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# EFFECTS OF ANTIPERSPIRANTS ON MAMMALIAN SKIN: GROSS AND HISTOLOGICAL

Anatomy

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## ABSTRACT

Worldwide the use of chemicals is a wide spread practice for enhancing and improving lifestyle. Along with the benefits of these products there is also the potential for adverse effects to the people and the environment. The most consistently used components since the commercial introduction of antiperspirants in 1903 are the aluminium salts, Aluminium Nitrate and Aluminium Chloride are the active ingredient in antiperspirants. The antiperspirants containing Aluminium nitrate and Aluminium chloride even in low dose concentration cause well defined histological damage to the skin and its appendages and the change almost revert back to normal after stoppage of application.

## KEYWORDS:

epidermis, dermis, hypertrophy, keratin,

## INTRODUCTION

The most consistently used components since the commercial introduction of antiperspirants in 1903 are the aluminium salts.[1]

Aluminium nitrate is a chemical compound. It is a salt of aluminium and nitric acid. Aluminum nitrate nonahydrate is used in the manufacture of incandescent filaments leather, antiperspirants, corrosion inhibitors, extraction of uranium, and as a nitrating agent.[2 3]

Aluminium chloride is used as a catalyst in the process of Friedel Crafts. It is a salt of aluminium and chloride. It is widely used in the manufacturing of many petrochemicals, in the manufacturing pharmaceuticals, dyes intermediates and other organic chemicals, in the production of rubber; lubricants and wood preservatives, and in cosmetics as an astringent; active ingredient in antiperspirants.

Worldwide the use of chemicals is a wide spread practice for enhancing and improving lifestyle. Along with the benefits of these products there is also the potential for adverse effects to the people and the environment.[4] Health concern has been raised with the daily transdermal exposure over long periods of time of metal containing compounds in personal care products .[5–7]

Consumers are introduced and used in various cosmetics and personal care products without full disclosure on its package labeling. The average person is misled to believe that such product ingredients have been adequately tested and are safe for public usage. Ironically, this is far from accurate.[8] Moreover, male and female individuals, from infancy to old age utilize a huge multivariate variety of shampoos, body soaps, antiperspirants and skin creams preparations, some with no known peer knowledge or concern of the preparation's hazardous potential.

The aim of research work is to study the gross and histological effects of aluminium nitrate and aluminium chloride on the skin of mammal, the albino rabbit.

## 2. Materials and methods

The present study was carried out on thirty six inbred adult albino rabbits. The numbers of animals used and procedures to minimize the suffering of the animals are in accordance with ethical committee on animal experiments of Government medical college Jammu. IEC no. pharma/2012/2818. The rabbits were procured from the Central Animal House of Government Medical College, Jammu. Animals were housed in with dust free rice husk as bedding material under laboratory conditions with controlled environment of temperature of 25±2°C , humidity (16% ± 10%) and 12 hours light/dark cycle ( 16- 18) as per committee for the purpose of control and supervision of experiment on animals (CPCSEA), Indian guidelines. They were provided standard feed and water ad libitum, before subjecting them for experimentation, animals were given a week's time to acclimatize with laboratory conditions.

The animals were divided into following three main groups (A, B and C) as follows:

Group A: Experimental group - 12 animals
Group B: Experimental group - 12 animals
Group C: Control group - 12 animals

These groups were further subdivided into group A1, A2, B1, B2, C1 and C2 containing 6 animals each.

Group A1 received 10% aluminium nitrate.
Group A2 received 20% aluminium nitrate.
Group B1 received 10% aluminium chloride.
Group B2 received 20% aluminium chloride.
Group C received distilled water.

Group C1 animals were taken as control for group A1 and B1 animals.
Group C2 animals were taken as control for group A2 and B2 animals.

About 2cm× 2cm area of skin on posterior surface of each ear of each rabbit was used as test areas. The test areas were shaved 24 hours prior to application of drug. Powdered form (98% purity) of aluminium nitrate and aluminium chloride was used. Solutions were prepared in distilled water at 10% and 20% concentrations daily before application.

0.5ml of solution was applied daily with the help of a clean glass rod to the test areas on experimental animals. After application, each experimental animal was kept in individual cages until the applied solution dried up at ordinary room temperature and atmosphere. Procedure was repeated daily for next fifteen days whereas only distilled water was applied to the control groups. On day sixteenth of the treatment right sided test areas of all groups were first examined using a hand lens. Then after proper local anesthesia with 1ml of 2% xylocaine subcutaneously, $1cm^2$ of the test areas were excised under proper aseptic conditions. The wounds left were properly cared for with povidine iodine till complete healing. Povidine iodine was applied twice a day locally on the wound. Left sided test areas were kept without treatment for next 30 days, on 46th day these test areas were first grossly examined using a hand lens and then $1cm^2$ of the test areas were excised after proper local anesthesia again using 1ml of 2% xylocaine subcutaneously and wounds were taken care of with povidine iodine applied locally twice a day till they healed completely.

The macroscopic or gross changes of the skin were seen with the help of hand lens while the histological changes were observed after the tissues were chemically fixed in neutral buffered formalin solution and later processed. Five-micron thick sections were sectioned and stained with Harris's Haematoxylin and eosin stain.

**Observations:-**

**In control groups (with the application of distilled water) immediate and delayed changes : -**

The skin of control subgroup animals on gross examination was silvery white in colour, smooth with moderate hairs. The epidermis of the control subgroup animals is 2-3 layered thick. Basal layer cells were cubical and upper layer cells were oval in shape. Cells of all the layers were closely packed. Dermis showed dense irregular arrangement of collagen fibers along with presence of sweat and sebaceous glands and same features are seen after one month of stoppage of distilled water (Fig1 and 2) (Table 1 and 2).

**In experimental groups with low and high (10% and 20%) concentration of aluminium nitrate and aluminium chloride after 15 days of treatment:-**

Skin colour changed to grayish brown, and surface became rough with slight hairs with low concentrations (Table 1). In addition moderate crusting and ulcers were also seen with 20% of aluminium nitrate and aluminium chloride (Table 1) Hyperplasia of epidermis along with hypertrophy of stratum corneum and intercellular oedema in epidermis was observed (fig. no. 3, 5, 9 and 11). Skin treated with 20% aluminium nitrate showed marked no. of cysts filled with keratin along with epidermal erosions (fig no. 5,6,and 7 ) (Table 3 and 4) .

Dermal oedema, widening of dermal papillae as well as vascular iz ation in dermis was marked with aluminium nitrate 20% as compared to that with aluminium chloride 20%. There was decrease in number of hair follicles, sweat glands and sebaceous glands but increase in number of inflammatory cells and fibroblasts was observed (Table 5 and 6).

**In experimental groups with low and high concentration (10% and 20%) of aluminium nitrate and aluminium chloride after one month of stoppage of treatment:-**

Skin colour remained grayish brown, but surface became smooth with moderate hairs (Table 2). Slight hyperplasia of epidermis and slight hypertrophy of stratum corneum persisted (Table 3 and 4). Dermis showed slight oedema. Hair follicles, sweat glands and sebaceous glands showed slight increase in number with decrease in number of fibroblasts and inflammatory cells(fig no. 4,8,10 and12 ) (Table 5 and 6).

**Discussion**

The aim of the present study was to evaluate if there are any changes in the skin after use of antiperspirants. The results of present study compares favorably with previous studies. The present study showed that the skin on gross examination becomes rough with fine crusts and colour changes to grayish brown following application of 10 % aluminium nitrate for 15 days and supports the findings of **Lansdown, Nasir *et al.*[9][10]**

**Lansdown[9]** observed erythema, thickening and scaling after 5 days of treatment with 10% aluminium chloride on skin of post scapular region of rabbit and mouse. The gross changes in the present study after 15 days of treatment with 10% aluminium chloride were fine crusting and brownish discoloration of the skin. The changes seen on gross examination with 20% aluminium chloride after 15 days of treatment were rough grayish brown coloured skin, ulceration and fine crusts whereas the findings put forth by **Lansdown[9]** with 25% aluminium chloride after 2 days of treatment on mice were severe erythema, thickening and scaling.

The present study showed grayish colour of the skin with fine crusts and hairs on gross examination after one month of stoppage of treatment with 10% aluminium nitrate and 20% aluminium nitrate and confirm the findings of **Nasir *et al.*[10]**

In addition to the hyperplasia of epidermis as observed by Nasir et al., with 10% aluminium nitrate for 15 days, present study also revealed hypertrophy of stratum corneum along with intercellular oedema and formation of keratin cysts in epidermis, as revealed by study of Lansdown.[9]

In the present study the left ear skin of albino rabbit showed slight epidermal hyperplasia after one month of stoppage of treatment with aluminium nitrate 10% correlating with the findings **Nasir *et al.*[10]**

The epidermis after treatment with 20% aluminium nitrate for 15 days showed marked hyperplasia of epidermis along with marked hypertrophy of stratum corneum, some foci of erosion of epidermis with intercellular oedema and cysts filled with keratin as compared to the findings of **Nasir *et al.*[10],** which were hyperkeratosis that is hypertrophy of stratum corneum and acanthosis that is hyperplasia of epidermis. After one month of stoppage of treatment with aluminium nitrate 20% epidermis showed slight hypertrophy of stratum corneum and slight hyperplasia of the epidermis, these findings are fully consistent with the findings of the **Nasir *et al.*[10]**

Findings in epidermis with 10% aluminium chloride are consistent with the findings of the **Lansdown[9], Nasir *et al.*[10]** After one month of stoppage of treatment with 10% aluminium chloride the epidermal changes reverted back to normal skin in the present study. Similar results have also been reported by **Nasir *et al.*[11]**

The epidermis showed marked hypertrophy of stratum corneum and hyperplasia of epidermis along with marked intercellular oedema and keratin cysts in the present study after 15 days of treatment with 20% aluminium chloride. These findings are almost in accordance with the findings of **Lansdown[9], Nasir *et al.*[11]**

After one month of stoppage of treatment with aluminium chloride 20% the changes reverted back to normal with almost complete reversion. Similar changes were also demonstrated by **Nasir *et al.*[11]**

In the present study the dermis showed slight oedema and vascularization, few hair follicles and fibroblasts along with inflammatory cells, rare sweat and sebaceous glands after 15 days of treatment with 10% aluminium chloride whereas dermis showed moderate oedema, vascularization, inflammatory cells, slight fibroblasts and rare sweat and sebaceous glands after 15 days of treatment with 20% aluminium chloride. In addition dermal papillae were elongated and widened. After one month of stoppage of treatment with 10% aluminium chloride and 20% aluminium chloride findings were moderate hair follicles, however sweat and sebaceous glands, vascularization and fibroblast were slight. But there is no literature available to compare these findings.

With 10% aluminium nitrate treatment for 15 days the findings in the dermis were slight oedema, vascularization, inflammatory cells, fibroblasts and hair follicles whereas sweat and sebaceous glands were rarely seen. The findings of the present study with 20% aluminium nitrate treatment for 15 days were marked oedema and vascularization, moderate inflammatory cells and fibroblast, slight hair follicles. Sweat and sebaceous glands were rarely present. These findings are fully consistent with the findings of **Nasir *et al.*[10]**

After one month of stoppage of treatment with 10% and 20% aluminium nitrate, except slight oedema, the changes in the dermis reverted back to normal. These observations coincide with the findings of **Nasir *et al.*[10]**

**Conclusion: -** The detailed study comparing effects of aluminium nitrate and aluminium chloride (the antiperspirants) was conducted and the observations were noted, critically analyzed and discussed with the findings of previous workers and hence it was concluded that maintenance of a healthy life style is crucial in daily well being. The antiperspirants containing aluminium nitrate and aluminium chloride even in low dose concentrations, may still cause well defined histological damage to the skin and its appendages, especially after a prolong time period of continuous application but the changes almost reverted back to normal after one month of stoppage of application. And if the duration of application is further continued permanent histological changes are eminent.

**Table-1: Gross changes in the mammalian skin after 15 days of treatment with aluminium nitrate and aluminium chloride as compared to control group.**

| TREATMENT | COLOUR | SURFACE | CRUSTING | SCALING | ULCER | HAIRS |
|---|---|---|---|---|---|---|
| **Aluminium Nitrate 10%** | Grayish brown | Rough | Slight | Nil | Nil | Slight |
| **Aluminium Nitrate 20%** | Grayish brown | Rough | Moderate | Nil | Moderate | Slight |
| **Control** | Silvery white | Smooth | Nil | Very Fine | Nil | Moderate |
| **Aluminium Chloride 10%** | **Grayish brown** | **Rough** | **Slight** | **Nil** | **Nil** | **Slight** |
| **Aluminium Chloride 20%** | Grayish brown | Rough | Moderate | Nil | Moderate | Slight |
| **Control** | Silvery white | Smooth | Nil | Very Fine | Nil | Moderate |

**Table-2: Gross changes in the mammalian skin after one month of stoppage of treatment of aluminium nitrate and aluminium chloride as compared to control group.**

| TREATMENT | COLOUR | SURFACE | CRUSTING | SCALING | ULCER | HAIRS |
|---|---|---|---|---|---|---|
| **Aluminium Nitrate 10%** | Grayish brown | Smooth | Slight | Nil | Nil | Moderate |
| **Aluminium Nitrate 20%** | Grayish brown | Smooth | Slight | Nil | Nil | Slight |
| **Control** | Silvery White | Smooth | Nil | Nil | Nil | Moderate |
| **Aluminium chloride 10%** | **Grayish brown** | | **Smooth** | **Slight** | **Nil** | **Nil** | **Moderate** |
| Aluminium chloride 20% | Grayish brown | | Smooth | Slight | Nil | Nil | Slight |
| Control | Silvery white | | Smooth | Nil | Nil | Nil | Moderate |

**Table-3: Epidermal changes in the skin as treated with aluminium nitrate.**

| | | After 15 Days of Treatment | | After One Month of Stoppage of Treatment | |
|---|---|---|---|---|---|
| **Different Histological Findings** | Control | Aluminium Nitrate 10% | Aluminium Nitrate 20% | Aluminium Nitrate 10% | Aluminium Nitrate 20% |
| **Hypertrophy of Stratum Corneum** | Nil | Moderate | Marked | Rare | Slight |
| **Hyperplasia of Epidermis** | Nil | Slight | Marked | Slight | Slight |
| **Intercellular Oedema** | Nil | Moderate | Marked | Nil | Nil |
| **Cysts filled with Keratin** | Nil | Moderate | Marked | Nil | Nil |
| **Erosion of Epidermis** | Nil | Nil | Present | Nil | Nil |

**Table-4: Epidermal changes in the skin as treated with Aluminium Chloride**

| | Control | After 15 Days of Treatment | | After One Month of Stoppage of Treatment | |
|---|---|---|---|---|---|
| **Different Histological Findings** | | Aluminium Chloride 10% | Aluminium Chloride 20% | Aluminium Chloride 10% | Aluminium Chloride 20% |
| **Hypertrophy of Stratum Corneum** | Nil | Moderate | Marked | Slight | Slight |
| **Hyperplasia of Epidermis** | Nil | Moderate | Marked | Nil | Nil |
| **Intercellular Oedema** | Nil | Moderate | Marked | Nil | Nil |
| **Cysts filled with Keratin** | Nil | Slight | Slight | Nil | Nil |
| **Erosion of Epidermis** | Nil | Nil | Nil | Nil | Nil |

**Table-5: Dermal changes in the skin treated with aluminium nitrate**

| | | After 15 Days of Treatment | | After One Month of Stoppage of Treatment | |
|---|---|---|---|---|---|
| **Changes** | **Control** | **Aluminium Nitrate 10%** | **Aluminium Nitrate 20%** | **Aluminium Nitrate 10%** | **Aluminium Nitrate 20%** |
| **Oedema** | Nil | Slight | Marked | Slight | Slight |
| **Vascularization** | Nil | Slight | Marked | Slight | Slight |
| **Inflammatory cells** | Nil | Slight | Moderate | Rare | Rare |
| **Fibroblasts** | Slight | Slight | Moderate | Slight | Slight |
| **Hair follicles** | Moderate | Slight | Slight | Moderate | Moderate |
| **Sebaceous glands** | Slight | Rare | Rare | Slight | Slight |
| **Sweat glands** | Slight | Rare | Rare | Slight | Slight |

**Table-6: Dermal changes in the skin treated with Aluminium Chloride.**

| Changes | | After 15 Days of Treatment | | After One Month of Stopping of Treatment | |
|---|---|---|---|---|---|
| | **Control** | **Aluminium Chloride 10%** | **Aluminium Chloride 20%** | **Aluminium Chloride 10%** | **Aluminium Chloride 20%** |
| **Oedema** | Nil | Slight | Moderate | Rare | Rare |
| **Vascularization** | Nil | Slight | Moderate | Slight | Slight |
| **Inflammatory cells** | Nil | Slight | Moderate | Rare | Rare |
| **Fibroblasts** | Slight | Slight | Moderate | Slight | Slight |
| **Hair follicles** | Moderate | Slight | Slight | Moderate | Moderate |
| **Sebaceous glands** | Slight | Rare | Rare | Slight | Slight |
| **Sweat glands** | Slight | Rare | Rare | Slight | Slight |

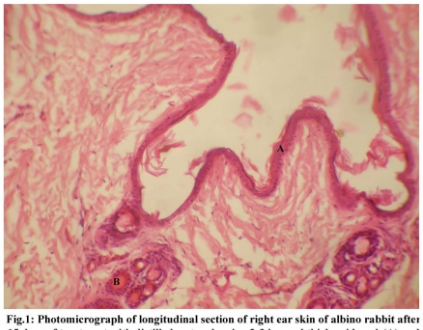


**Fig.1: Photomicrograph of longitudinal section of right ear skin of albino rabbit after 15 days of treatment with distilled water showing 2-3 layered thick epidermis(A) and sweat glands(B).**

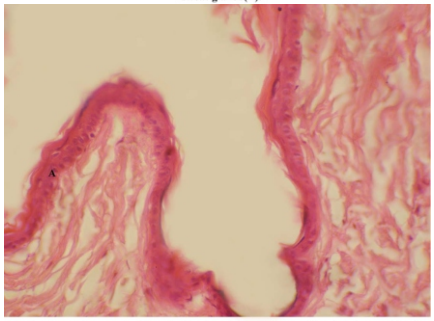


**Fig. 2: Photomicrograph of longitudinal section of left ear skin of albino rabbit after one month of stoppage of treatment with distilled water showing 2-3 layered thick epidermis(A).**

**(H&E Stain 400X**

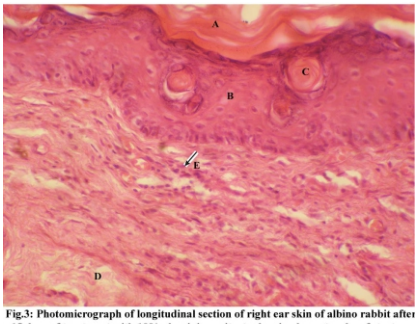


Fig.3: Photomicrograph of longitudinal section of right ear skin of albino rabbit after 15 days of treatment with 10% aluminium nitrate showing hypertrophy of stratum corneum(A), hyperplasia of epidermis(B), keratin cyst(C), dermal oedema(D) and inflammatory cell(E).

(H & E Stain 400X)

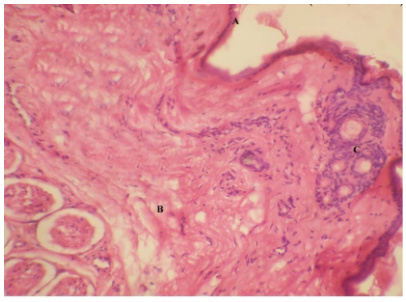


Fig.4: Photomicrograph of longitudinal section of left ear skin of albino rabbit after one month of stoppage of treatment with 10% aluminium nitrate showing normal thickness epidermis(A), slight dermal oedema(B) and sweat glands(C).

(H &E Stain 200X)

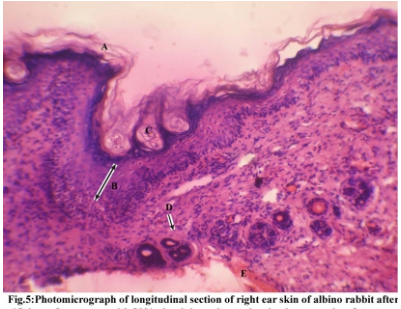


Fig.5:Photomicrograph of longitudinal section of right ear skin of albino rabbit after 15 days of treatment with 20% aluminium nitrate showing hypertrophy of stratum corneum(A), hyperplasia of epidermis(B), keratin cyst(C), periappendageal inflammation (D) and vascularization(E).

(H &E Stain 200X

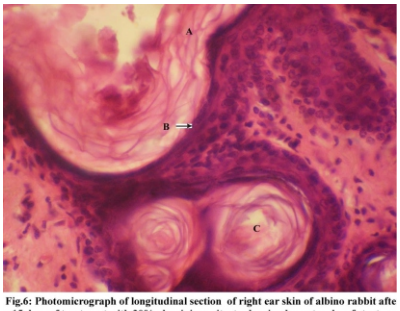


Fig.6: Photomicrograph of longitudinal section of right ear skin of albino rabbit after 15 days of treatment with 20% aluminium nitrate showing hypertrophy of stratum corneum(A), prominent granular layer(B) and keratin cyst(C).

(H&E Stain 400X

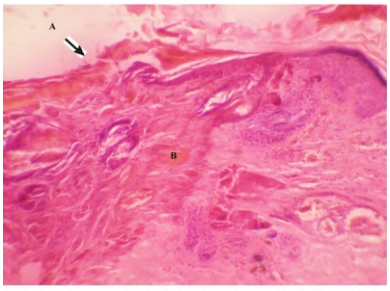


Fig.7: Photomicrograph of longitudinal section of right ear skin of albino rabbit after 15 days of treatment with 20% aluminium nitrate showing epidermal erosion(A) and vascularization(B).

(H&E Stain 100X)

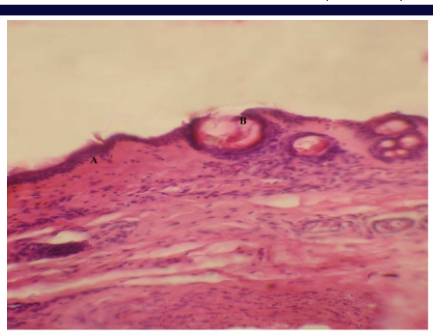


Fig.8: Photomicrograph of longitudinal section of left ear skin of albino rabbit after one month of stoppage of treatment with 20% aluminium nitrate showing slight epidermal hyperplasia(A) and keratin cyst(B).

(H&E Stain 100X)

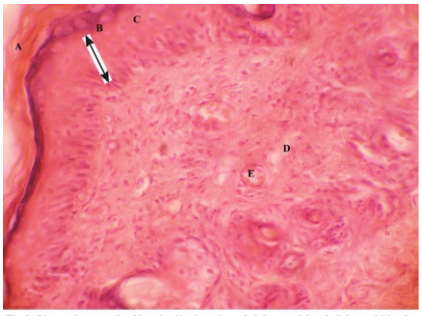


Fig.9: Photomicrograph of longitudinal section of right ear skin of albino rabbit after 15 days of treatment with 10% aluminium chloride showing hypertrophy of stratum corneum(A), epidermal hyperplasia(B), intercellular oedema in epidermis(C), dermal oedema(D) and vascularization (E).

(H&E Stain 200X

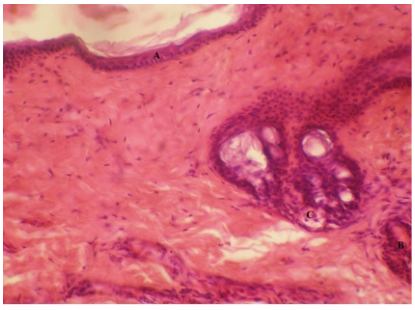


Fig.10: Photomicrograph of longitudinal section of left ear skin of albino rabbit after one month of stoppage of treatment with 10% aluminium chloride showing normal thickness epidermis(A), sweat gland(B) and sebaceous gland(C).

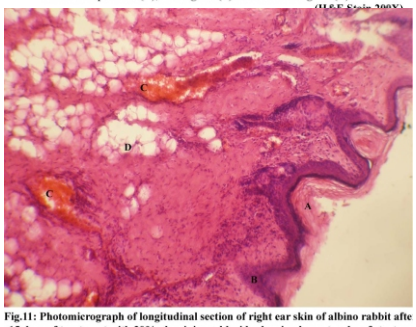


Fig.11: Photomicrograph of longitudinal section of right ear skin of albino rabbit after 15 days of treatment with 20% aluminium chloride showing hypertrophy of stratum corneum(A), epidermal hyperplasia(B), vascularization in dermis(C) and dermal oedema(D).

(H&E Stain 200X

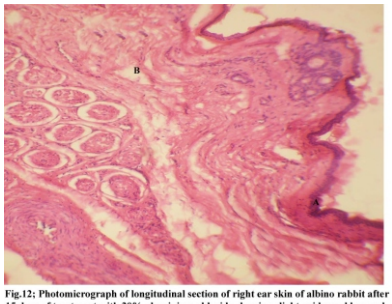


Fig.12; Photomicrograph of longitudinal section of right ear skin of albino rabbit after 15 days of treatment with 20% aluminium chloride showing slight epidermal hyperpl--asia(A) and dermal oedema(B).

(H&E Stain 100X)

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