

Comparative Effect of Aqueous *Datura (Datura metal)* Extract and A Gentamicin on The quick Healing of The Wound in Thigh Region in Laboratory Rats

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Abstract

The present study is achieved for observing and evidence the effect of *Datura* Aqueous Extraction (DAE) on the success the quick repair the cutaneous wound in thigh region in lab rats which are infected with *Saphylococcus aureus*. The study is achieved in 28 mature rats ,those same age and weight (250 ± 25 g), 8 month age and live at similar condition. The animals are divided into three groups, 1st and 2nd was contain 10 rat (5mal+5femal) in treated groups while control group was contain 4 rat which subdivided into (4male+4female) and in all groups were made surgical wound (superficial) in thigh region. The 1st (*Datura*) and 2nd (*Gentamicin*) groups were infected by *staph. aureus* with 10^{-8} titration of germs. After 48 hr the both groups are treated the 1st by DAE 10 mg/kg b.w. and 2nd by Gentamicin 5% w/w as well as sham-control subgroup is leave. The control group was subdivided into two subgroup, the 1st subgroup (standard subgroup) was leaved stay sterile wound without infection (superficial disinfection), recovery without treatment (self cure), the other were infected with *staph. aureus* and leaved without treatment. The experiment is evidencing the quick healing of wound that is treated with (DAE), compare with the wound that treated with Gentamicin. There are no destructive change in microscopic histopathology due to less toxic effect of *datura*, as well as blood parameter is good evidence in *datura* group. Statically analysis was achieved by SPSS v. 16.0 , there are significant results of *datura* compare with other groups, no significant results of gentamicine group and standard control subgroup while significant with sham-control subgroup $P < 0.05$.

Key word : *Datura*, wound healing , gentamicine

Introduction

Datura metal (saolanacae) is a popular or widely medicinal uses include of dried leaves of plant as anti-cholenergic agent^{1,2,3}. As well as it use as an anti-ulcer, pain relief, and nervous system stimulator, hyoscine^{4,5}. Phytochemical detected in plant alkaloid atropine and scopolamine and other chemical composition⁶. This plant was tested as anti-microbial by Weckbach and Langlois⁷, they use *Staphylococcus aurous* betray-dish with plant extraction disc, the diameter of inhibition zone were measured by using ruler and size of inhibition were by Kirby-Baur method⁸. wound infection have been thought of as infection in surgical wound occurring between the skin and deep soft tissue. There are three factors effect on wound healing: 1-bacteria or infectious agents, 2-host defense mechanisms, 3-

surgical sites⁹. Antimicrobial or antibiotic should be taken by inconsideration the side effect and cellular toxicity, *Datura metal* extraction either plant or seed are toxic in human or animal have been reported¹⁰. therefore, the use of *D. metal* extraction have many risk and the using of plant as skin ointment is suitable¹¹. The effect of *D. metal* on the liver and kidney function were estimated by Gidado *et al.* , they showed increase total billiorubin, urea, creatinine, liver enzymes and plasma ions (Cl^- , Na^+ , K^+), when they used the plant with food intake (200mg/kg.bw) daily¹⁰. Other effect of *D. metal* in the body was reported in lung, heart, and nervous system by Knight and Walter, Francis, Beasly , Artoud and Langdon^{12,13,14,15}. Other researches were study the analgesic effect of *D. metal* such as Wannang *et al.*

because the plant have pre-medication anesthesia such as atropine and hyoscine¹⁶. Gentamicin is bactericidal aminoglycoside antibiotic with wide clinical uses but distributing toxicity, nephrotoxicity, and ototoxicity are most common adverse reaction. Gentamicin is a specify to Gram+ bacteria²³. attempts to further the incidence of postoperative wound sepsis have included gentamicin irrigation prosthesis implantation²⁴. Numbers of risk factors associated with

treatment by gentamicin such as volume depletion, metabolic acidosis, hypokalemia, Ca^{++} deficiency, free radical generation, and ascorbic acid deficiency^{23,25}. there are many attempts to reduce the gentamicin toxicity by addition some agent or drugs such as sodium bicarbonate, high calcium diet, vitamin B-complex, and vitamin C^{23,25,27}. The aim of present study to knowledge of herbal extract (Datura) to treatment wound infection and its side effects.

Material and Methods

28 adult rats (male + female 50:50), they a live in similar condition (feeding, temperature, and water). Aqueous Datura extraction (10%)¹², gentamicine ointment (5%) w/w. [RBCs, WBCs, HB, and PCV] apparatuses. Anesthesia was used the mixture of ketamine+xylazine (100mg+10mg) with dose (30mg/kgbw)¹⁹, as well as surgical instruments. Prepare the animals to shaving the thigh region at lateral view by hair removal cream, after 5 minutes the area wash by water and covered with cotton saturated by 70% ethyl

alcohol to each animals, after than made incision 2cm in thigh region by surgical blade¹⁷. Animals are massacrred by cervical dislocation method and the blood is drowned by DETA test tubes for blood examinations and knowledge the effect of therapy on blood parameters. The rats were divided into three groups, Datura group, Gentamicin group, and control group, the last was subdivided into two subgroups: standard-control and sham.

| total animals 28 | | | |
|----------------------|--------------------------|----------------------------------|-------------------------------|
| Datura group (10) | Gentamicin group (10) | Control group (8) | |
| | | Standard-control subgroup (4) | Sham- control subgroup (4) |

All rats were exposed infection by *Staphylococcus aureus* 10^{-8} except standard control subgroup, after 48hr when inflammation signs appears on the wound, they were treated by Datura extraction at Datura group, and Gentamicin at Gentamicin group, while the sham-control subgroup was leaved without treatment. Blood examination were estimated by classical method, HB was estimated by Salhi' method, RBCs and

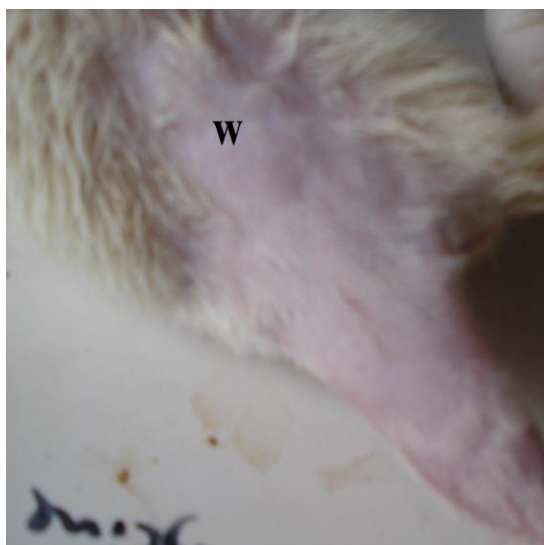
WBCs were estimated by counter chambers slid under light microscope and PCV was estimated by micro-centrifuge²⁰. Skin wound pictures were toke by digital (12 migapixel) china origin, Canon. Were prepared from skin wound healing by toke specimens from skin contain wound edges and maintain in formalin solution 10% and prepared slides, stain with eosin and heamatoxylin staining technique¹⁸.

Results

The clinical signs of surgical wounds incision are normal in two days post surgery, but after 3 days the sings of inflammation were began except standard subgroup control were leaved without infection. The severity of inflammation after treatment were vary from group to other, in Datura group were showed faster

healing, while Gentamicin group were less than the first, but both group were faster than two subgroup control fig(1). The effect of Datura, Gentamicin on the internal organs were showed histopathologically in the fig(2) compare with two subgroups control. The blood parameters were summarized in this table (1)

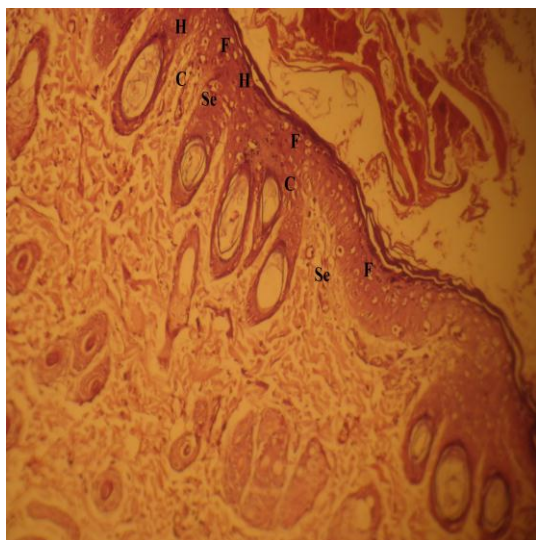
Fig (2) wounds gross pictures

Gross wound in rat in *Datura* group w

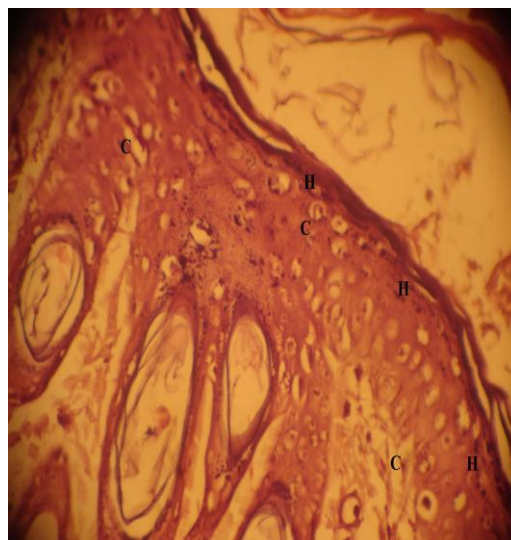
Gross wound in rat in gentamicin group w

Gross wound in rat in standard-subgroup
control group wGross wound in rat in sham-subgroup
control group w

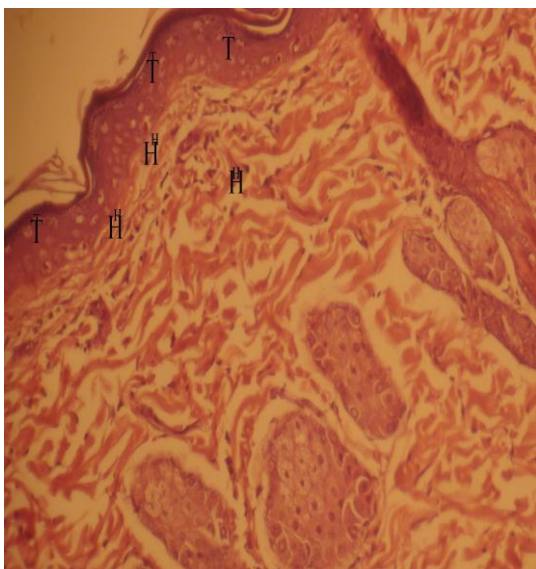
Fig (3) histopathological section in skin of rat groups



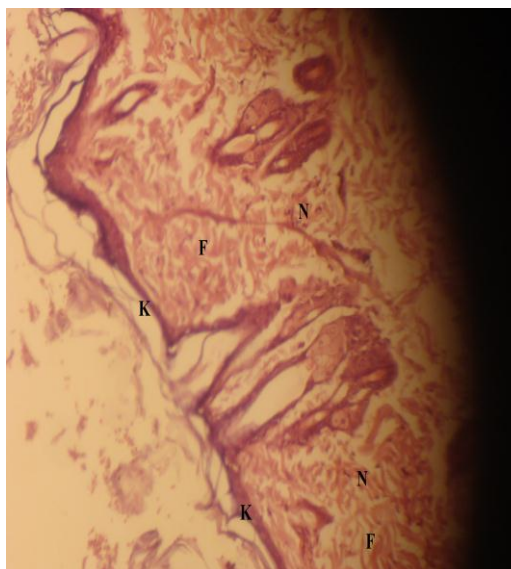
Hyperkeratosis H, collagen infiltration C, number of hair follicular F, prominent sebaceous gland Se (H&EX10) *Datura group*



Hyperkeratosis H, minimal of collagen infiltration C, no evidence of sebaceous gland Se (H&E stain X10) *Gentamicin group*

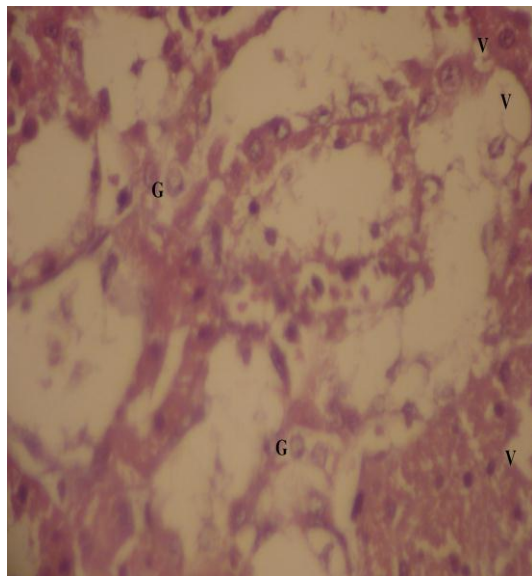


Hyperkeratosis H, and thick epidermis T, micro erosion M (H&E stain X40) *Standard-control subgroup*

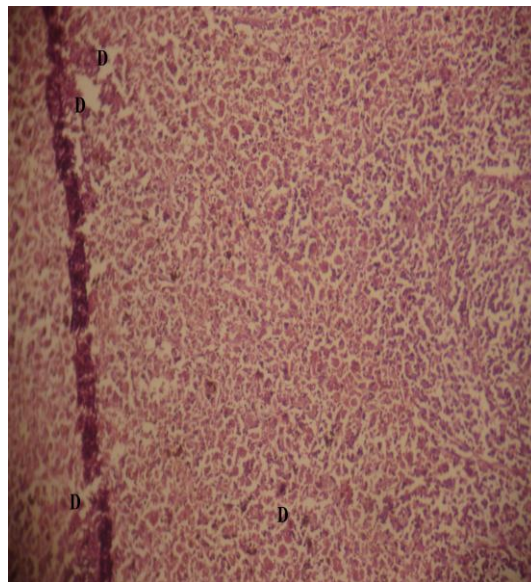


Inflammatory cells N, kerosis K, thick epidermis T, fibrosis F (H&E stain X40) *sham-control subgroup*

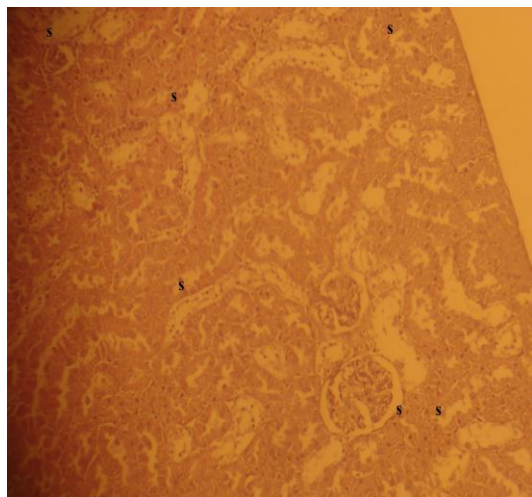
Fig (4) histopathological section in kidney of rat groups



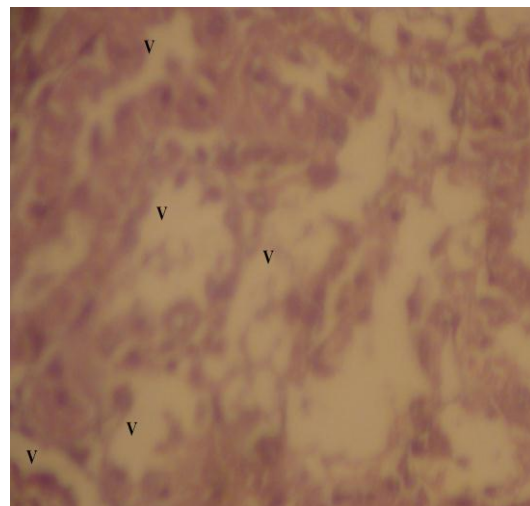
Vacuolation of sub capsular renal cortical tubules V,
atrophy of glomerulus's G (H&E stain X40) *Datura*
group



Dilated central tubules D(H&E stain X40)
Gentamicin group

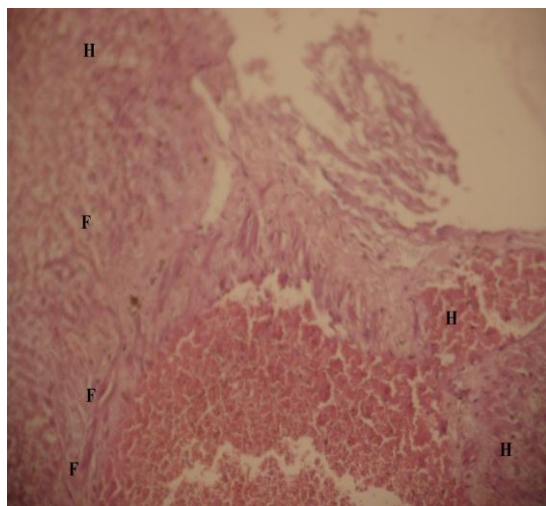


Sub-cortex-sub capsules vacuolation S
(H&E stain X40) *Standard-control subgroup*

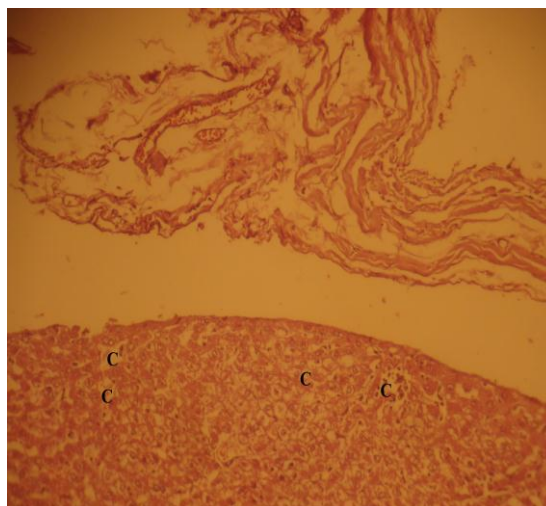


Vacuolation of cortical tubules V
(H&E stain X40) *sham-control subgroup*

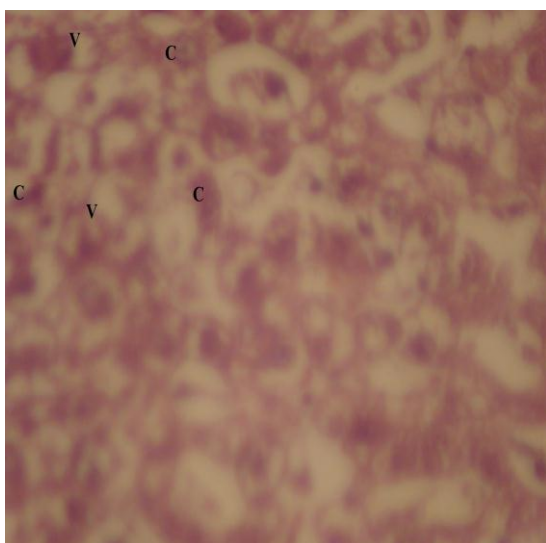
Fig (5) histopathological section in liver of rat groups



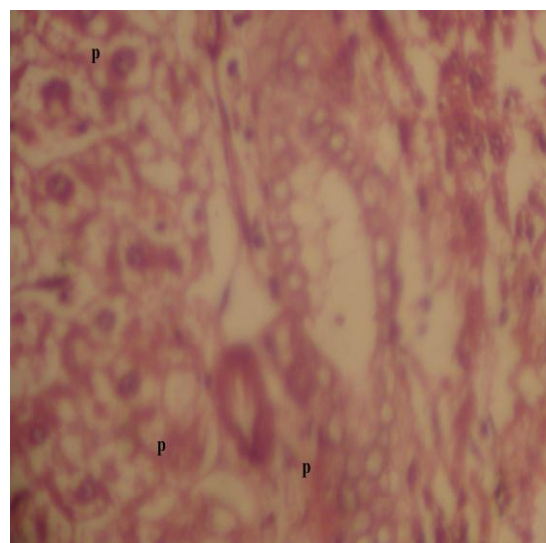
Sub-capsular hemorrhage with pericapsular congestion H, fibrosis F (H&E stain X40)
Datura group



Centrally lobular vacuolation of hepatocytes C
(H&E stain X40) *Gentamicin group*

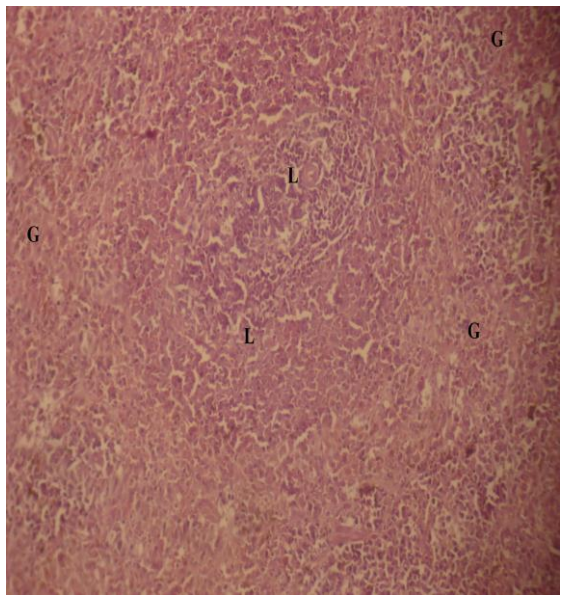
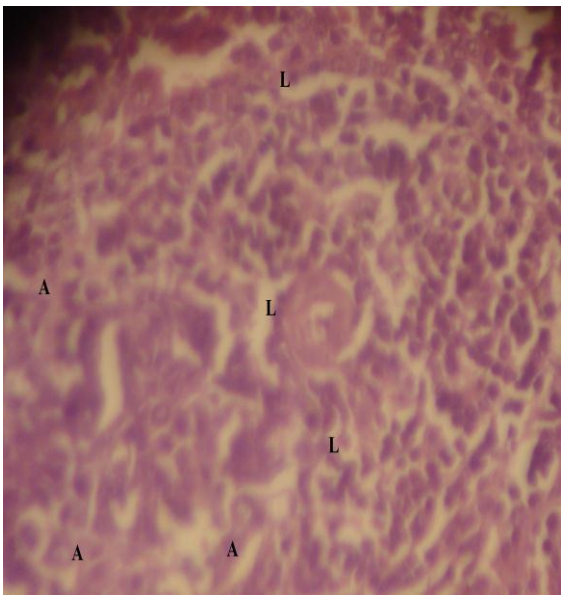
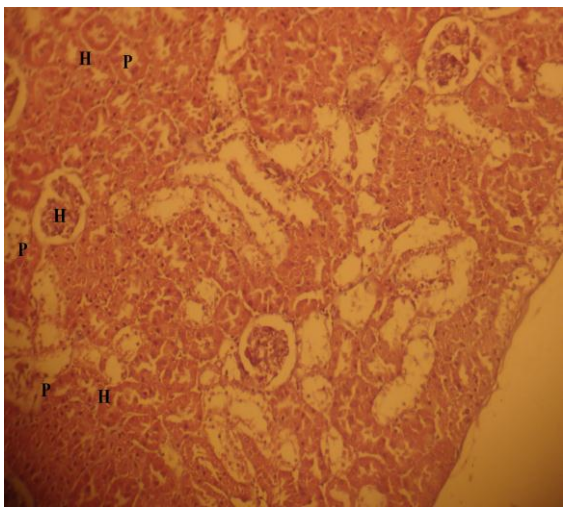
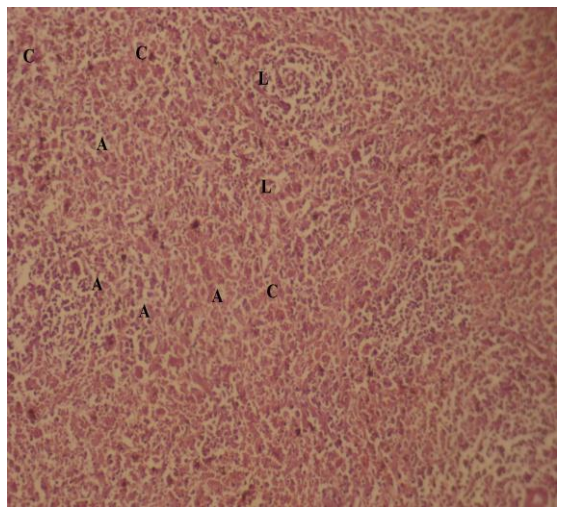


Minimal vacuolation of hepatocytes and general congestion (H&E stain X40) *Standard-control subgroup*



Portal triad bile duct proliferation P
(H&E stain X40) *sham-control subgroup*

Fig (6) histopathological section in spleen of rat groups

| | |
|---|---|
|  <p>Lymphoid hyperplasia L, germinal central formation G (H&E stain X40) <i>Datura group</i></p> |  <p>Atrophy of white pulp A, lymphoid hyperplasia L (H&E stain X40) <i>Gentamicin group</i></p> |
|  <p>White pulp lymphoid hypertrophy H and hyperplasia p (H&E stain X40) <i>Standard-control subgroup</i></p> |  <p>White pulp atrophy A, lymphoid hyperplasia L, general congestion C (H&E stain X40) <i>sham-control subgroup</i></p> |

Discussion

Datura metal herb is most common herbal medicine as alkaloid pharmacology, and there are agrees and disagrees with other researchers in the internal medicine and microbiology field, but in surgery there are no researches discuss yet. *D. metal* have anti microbial effect against several bacteria such as *Staph. aurous*, *E. coli*, *K. pneumonia* and other bacteria. This properties to regard to tannin, glycosides compounds, and alkaloid compounds, these agree with other such as Wannang¹⁰ *et al.*, Gidado¹¹ *et al.*, and other^{12,15}. Toxological effect of *D. metal* don't showing clearly and significant changes in organs and blood parameters, the reason may regard to the few amount which used in experiment or the rout of administration (skin ointment 10%) or both, while other researchers were used in oral administration, they show changes in blood organs, therefore this herb should be don't use orally alone in spite of the professional of herbal medicine regard *D. metal* is safe herbal medicine compare with other species¹⁵. The factors which effect on wound healing are antimicrobial activity of *D. metal* extraction, atropine effect on blood vessels, irritation effect of tannic acid which accelerate the wound healing, and formation of scar tissues²¹, as well as

the analgesic effect of *D. metal* on wound healing also help the animals to don't crushing the edges of wound²². Gentamicin activity against bacteria (wound infection) and also the role of gentamicin in wound healing were agreed with other studies such as Souza *et al.*²⁷, Sorger *et al.*²⁸ and Fallahzadeh *et al.*²⁶, while the side effect of gentamicin in our study were showed more severity compare with *D. metal*, this result also agreed with other searchers such as Lee *et al.*²⁵, Lee *et al.*²⁴, Bello *et al.*²³; they showing significant changes pathologically and physiologically while our study used skin infiltration as skin ointment also show pathological changes, therefore they used other agent to advantaged the gentamicin effect such as vitamin B-complex, vitamine C and soduium bicarbonate^{23,24,25}. The genera of animals no effect in wound healing significantly, and all animals in resent study were similar age, while in other studies were showed different response to wound healing in young and older rats such as Gidado *et al.*¹¹, Beasley *et al.*¹⁴. The effect of *D. metal* on the normal physiology of rats no significant in present study because of few dose was absorbed in body, while Bello *et al.*²³ and Lee *et al.*²⁵ were showed toxic nephrosis.

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تأثير مقارن لمستخلص الداتورا المائي (*Datura metal*) والجنتاميسين على سرعة التأم الجروح الجلدية في منطقة الفخذ للجرذان المختبرية

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الخلاصة

اجري البحث بهدف دراسة تأثير مستخلص الداتورا المائي على نجاح سرعة التأم الجروح الجلدية لمنطقة الفخذ للجرذان المختبرية الملوثة بجرثومة *Staphylococcus aureus*. أنجزت الدراسة على 28 حيوان (جرذ مختبري) ذوي أعمار متقاربة وناضجة ونفس الأوزان (25 ± 250) وبعمر 8 أشهر، وتحت نفس ظروف المعيشة. ثم قسمت الحيوانات الى ثلاثة مجاميع رئيسية، مجموعة المعالجة الأولى (الداتورا) ومجموعة المعالجة الثانية (الجنتاميسين) تحوي 10 جرذان (5 ذكور++ 5 إناث)، بينما مجموعة السيطرة تحوي (2 ذكر+2 أنثى) جرحت جميع المجاميع الثلاث في منطقة الفخذ. المجموعة الأولى والثانية أصيبت تجريبيا بالجراثيم وجرعة تخفيف 10^{-8} ، وبعد 48 ساعة عولجت المجموعة الأولى بمستخلص الداتورا المائي والمجموعة الثانية بالجنتاميسين 5% وزن/وزن، بالإضافة الى مجموعة السيطرة الصورية. أما مجموعة السيطرة فقسمت إلى مجموعتين ثانويتين، المجموعة الأولى بقي الجرح نظيف ومعقم لمنع التلوث وشفيت تلقائيا وبدون علاج، أما المجموعة الأخرى أصيبت بالجراثيم وتركزت بدون علاج (هلكت). أظهرت النتائج سرعة شفاء الجروح المعاملة بالداتورا مقارنة بالجروح المعاملة بالجنتاميسين. لم تظهر الصور النسجية المرضية تغيرات تدميرية للنسيج نتيجة لقلة امتصاص مستخلص الداتورا. التحليل الاحصائي لعينات الدم انجز باستخدام البرنامج الاحصائي الجاهز SPSS 16.0، هناك فرق معنوي بين مجموعة الداتورا وبقية المجاميع، بينما مجموعة الجنتاميسين لم تظهر فرقا معنويا مع مجموعة السيطرة القياسية واطهرت فرقا معنويا مع مجموعة السيطرة الصورية $p < 0,05$.