling plant pests, since the latter are well known harmful to human and animals and their use is frequently described as a cause of cancer in addition to its harmful effect to the environment. Biocides are natural products derived from selective microorganisms like fungi and bacteria (1). The process of wound healing takes place in four stages: hemostasis, inflammation, proliferation, and remodeling or resolution (2). These stages occur in a fixed sequence and at a specific time and continue for a specific duration at an optimal intensity (3). These stages are driven and controlled by many factors such as wound oxygenation, presence of infection, age and gender of individual, hormones, stress and nutrition (4). Some studies in animal like rats, mice and rabbits about wound, side effect of chemical and biocides. Al-jenabi (5) had found that there were no any changes in biochemical and physiological blood standard measurements beside no alteration in treated animals in hepatic and renal tissues when he use biocide of Pseudomonas fluorescens CHAO. Also Al-Ashur (6) prepared the biocide from Bacillus cereus named Bacillin and tested in white rabbits, the results appeared that no effect in biological blood standard measurements and no changes in the liver and kidney tissue. Alwan (7) prepared an oil bioformulation from the fungus Trichoderma harzianum, then tested in white rabbits, the results appeared that no variation in physiological blood standard, haemoglobin, deposition of blood measurements. He also evaluated the compound on wound healing when exposure to magnetic field at 50 gauss for 15 minute daily for seven days (8). Glavic et al. (9) found that the pesticides delay wound healing. Which is confirmed by histopathological examination of the liver which revealed (lipofuscinosis) of liver cell and adaptive reactions (hypertrophy of endoplasmic reticulum) (10). Beside the liver, the kidney were also affected, and histomorphological changes were reported in rats treated with pesticide-cholopyrifos (11). This study was designed to investigate the effect of some bioformulations on wound healing, and also to evaluate possible side effect on end organs as the liver and kidney.

Materials and Methods

Animals:

In present study twelve male rabbits were used (Lepus cuniculus), aged 6-8 months, live in similar condition fed with bread and vegetable, which were divided into three groups, 1st and 2nd groups were treated while the 3rd group was a control. All rabbits were anesthetized with intramuscular administration of 10 mg/kg body weight xylazine hydrochloride (SANOFI SANTE NUTRITION, LaBallastere 33011: bonne Cedex, France) and 50 mg/kg body weight ketamine hydrochloride (ROTEx-NEDTCR-GMBH-Germany).

The area of gluteal region shaved and cleaned. Then a longitudinal incision in skin were made by sharp dissective involving epidermis, dermis and hypodermis. The length of each incision was 3cm (8).
1\textsuperscript{st} group was dosage with 1gm/kg weight of rabbit from bioformulation-1 \textit{(Trichoderma harzianum} carried on CaCO\textsubscript{3}) every day for ten days , 2\textsuperscript{nd} group was dosage with 1gm/kg weight of rabbit from bioformulation-2 \textit{(Trichoderma viride} carried on CaCO\textsubscript{3}) every day for ten days, the 3\textsuperscript{rd} group was dosage with 9ml/kg weight of rabbit distill water every day for ten days (6).

**Histopathological examination**

After 7 days the experimental animals were killed by air bubbles in the heart of rabbits , then liver , kidney were removed and wounds were excised at the determined around the wound , then these organs were immediately placed in 10\% formalin .Following fixation , routine tissue processing , the tissues section were stained with hematoxylin and eosin (12). The histopathological sections prepared from the liver , kidney and wounds were examined by compound microscope at college of veterinary medicine university of Basra ,Iraq .

**Results and Discussion**

**Wound healing**

Results indicated that the wound Skin showed congested blood vessels in the dermis with area of hemorrhage and atrophy of hair follicles (Fig.1A), The re - epithelization of epidermis layer with some infiltration of inflammatory cells and atrophy of hair follicles, (Fig.1B,C) , also the full re epithelization of epidermis layer with some infiltration of inflammatory cells and congestion of blood vessels (Fig.1D).

The loss of epithelial layer (epidermis) with congestion of blood vessels in the dermis layer, epidermis layer and dermis beneath it also there is infiltration of inflammatory cells in the dermis layer, there is complete regeneration of skin layers (Fig.2A,B) , Also loss of some area of epithelial layer (epidermis) with congestion of blood vessels and hemorrhage in the dermis layer also there is area of re epithelization in the left side , with congestion of blood vessels and hemorrhage in the dermis layer (Fig.2C,D),while the control appeared that scab formation above the epidermis layer, also there is atrophy of hair follicles in the dermis layer with thin layer of epidermis above the dermis (Fig.2E).There was not effect happened .

**Liver:**

The results of the present study appeared that the liver showed a centrilobular vacuolation of hepatocytes around congested central vein (Fig.3A,C) , also a centrilobular necrosis of hepatocytes around dilated central vein(Fig.3B) . Then a pyogranulomatous lesion around dilated central vein with marked vacuolation of hepatocytes and marked vacuolation of hepatocytes (Fig.3D,E).While many researchers had not shown side effects in animals treated with biocides prepared from \textit{Pseudomonas fluorescens} CHAO in white rabbits, in thier studies there were no changes in liver and kidney tissue of their animals(5).

Lack of effect on liver tissue in rabbits was also observed by Alwan (7) who prepared an oil bioformulation from the fungus \textit{Trichoderma harzianum} .Toxic effect on the liver observed in the present study may raise possibilities attributed to the process of synthesis and formulation of the biocides prepared in our laboratory .

**Kidney:**

The results appeared that showed a severe hemorrhagic nephritis in the cortical area between glomeruli and renal tubules(Fig.4A) , also a severe
atrophy of glomeruli in the cortical area with some congested blood vessels in the renal parenchyma(Fig.4B,C) . Then a severe atrophy of glomeruli in the cortical area with some congested blood vessels in the renal parenchyma and some degree of dilation of renal tubules(Fig.4D), also a severe hemorrhage in the renal parenchyma dilation of renal tubules(Fig.5A,B). In the Fig.5 C,D, E showed severe atrophy of glomeruli in the cortical area with some congested blood vessels in the renal parenchyma and some degree of dilation of renal tubules. Kidney showed severe congestion of blood vessels between renal tubules with degree of vacuolation in the renal tubules. Alwan (7) prepared an oil bioformulation from the fungus *Trichoderma harzianum*, then tested in white rabbits, the results appeared that no changes in treated animal in kidney tissue.
Figure 2: (A) loss of epithelial layer (epidermis) with congestion of blood vessels in the dermis layer, (B) epidermis layer and dermis beneath it also there is infiltration of inflammatory cells in the dermis layer, there is complete regeneration of skin layers, (C,D) loss of some area of epithelial layer (epidermis) with congestion of blood vessels and hemorrhage in the dermis layer also there is area of re-epithelization (E) (control) scab formation above the epidermis layer, also there is atrophy of hair follicles in the dermis layer with thin layer of epidermis above the dermis. (H&E stain).
Figure (3): Liver showed (A) centrilobular vacuolation of hepatocytes around congested central vein, (B) Liver showed centrilobular necrosis of hepatocytes around dilated central vein, (C) centrilobular vacuolation of hepatocytes around central vein, (D) pyogranulomatous lesion around dilated central vein with marked vacuolation of hepatocytes, (E) marked vacuolation of hepatocytes. (H&E stain).
Figure (4): kidney showed  (A) severe hemorrhagic nephritis in the cortical area between glomeruli and renal tubules , (B) severe atrophy of glomeruli in the cortical area with some congested blood vessels in the renal parenchyma , (C): kidney showed severe atrophy of glomeruli in the cortical area with some congested blood vessels in the renal parenchyma , (D) severe atrophy of glomeruli in the cortical area with some congested blood vessels in the renal parenchyma and some degree of dilation of renal tubules , (H&E stain.)
Figure (5): Kidney showed (A) severe hemorrhage in the renal parenchyma, dilation of renal tubules, (B) kidney showed dilation of renal tubules, (C) kidney showed severe atrophy of glomeruli in the cortical area with some congested blood vessels in the renal parenchyma and some degree of dilation of renal tubules, (D) Kidney showed severe congestion of blood vessels between renal tubules with degree of vacuolation in the renal tubules, (E) Kidney showed dilation of renal tubules with degree of vacuolation in the renal tubules. (H&E stain.)
References