Antipyretic, anti-inflammatory and antibacterial effects of *Datura innoxia* leave hydroalcoholic extract in rabbits

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Summary

Datura innoxia was tested as a proposed formula at concentration of 3% that evaluated in-vivo by using 12 rabbits divided equally into 3 groups (plant extract ointment, Tetracycline ointment, Vaseline vehicle). By inducing four abraded skin wound on the back of each group of rabbits by using three special scalpel device, two of the wounds were infected artificially with 250×10^8 cfu pathogenic *Staphylococcus aureus* while other two wounds were not infected. Two abraded wounds were treated topically once daily and for two weeks with 3% *D.innoxia* ointment or 3% tetracycline ointment and one abraded wound was left not treated and the other one was applied with Vaseline as control. Antipyretic effect was also evaluated by taking rectal temperature each 2 days. The result showed the superiority of the extract formula in the therapy of induced skin infection with *S.aureus* over that of tetracycline ointment at same concentration (3%), since it showed better healing activity, more anti-inflammatory and also better antipyretic effects than tetracycline and control groups. This must be due to its good pharmaceutical content that posses analgesic, anti-inflammatory, antipyretic as well as antibacterial effect than tetracycline that contain only antibacterial activity.

Keywords: Antipyretic, Anti-inflammatory, Antibacterial, Ointment.

Introduction

One of the most important medicinal plants is *D.innoxia* mill (thorn apple). It is an annual herb that belongs to the family Solanaceae (1). *D.innoxia* Mill. (Solanaceae) is native to central and South America and introduced in Asia, Africa, Australia and Europe (2). The family was of great economic importance as a source of drug in medicine and pharmacology but can be poisonous if taken in excess (3). This medicinal plant is considered as a major source of tropane alkaloids and used as a commercial source of hyoscyamine and scopolamine (2). Being anticholinergic agents, they were used in veterinary and human Medicine as antispasmodics, preoperative medication and sedative (4). The other phytochemicals that documented to be present in the plant like phenols, flavonoids, saponins, tannins, glycosides and resins, they reported to have analgesic, anti-inflammatory, antibacterial activity and antipyretic activities (5-7). The present study aimed to evaluate anti-inflammatory, antibacterial and antipyretic effects of *D.innoxia* leaves hydroalcoholic extract in rabbits and evaluating the prepared *D.innoxia* extract ointment as possible skin therapeutic drug.

Materials and Methods

Fresh *D.innoxia* leaves were collected from agricultural field in the College of Agriculture/University of Baghdad, and authenticated by Ministry of Agriculture/ State Board for Seeds Testing and Certification (S.B.S.T.C) in Abu Graib /Baghdad. The collected leaves were washed under tap water 3 times then dried under shade in room temperature then powdered by electrical grinder. Hydroalcoholic extract 30% was carried out according to (8) in which 50 grams of leaves powder was put in the flask, then upto1000 ml of 30% ethanol was added and mixed by using magnetic stirrer apparatus at 40°C for 48hours then filtered by gauze and extra filtrated by whatman paper and Millipore paper (0.5mm). The filterate dried up by using incubator at 40 °C then weighed and kept stored in airtight container till used.

Plant extract was formulated as ointment according to w/w concentration ratio of 3% of the extract and other constituent. Preparation of formula was done under sterilized condition in UV cabinet with sterilized materials. Plant extract ointment at concentration 3% was evaluated by dissolving the extract ointment by DMSO (dimethyl sulphoxide) and testing

its minimum inhibitory concentration (MIC) according to tube dilution test for the concentrations (32, 16, 8, 4, 2, 1, 0.5 and 0.25) mg/ml, and at concentrations (256, 128, 64, 32, 16, 8, 4 and 2 µg/ml) for Tetracycline, and sensitivity test against *S.aureus* according to agar well diffusion test (9 and 10). Also stability of the formulae were examined morphologically and by measuring MIC at different storage environment periods and temperature at (15-40 °C) after nine months.

Preparation of Standardized Bacterial Suspension: Serial ten dilution tubes were prepared by adding 9 ml of prepared pepton water in each tube for dilution, then brain heart infusion agar slant cultured with loopful pathogenic *S.aureus* and incubated 18 hours at 37 °C, after incubation period the slant was washed with 5 ml of brain heart infusion broth to obtain standard bacterial suspension. After that 1 ml of bacterial suspension was taken and put it in first tube that containing 9 ml of pepton water so the dilution of bacteria in the first tube was 1/10, then the first tube was shaked well and 1 ml from it was taken and added to the second tube also containing 9 ml of pepton water and the dilution of bacteria in the second tube was 1/100, this process of dilution was applied in all remaining tubes. Then the nutrient agar was prepared and ten petridishs were prepared and marked with a number represented the same number on tubes. After that 1ml from each diluted tubes were poured in the petridishes to which 10 ml of nutrient agar in each petridish plate was added and shaked gently in a circular motion then these petridishes were left to solidify and then put in incubator 18-24 hours at 37 °C. In the second day counting of bacterial colonies was done by choosing the petridish that contained about 30-300 colonies (11).

The animals selected were 12 rabbits of either sex weighing between 1.5-2 kg, the animals were housed and maintained at standard condition of temperature and ventilation, and each rabbit was kept in individual cage. The animals were fed on special formula feed pellets and given water ad libitum. This work was carried out at approved of Collage of Veterinary Medicine of Baghdad University in accordance with international ethical standard of research of work with laboratory animals.

Anti-inflammatory, antibacterial and antipyretic effects of hydroalcoholic leaves extract in rabbits were tested by using modified design of draize test which is performed according to (12), in which 12 rabbits divided into 3 equal groups each consist of four rabbits by inducing four abraded skin wound by using device of three surgical blades at same diameter ($3cm^2$) and same depth on the back of each 4 animal/group, two of the wound were infected artificially with pathogenic *S.aureus* 250×10^8 cfu (a and c) while other two wounds were not infected (b and d). Bacteria was supplied from zoonotic disease department, and was tested morphologically and biochemical test was done for identification of bacteria. The induced wounds applied topically with 1.5gm of ointment for each treated wound once daily after the appearance of inflammation and skin infection signs after 2 days of abrasion or infection with the following treatment for two weeks according to the following divided groups:

D.innoxia treated group: Areas of (a) infected wound and (b) not infected wound treated with 3% D.I ointment topically, (c area) infected wound without treatment, while (d area) not infected treated with Vaseline as negative control.

Tetracycline treated group: Areas of (a) infected wound and (b) not infected wound treated with 3% tetracycline ointment topically, (c area) infected wound without treatment, while (d area) treated with Vaseline as negative control.

Vaseline vehicle treated group: All four abraded wounds applied with Vaseline in which two infected and two not infected. The treatment continued for two weeks with monitoring of healing process in all groups, recording of all signs of healing on animals daily as positive and negative control.

Antipyretic effect: Body temperatures were measured rectally by putting the rabbits of all groups in restriction box to take rectal temperature by Medical thermometer before and after infection and each two days during treatment.

Statistical analysis was applied by two ways ANOVA with least significant differences (LSD) to compare groups means. Probability level $P<0.05$ was considered statistically significant by using statistical package for social sciences (SPSS).

Results and Discussion

Minimum Inhibitory Concentration result indicate that MIC of plant extract against *S.aureus* growth was 2 mg/ml (Table, 1), while MIC of Tetracycline was 128 µg/ml. The result of antibacterial activity of *D.innoxia* extract showed increased *S.aureus* growth inhibition positively proportional with increase of extract concentration (Table, 2) indicating that plant contains possibly a lot of components with active antibacterial effect.

Table, 1: MIC of *D.innoxia* extract in broth and agar media.

Conc . of *D.innoxia* mg/ml	Bacterial Growth	
	Dilution test	Mannitol salt agar
0.25	Very opaque brown color	Highly growth
0.5	Opaque brown color	Moderate growth
1	Brown with slight opacity	Mild growth
2	Transparent brown color	No growth
4	Transparent brown color	No growth
8	Transparent brown color	No growth
16	Transparent brown color	No growth
32	Transparent brown color	No growth

Table, 2: Antibacterial activity of *D.innoxia* extract at different concentrations on growth of *S.aureus* (agar well diffusion).

Concentration mg/ml	*Staph.aureus* (inhibition zone-mm)
2	8.5
4	12.3
8	13.6
16	16.9
24	18.6
32	20.5

Stability and antibacterial activity of formula: No change in stability over nine monthes storage at different room temperature (20-40 °C) since no clear change in color (yellowish to brown color) or MIC that remeasured at the end of storage period and give nearly same MIC as that of beginning use of formula 2 mg/ml.

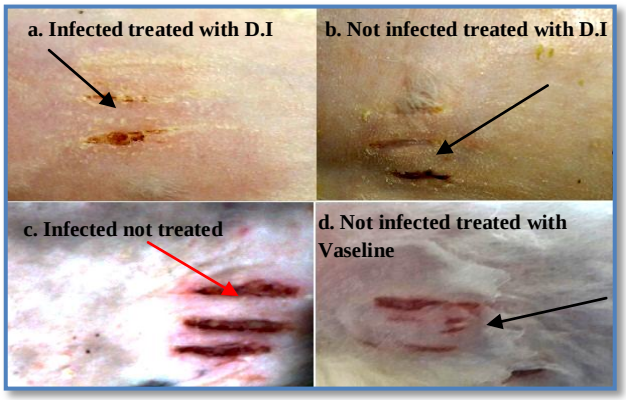
Plant formulae therapeutic effect: Infection was developed on wounds infected experimentally with pathogenic *Staph.aureus* and inflammatory signs appeared which manifested by (erythema, mild edema and pus formation) after 2 days of application of bacterial pathogenic *S.aureus* swab at growth conc. of 250×10^8 cfu/ml on skin wounds with elevation of body temperature in all infected rabbits ranged from (41.6- 41.8 °C). Sings of healing activity of *D.innoxia* extract ointment at 3% during time course of treatment were listed (Table, 3 and Fig. 1). The result of treatment by D.I extract ointment at conc. 3% showed that *Datura* ointment group was more potent and faster healing with better anti-inflammatory, antibacterial and antipyretic effects than group of tetracycline, and in comparison with control group applied with Vaseline vehicle which showed lesser healing signs during treatment time course. The healing activity of our study in *D.innoxia* group required 15 days in their treated abraded wounds in comparison with Tetracycline group that required 17 days in treated abraded wounds.

In the second group treated with Tetracycline ointment 3%, the results showed that tetracycline had healing effects but less than that of *D.innoxia* extract 3% (Fig. 2). While the group treated with Vaseline vehicle, the result showed that the healing effect of area b and d (not infected treated with Vaseline) were similar to area (d) of *D.innoxia* or tetracycline treated group, while wound area (a and c) that infected and treated with Vaseline vehicle the inflammatory reaction was still obvious with wound scar formation and highly redness of skin (Fig. 3). One of major process involved in the early stages of healing was related to the inflammatory response (13). The wound-healing property of *D.innoxia* leaves extract ointment may be attributed to the phytoconstituents present in the plant and the faster process of wound healing could be a function of this phytoconstituents. Flavonoids may be one of the phytoconstituents which contribute with healing process, (14) reported in his study that phytochemical constituents like flavonoids were known to promote wound healing process mainly due to their astringent and

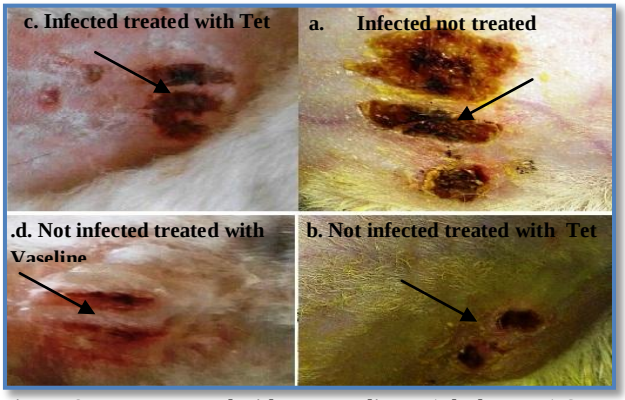
antimicrobial properties, which appeared to be responsible for wound contraction and increased rate of epithelization and healing of bacterial infection. Also tannins were astringent and have antimicrobial property and it is one of phytoconstituent found in *D.innoxia* leave extract hence it can be referred that the wound healing activity of the leaf extract of *D.innoxia* observed in wounds (a and b) were due partly to its tannins and flavonoids contents, this is in agreement with (15) who reported in his study that tannins were responsible for wound contraction and increased rate of epithelization. Flavonoids one of the phytoconstituent found in our D.I leaves extract, and thought it may contribute as antibacterial effects as recorded in different studies that flavonoids possess antibacterial activity (16 and 17) also it reported that flavonoids have direct antibacterial activity, synergistic activity with antibiotics, and the ability to suppress bacterial virulence factors in numerous in vitro and a limited number of in vivo studies. Another phytochemical which may exert its activity as antibacterial is tannins which have been reported by (18) to be bacteriostatic or bactericidal against *S.aureus*. Saponins is one of the component that have antibacterial effects that was found in our D.I leaves extract, it may exhibited its activity by different antibacterial mechanisms, as reported by (19) who mentioned that the antibacterial effect of saponin, appear to involve memberanolytic properties, rather simply altering the surface tension of extra cellular medium, thus being influenced by microbial population density. On other hands (20) reported that saponins can penetrate the pathogenic proteins molecules leading to degradation the pathogenic invade.

Table, 3: Healing sings after treat. with D.I extract on different period

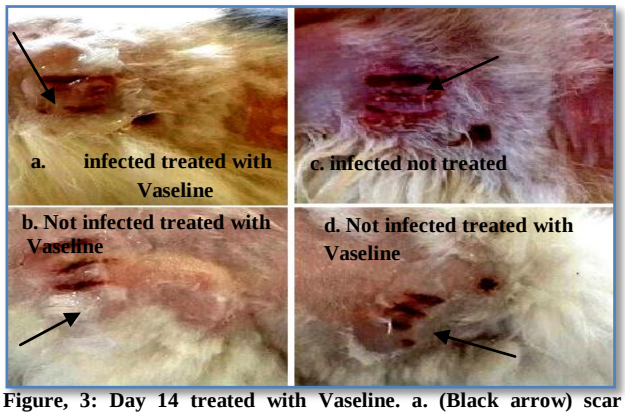
Days / Treatment	Day 3	Day 7	Day 9	Day 14
a. Abraded infected treated D.I	Beginning Scar formation with redness of skin	Shrinkage of wound and fewer redness of wound	The scar begin to elevate from skin wound and begin to detached	Wound healing was progressed and detachment of scar form wound
b. Abraded not infected treated D.I	Beginning Scar formation	Shrinkage of wound with presence of scar in all wound size	The scar elevated from the wound and began to detached without harmless of skin	Wound healing was progressed and detachment of scar form wound
c. Abraded infected not treated	Highly redness of wound without scar formation, pus formation	Different redness of skin wound with some pustulation	Redness of wound without pustulation	Scar formation with redness of wound
d. Abraded not infected treated Vaseline	No scar with Redness of wound	Redness of skin wound without pustulation	Scar begin to formed with some redness	Scar formation appeared on wound



Figure, 1: Healing at day 14 treated with D.I extract ointment. Day 14 treated with D.I extract ointment at 3%. a. (Black arrow) nearly complete healing detached scar without signs of inflammation. b. (Black arrow) complete healing detached scar. c. (Red arrow) Redness with scar formation. d. (Black arrow) Scar formation without redness sings.



Figure, 2: Day 14 treated with Tetracycline a. (Black arrow) Scar formation with redness of wound. b. (Black arrow) Detached scar without inflammation. c. (Black arrow) Scar formation with redness of wound. b. (Black arrow) Scar with redness of wound.



Figure, 3: Day 14 treated with Vaseline. a. (Black arrow) scar formation with highly inflammatory reaction. b. (Black arrow) scar formation with slight redness of skin. c. (Black arrow) highly inflammatory reaction with scar formation. d. (Black arrow) scar formation with slight redness of skin.

Antipyretic effect: The change in body temperature after treatment in all groups was listed in (Table, 4).

Table, 4: Body temperature (°C) of artificilly infected rabbit in different periods.

Groups / Days	*Datura.innoxia* 3% M ± S.E	Tetracyclin 3% M ± S.E	Vaseline M ± S.E
Before infection	38.1 ± 1.9 C a	37.6 ± 1.4 C a	37.8 ± 1.5 C a
Zero time (at infection)	41.4 ± 3.1 A a	41.6 ± 3.7 A a	41.7 ± 3.9 A a
3 days	40.1 ± 2.1 A b	40.4 ± 2.8 B b	41.4 ± 3.1 A a
7 days	39.3 ± 1.5 B a	40.1 ± 2.2 B a	40.0 ± 2.1 B a
9 days	39.1 ± 1.7 B a	39.5 ± 1.7 B a	40.1 ± 2.3 B a
14 days	38.0 ± 1.6 C b	38.2 ± 1.2 C b	40.5 ± 2.4 B a

***Different capital letters denote significant differences (P<0.05) within groups. Different small letters denote significant differences (P<0.05) between groups. L.S.D. = 0.9, N= 4.**

The result of antipyretic effect listed in (Table, 4) showed significant reduction (P<0.05) in body temperature in group treated with *D.innoxia* extract ointment 3%, and *D.innoxia* treated group appeared it's superiority in reducing of body temperature in all treatment days when compared with tetracycline which showed antipyretic effect less than *D.innoxia* treated group and control Vaseline treated group which showed no antipyretic effects in all days of treatment. An inflammatory response may be associated with elevation of body temperature and this symptom of elevation of body temperature appeared on rabbits due to inflammatory process after making abraded infected and not infected wounds on the skin. Suppression of elevating body temperature in extract treating group might be due to the combination of antibacterial, anti-inflammatory and antipyretic effects of the *D.innoxia* leave extract due to its phytochemical contents while tetracycline only posses antibacterial activity. This present results suggested that the plant possibly has some influence on prostaglandins (PGs) biosynthesis because PGs are believed to be regulators of body temperature (21).

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التأثيرات الخافضة للحرارة والمضادة للالتهاب والمضادة للجراثيم لأوراق نبات الداتورا أنوكسيا في الأرانب

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الخلاصة

اختُبر نبات الداتورا انوكسيا كتركيبة مرهم بتركيز 3% والتي قُيمّت على الجسم باستعمال 12 أرنب قسمت على ثلاث مجاميع متساوية (مرهم المستخلص النباتي، مرهم التتراسايكلين وتركيبة الفازلين) وذلك عن طريق إحداث أربعة جروح لكل أرنب بوساطة المشرط على ظهر الحيوانات المستعملة في التجربة باستعمال أداة خاصة ثلاثية المشرط، اثنان من الجروح أصيبت تجريبيا بواسطة المكورات العنقودية الممرضة بجرعة 250×10^8 وحدة تكوين مستعمرة، أما الجرحان المتبقيان فلم تُصَب. عولج اثنان من الجروح موضعياً مرة واحدة يوميا ولمدة أسبوعين بمرهم المستخلص النباتي وبتركيز3% أو بمرهم التتراسايكلين وبتركيز 3% أيضا وقد ترك أحد الجروح بدون علاج أما الجرح المتآكل الرابع فقد عولج بالفازلين واعتبر جرح سيطرة. وكذلك قُيّم التأثير الخافض للحرارة من أخذ درجات الحرارة بمقياس طبّي كل يومين عن طريق المستقيم. أظهرت النتائج تفوق تركيبة المستخلص النباتي في علاج الإصابات الجلدية المستحدثة بوساطة المكورات العنقودية عما أظهرته نتائج مرهم التتراسايكلين وبنفس التركيز 3%. وذلك من إظهار نشاط أفضل لالتئام الجروح وأكثر كمضاد للالتهاب وكخافض للحرارة أفضل من مجموعة التتراسايكلين ومجموعة السيطرة. من المحتمل أن تكون بسبب محتواها الصيدلاني الجيد والتي تمتلك تأثيرات مسكنة ومضادة للالتهاب وخافضة للحرارة وكذلك تأثيرات مضادة للجراثيم أكثر من التتراسايكلين الذي يحتوي على نشاط مضاد جرثومي فقط.

الكلمات المفتاحية: مضاد الحرارة، مضاد للالتهاب، مضاد للجراثيم، مرهم.