

## A study to Assess the Effects of Antiperspirant Aluminium Nitrate on the Rabbit Skin

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### Abstract

This present study was aimed to observe under the research grade compound light microscope the histo-toxicological effects manifested post-application of 10% and 20% solutions of aluminium nitrate that was topically applied onto the outermost surface of the ear integument of 24 Albino rabbits treated daily. The animals were then divided into two groups comprising of 12 experimental and 12 controls. Initially right sided test areas were excised of the experimental and control animals after 15 days of application to observe immediate effects, whereas the left sided test areas of the animals were kept for another month without any further treatment and then excised on day 45 to elucidate its delayed effects. The results revealed histological features of a thickened epidermis with hyperkeratosis, acanthosis and the presence of epidermal micro-abscesses. The dermis showed features of edema, increase in vascularity, decrease in sweat and sebaceous glands and infiltration of inflammatory cells. It was significantly noted that the histological features were far more severely manifested in the integument applied with 20% w/v solution as compared to that applied with 10% w/v solution. However these changes persisted in a moderate form following discontinuation of aluminium nitrate application.

### Keywords

Aluminium nitrate, Toxicity, Bromhidrosis, Nonahydrate

### Introduction

Skin is a structurally and physiologically specialized boundary between the body and its environment. It forms about 8% of the total body mass and has an area between 1.2 - 2.2 m<sup>2</sup>.(1,2)

Body odor or bromhidrosis is the smell of bacteria that multiply rapidly on skin in the presence of sweat but sweat itself is completely odorless.(3) It becomes problematic when there is excessive odor or it is unpleasant. Therefore, interest has centered on chemicals which might produce anhidrosis.

The most consistently used components since the commercial introduction of antiperspirants in 1903 are the aluminium salts.(4) Electrolytes in the sweat react with aluminium complexes to form a gel plug in the duct of the sweat gland which prevent the gland from

excreting liquid and plugs are removed with time by the natural sloughing of the skin.(5) Aluminium salts also have a slight astringent effect on the pores; causing them to contract, further preventing sweat from reaching the surface of the skin.(6)

Aluminum is third most abundant element, constitutes approximately 8% of the earth's crust, and is ubiquitous in soil, water and air. Aluminum is very important in world economy.

Aluminium nitrate is a salt of aluminium and nitric acid and is a strong oxidizing agent. It is used in the preparation of insulating papers, on transformer core laminates and in cathode-ray tube heating elements; as a tanning agent, antiperspirant, nitrating agent, corrosion inhibitor, catalyst in petroleum refining and in uranium extraction; and in

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the manufacture of incandescent filaments leather, antiperspirants, corrosion inhibitors, extraction of uranium, and as a nitrating agent.(7,8)

According to U.S Food and Drug Administration, antiperspirants, affect odor as well as prevent sweating by affecting sweat glands.(9) The antiperspirants are the daily household products used globally by both the genders. Approximately about 90% of U.S population regularly uses antiperspirants. But along the benefits, these products have the potential for adverse effects on the people and the environment.(10) Some health concerns have been raised over the transdermal exposure for long periods of time of metal containing compounds in personal care products.(11,12,13) These metallic compounds do have some toxic effects on skin resulting into thickening, hardening, hypersensitivity and hyperpigmentation of the skin.(14)

Therefore the present study was conducted as an advancement of knowledge and as an evidence-base study of the toxicological effects of aluminium nitrate especially on the mammalian skin vis to vis in regulating complications manifested by exploitation by cosmetics producers.

**Material and Methods**

After taking clearance from the institutional ethical committee vide IEC number Pharma/2012/2818, present study was conducted in Postgraduate Department of Anatomy, Government Medical College, Jammu. This study was carried out on 24 inbred adult albino rabbits which were procured from the Central Animal House of Government Medical College, Jammu. The animals were divided into two main groups - Group A: Experimental

group - 12 animals Group B: Control - 12 animals. These groups were further subdivided into group A1, A2, B1 and B2 containing 6 animals each. Group B1 animals were taken as control for group A1 animals and Group B2 animals were taken as control for group A2 animals. A humane approach was kept towards the rabbits throughout the study and they were group housed in the rabbit section of the animal house {in accordance to the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines for laboratory animal facility}.(15) A standard feed and water ad libitum and 12 hour light-dark cycle was provided.

About 2 cm × 2 cm test area of skin on posterior surface of each ear of each rabbit was shaved 24 hours prior to application of drug. Using powdered form (98% purity) of aluminium nitrate and distilled water 10% and 20% solutions were prepared daily before application. For 15 consecutive days, Group A1 received 10% aluminium nitrate and Group A2 received 20% aluminium nitrate while Group B1 and B2 both received distilled water. 0.5ml of solution was applied daily with the help of a clean glass rod to the test areas. After application, each animal was kept in individual cages until the applied solution dried up. On 16th day, 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<sup>2</sup> of the test areas were excised under proper aseptic conditions. Till complete healing povidone 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<sup>2</sup> of

*Table 1. Gross changes in the mammalian skin after treatment with aluminium nitrate*

Gross findings	After 15 Days of Treatment			After One Month of Stoppage of Treatment		
	CONTROL	ALUMINIUM NITRATE 10%	ALUMINIUM NITRATE 20%	CONTROL	ALUMINIUM NITRATE 10%	ALUMINIUM NITRATE 20%
Color	Silvery white	Grayish brown	Grayish brown	Silvery white	Grayish Brown	Grayish Brown
Surface	Smooth	Rough	Rough	Smooth	Smooth	Smooth
Crusting	Nil	Slight	Moderate	Nil	Slight	Slight
Scaling	Very fine	Nil	Nil	Nil	Nil	Nil
Ulcer	Nil	Nil	Moderate	Nil	Nil	Nil
Hairs	Moderate	Slight	Slight	Moderate	Moderate	Slight

the test areas were excised after proper local anesthesia again using 1ml of 2% xylocaine subcutaneously and wounds were taken care of with povidone iodine applied locally twice a day till they healed completely. The histological changes were observed after the tissues were chemically fixed in neutral buffered formalin solution and later processed. 5 $\mu$  thick sections were sectioned and stained with Harris's haematoxylin and eosin stain.

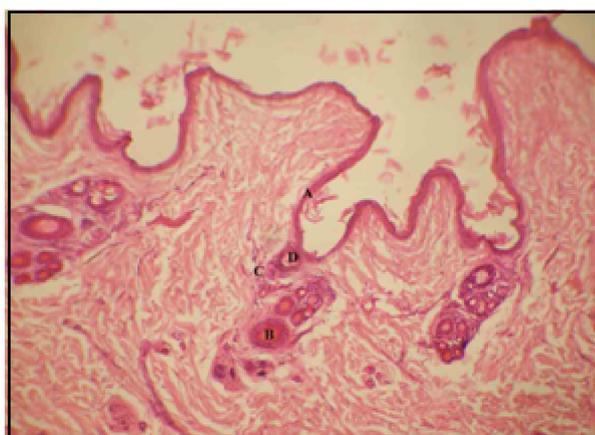
**Results**

After 15 days of treatment with 10% aluminium nitrate, skin appeared grayish brown in colour and the surface was rough. On light microscopic examination, diffuse epidermal hyperplasia in epidermis and slight oedema in dermis was observed. With 20% aluminium nitrate treatment for 15 days, similar but more marked results were seen. (Table 1, 2 and 3)

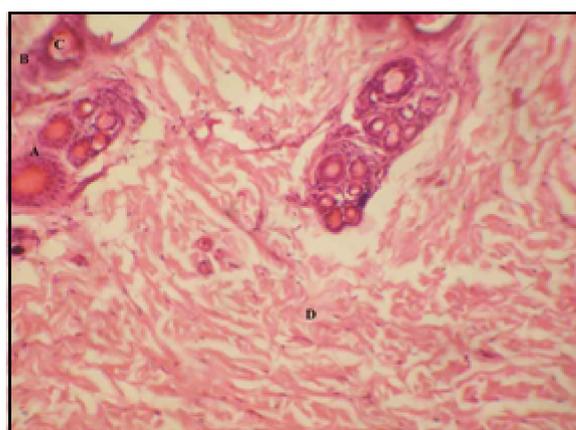
After 1 month of stopping the treatment, all the changes (gross and histopathological) seemed to revert back in both the groups (A1 and A2).

**Table 2. Epidermal changes in the skin treated with aluminium nitrate**

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



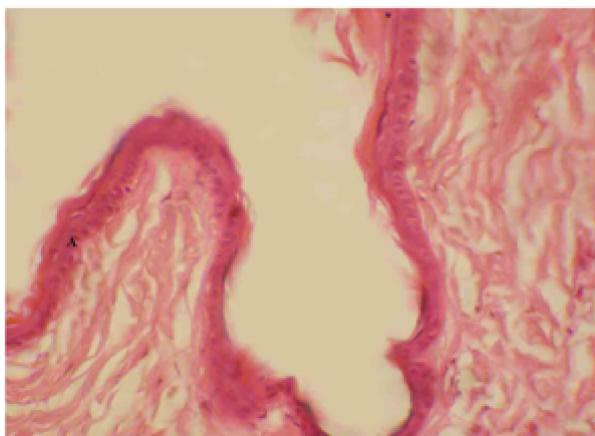
**Fig 1. Photomicrograph of longitudinal section of right ear skin of Albino rabbit after 15 days of treatment with distilled water showing epidermis (A), sweat glands (B), sebaceous glands (C) and hair follicle (D). (H & E Stain 200X)**



**Fig 2. Photomicrograph of longitudinal section of right ear skin of Albino rabbit after 15 days of treatment with distilled water showing sweat glands (A), sebaceous glands (B), hair follicle (C)**

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

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



**Fig 3. Photomicrograph of longitudinal section of left ear skin of Albino rabbit after 1 month of stoppage of treatment with distilled water showing 2-3 layered thick epidermis (A). (H & E Stain 400X)**

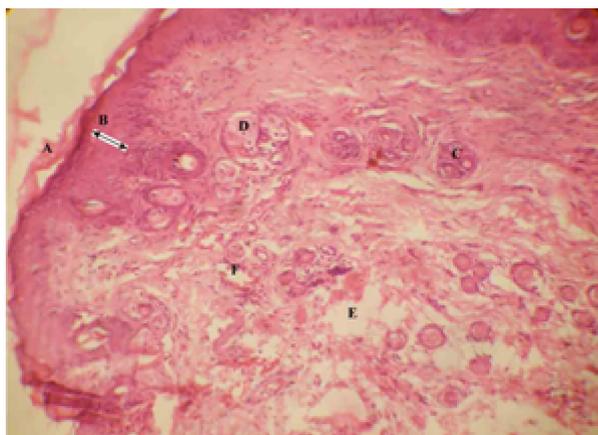
**Discussion**

Aluminium salts are widely used commercially in cosmetics and other medicinal preparations due to their antiperspirant and astringent properties. These salts irritate the mammalian skin immediately once applied however the intensity of their irritancy varies according to the concentration used in antiperspirant preparations. Commercially, aluminum nitrate is available as the form

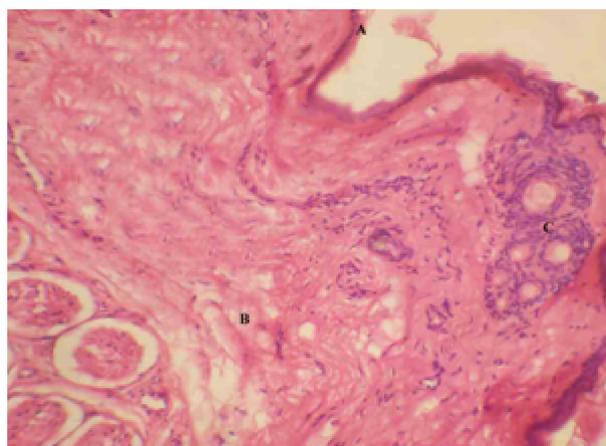


**Fig 4. Photomicrograph of longitudinal section of left ear skin of Albino rabbit after 1 month of stoppage of treatment with distilled water showing 2-3 layered thick epidermis (A), sweat glands (B), sebaceous glands (C) and hair follicle (D). (H & E Stain 400X)**

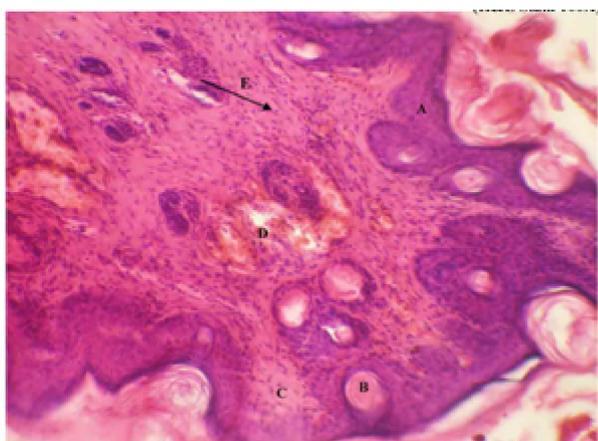
of nonahydrate and is more irritating as compared to chloride, chlorhydrate and citrate salts.(7,8) Aluminium based antiperspirants work by occluding the sweat ducts, thereby reducing the amount of sweat that is able to reach the skin's outer surface. The production of epidermal damage is due to the ability of the aluminium cations to denature epidermal keratin and the action of



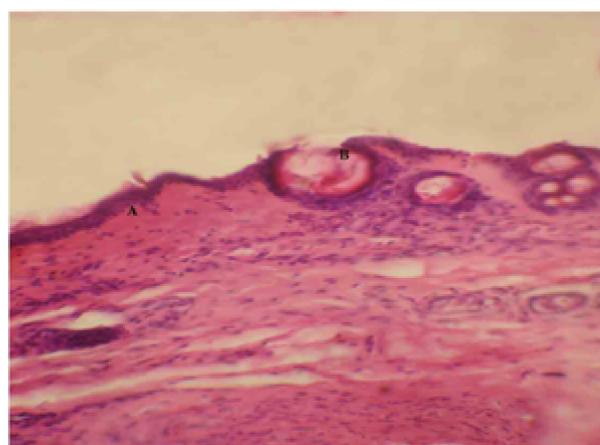
**Fig 5. Photomicrograph of longitudinal section of right ear skin of Albino rabbit after 15 days of treatment with 10% aluminium nitrate showing hypertrophy of stratum corneum (A), hyperplasia of epidermis (B), sweat glands (C), sebaceous glands (D), dermal oedema (E) and vascularisation (F). (H & E Stain 200X)**



**Fig 6. Photomicrograph of longitudinal section of left ear skin of Albino rabbit after 1 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 7. Photomicrograph of longitudinal section of right ear skin of Albino rabbit after 15 days of treatment with 20% aluminium nitrate showing hyperplasia of epidermis (A), keratin cyst (B), widening of dermal papillae (C), vascularisation (D) and inflammatory cells (E). (H & E Stain 200X)**



**Fig.8 Photomicrograph of longitudinal section of left ear skin of Albino rabbit after 1 month of stoppage of treatment with 20% aluminium nitrate showing slight epidermal hyperplasia (A) and keratin cyst (B). (H & E Stain 100X)**

free mineral acid formed by hydrolysis of compound in water.

In our study, we observed that the skin on gross examination becomes rough with fine crusts and color changes to grayish brown following application of 10% and 20% aluminium nitrate for 15 days. Similar results were reported by Lansdown in 1973 in a study conducted on mouse and rabbit skin after treatment with 10% aluminium nitrate for five consecutive days on post scapular region.<sup>16</sup> These gross findings in the skin were

also reported by Nasir et al in 2008 after 15 days of treatment with 10% aluminium nitrate.<sup>(17)</sup>

Present study showed that after treatment with 10% and 20% aluminium nitrate for 15 days there was hyperplasia of epidermis along with marked hypertrophy of stratum corneum, some foci of erosion of epidermis with intercellular oedema and cysts filled with keratin. Nasir et al (2008) also observed hyperplasia of epidermis after 15 days treatment with 10% aluminium nitrate on albino rabbit skin and Lansdown (1973) made similar observations.<sup>(16,17)</sup>

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 were fully consistent with the findings of Nasir *et al* (17)

In our study, after one month of stoppage of treatment with 10% and 20% aluminium nitrate, findings were

grayish color of skin with fine crusts and hairs; slight epidermal hyperplasia and in the dermis, all the changes reverted back to normal except slight oedema. Nasir *et al* observed similar findings after 1 month of stoppage of treatment.(17)

This study concludes that antiperspirants containing aluminium nitrate even in low dose concentrations, may still cause well defined histological damage to the skin and its appendages. These changes revert back if use is discontinued but more serious and permanent damage is expected if these aluminium salt containing antiperspirants are used over prolonged period of time.

### References

1. Montagna W. The Structure and function of skin 2nd edition. New York, London: Academic Press; 1968. Cited by Gray's Anatomy 37th edition: p. pp. 70-1.
2. Jakubovic HR and Ackerman AB. Structure and function of skin. Development, morphology and physiology. In: Moschella SL and Hurley HJ (eds) Dermatology 2nd edition Philadelphia: Saunders. 1985; 1: .pp.1-74.
3. Williams J, Timothy B and Dirk E. Andrews' Diseases of the skin. Clinical Dermatology.2005; 10<sup>th</sup> edition: 1-12.
4. Laden K. Antiperspirants and deodorants: history of major HBA market. In: Laden K (ed): Antiperspirants and deodorants. New York: Marcel Dekker.1999; .pp.1- 15.
5. Rados and Carol. "Antiperspirant Awareness: It's Mostly No Sweat." FDA consumer Magazine Jul-Aug 2005. Food and Drug Administration. 4 Jan 2008.
6. Draelos ZD."Antiperspirantsandthe hyperhidrosis patient". *Dermatol Ther*.2001; 14 (3): 220-24.
7. Grams GW. Aluminum compounds: aluminum halides and aluminum nitrate: aluminum nitrate. In: Kirk-Othmer encyclopedia of chemical technology John Wiley and Sons 4th ed.1992; 2: .pp.289.
8. Budavari S. The Merck index: an encyclopedia of chemicals, drugs, and biologicals.12<sup>th</sup> ed.1996 Merck and Co., Inc.,
9. Food and Drug Administration (U.S). Cosmetics Q&A:"Personal Care Products".
10. Globally Harmonized System of Classification and Labelling of Chemicals. Purpose, Scope and Application of the Globally Harmonized System of Classification and labeling of Chemicals. 2009; 3.
11. Hostynek JJ, Hinz RS, Lorence CR, Price M, Guy RH. Metals and the skin. *Crit Rev Toxicol*. 1993; 23: 171-235.
12. Exley C. Does antiperspirant use increases the risk of aluminum-related disease, including Alzheimer's disease? *Mol Med Today Mr*.1998; 107-9.
13. Drabre PD. Hypothesis. Underarm cosmetics are a cause of breast cancer. *Eur J Cancer Prev*.2001; 10: 389-93.
14. Abreo V. "The Dangers of Aluminum Toxicity"<http://www.bellaonline.com/articles/art7739.asp>. Retried 2009-05-05.
15. CPCSEA guidelines for laboratory animal facility. *Indian Journal of Pharmacology* 2003; 35: 257-274.
16. Lansdown ABG. Production of epidermal damage in mammalian skins by some simple aluminium compounds. *British Journal of Dermatolog*.1973; 89: 67-76.
17. Nasir A, Rabail NA. A Gross Anatomical and Histological Inspection of the mammalian integument post-treated with aluminium nitrate. *Annals of Microscopy*.2008; 8: 04-13.