Original Article

Pomegranate Protects Minocycline Induced Epidermal Pigmentation in the Extremities of Guinea Pigs

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ABSTRACT

Objective: To investigate the protective effects of Pomegranate on Minocycline induced epidermal pigmentation on the extremities of guinea pigs.

Study Design: An experimental observational study.

Place and Duration of Study: This study was conducted at the Anatomy Department, BMSI, J PMC, Karachi.

Materials and Methods: 60 adult guinea pigs were divided into 3 groups A B and C, A served as control, B given Minocycline, while C given Minocycline with Pomegranate for 8 weeks, after which their skin was processed for histological examination and pigmentation was observed in Masson Fontana stained sections under light microscope.

Results: The melanin pigmentation deposition observed in Minocycline treated group B, distributed densely and extended till stratum corneum as compared to the control group A, while in the Pomegranate treated group C along with Minocycline, the melanin pigmentation was considerably reduced and was observed to be distributed sparsely extended till stratum spinosum.

Conclusion: Based on the present study it is conducted that pigmentary changes induced by Minocycline can be protected by taking pomegranate.

Keywords: Pomegranate, Minocycline, Epidermal Pigmentation, Guinea pigs.

INTRODUCTION

Skin pigmentary abnormalities are seen as aesthetically unfavorable and have led to the development of cosmetic and therapeutic treatment modalities of varying efficacy[1]. Unwanted cutaneous hyperpigmentation can also produce a significant psychological stress[2]. Drug induced pigmentary alteration are quite common[3]. A variety of drugs have been reported to induce hyperpigmentation[4]. Regarding this, minocycline, a synthetic broad spectrum antimicrobial tetracycline whose common application in the treatment of pneumonia, acne infections of skin genital and urinary systems[5] and rheumatoid arthritis[6] has been associated with cutaneous hyperpigmentation with its prolonged use[7,8].

Hyperpigmentary disorders are often treated with hydroquinones, retiniods and tyrosinase inhibitors[9]. Increasing consumer interest in skin care and treatment products derived from natural sources has driven increased research into novel skin depigmentating agents[10].

Pomegranate (Punica granatum, Punicaceae) a traditional Chinese medicine[11] has been extensively used in the folk medicine of many cultures[12-14]. Some previous studies cited by Moawad et al[15] had proved that the products of pomegranate tree (Including peels, juice, leaves, seeds, flowers etc) have medicinal and industrial importance. Pomegranate is a rich source of polyphenoic compounds anthocyanins (such as cyanidine, delphinidin) and hydrolysable tannins (such as ellagic acid, gallagic acid), which account for 92% of antioxidant activity of the whole fruit[16]. Its main constituent, ellagic acid, a naturally occurring polyphenol, when applied topically suppresses U-V induced pigmentation in brownish guinea pigs, as it has a high affinity for copper at the active site of tyrosinase enzyme[17-19]. Tyrosinase is the rate limiting enzyme for melanin synthesis[10] which synthesized by melanocytes situated in the basal epidermis and are transferred to the surrounding epidermal keratinocytes.[19,20]The type and amount of melanin synthesis and its distribution pattern in the surrounding keratinocytes determines the actual color of the skin[10]. Therefore, chelating copper at the active site of tyrosinase enzyme inhibits the activity of it[22], which is also being proved by Zho and Goa[23].

In the light of above mentioned studies, this study was conducted to observed the protective effects of pomegranate against the minocycline induced pigmentation in the epidermis and note down the histological changes occurring in this regard.

MATERIALS AND METHODS

This study was conducted in the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, on 60 adult male guinea pigs weighing between 450 – 650 grams, and were divided into 3 main groups A, B and C.

Group A animals were served as control, group B received Minocycline 0.02mg/G body weight/ day orally, and group C received Minocycline 0.02mg/G/day orally along with pomegranate 0.9mg/G body weight/day orally for 8 weeks.

After completion of the experimental period all the guinea pigs were sacrificed under ether anesthesia in a glass container and one skin fragment of two inches size, from both upper and lower limbs and of abdomen were taken from each animal. The fragment from each extremity was fixed in 10% formalin for 24 hours and processed for paraffin embedding, and then paraffin blocks were made in the tissue embedding system. 4 to 5 micron thick sections were cut on rotatory microtome and mounted on albumenized glass slides. After that, they were stained with the Haematoxylin and Eosin and Masson's Fontana stains for histological studies under light microscope and the results were graded and recorded.

RESULTS

In the haematoxylin and eosin stained sections of control group A animals, the epidermis of abdomen and both extremities observed under 40 X showed 3 to 5 layers of cells in addition to stratum corneum and their cytoplasm with nuclei were visualized normal in appearance. The cells in all layers were stained uniformly.

Table No. 1: Distribution of melanin deposition in all epidermal layers indifferent groups

Group	Period 0f Treatment	Treatment Given	Sparse*	Patchy**	Dense***
A Control	8 Weeks	Laboratory diet ad libitum	+		
B Minocycline treated	8 Weeks	0.02mg/G body weight/day(Orally)			+++
C Minocycline + Pomegranate treated	8 Weeks	0.02mg/G body weight/ day + 0.09mg/G body weight/ day(Orally)		++	

[8]Sparse: scatteredly distributed(+),Patchy: patchy distributed,***Dense: uniformly distributed**

Table No. 2: Extension of melanin deposition in all epidermal layers in different groups

Group	Period of Treatment	Treatment Given	Normal*	Grade I**	Grade II***
A Control	8 Weeks	Laboratory diet ad libitum	+		
B Minocycline treated	8 Weeks	0.02mg/G body weight/day(Orally)			+++
C Minocycline + Pomegranate treated	8 Weeks	0.02mg/G body weight/ day + 0.09mg/G body weight/ day(Orally)		++	

***Normal: extended till stratum basale (+) **Grade-I: extended till stratum spinosum (++) ***Grade-II: extended till stratum corneum (+++)**

In the Minocycline treated group B animals, tissues were observed under 40 X and showed all the layers of epidermis of both extremities bearing the same morphology as that of control group A animals, but there is black colored pigment deposition was seen within the cells as well as outside the cells in all the layers of the epidermis.

Meanwhile in the protective group C treated with pomegranate along with Minocycline, the morphology is same as observed in control group A and there is no deposition of any pigment within or outside the cells.

The Masson Fontana stained sections were observed under 40 X. In the control group A animals tissues, the melanocytes were dark black and brown black in color. They were in scattered manner; located in the epidermal basal layer. They were oval to irregular in shape with pale staining cytoplasm. The distribution of melanin pigment deposition was of sparse pattern (+) and extension was of normal grade. (Table 1 and Table 2, Fig 1)

In the Minocycline treated group B animals, the morphology shows no significant changes but the deposition of melanin pigment was of dense pattern (+++) and extension was of grade II. (Table 1 and Table 2, Fig 2).

In the Minocycline and pomegranate treated group C animals, the morphology shows no significant changes as compare to control group A but the deposition of melanin pigment was of Patchy to sparse pattern (++)

and extension was of grade I.(Table 1 and Table 2, Fig 3).

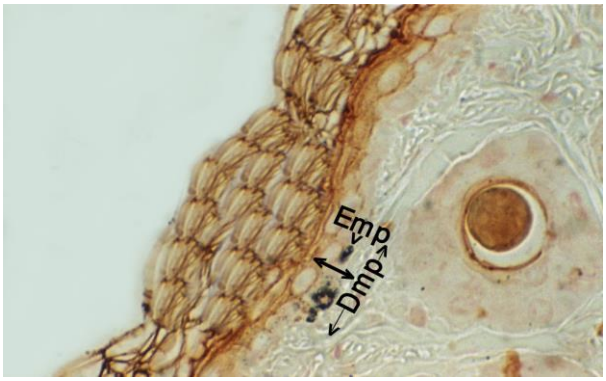

Figure No. 1: Epidermis of group A control animals showing distribution of melanin pigment (Dmp) and extension of melanin pigment (Emp) in Masson Fontana stain, (Photomicrograph 40 X).

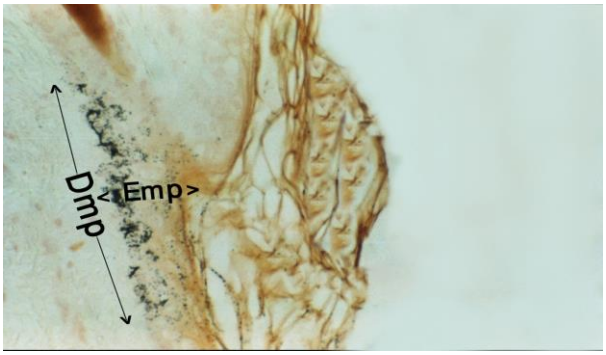

Fig 2: Epidermis of group B Minocycline treated animals showing distribution of melanin pigment (Dmp) and extension of melanin pigment (Emp) in Masson Fontana stain,(Photomicrograph 40 X).

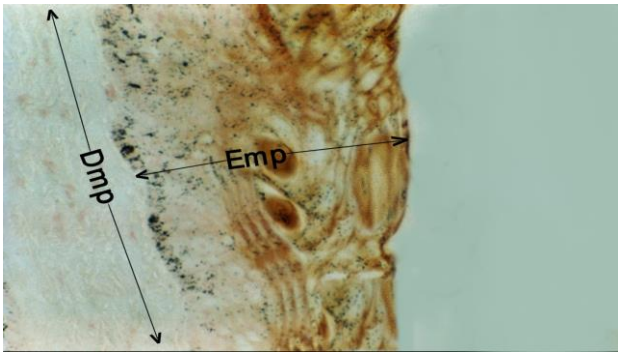

Figure No. 3: Epidermis of group C Minocycline and Pomegranate treated animals showing distribution in melanin pigmentation (Dmp) and extension of melanin pigment (Emp)deposition in Masson Fontana stain.(Photomicrograph 40 X).

DISCUSSION

Pomegranate (punica granatum) has been used extensively in traditional medicines in many countries[12,14,16,17,19]. Its main constituent, ellagic acid, a naturally occurring polyphenol, is reported to have the high affinity for copper at the active site of tyrosinase and inhibits its activity by binding to the copper[17-23]. The tyrosinase is the rate limiting enzyme in the biosynthesis of melanin. Thus inhibiting tyrosinase, inhibit the melanin synthesis proven in various international studies[10,17,18,19,23].

Melanin is synthesized in the melanocyte in which tyrosinase played an important role. As a result of tyrosinase activity, tyrosine is transformed first into 3, 4-dihydrooxyphenylalanine (dopa) and then into dopaquinone, which is converted, after a series of transformations, into melanin. Once formed, melanin within the membrane bounded granules called melanosomes, migrates within the dendrites of melanocytes, and is transferred to the cells of all epidermal layers, whish act as a depot and create the pigmentation of skin[20].

The histological changes in the Minocycline treated group B animals showed densely distributed black brown pigment deposition as compared to the control group A. This black brown pigment is melanin as it is being stained by the Masson's Fontana stain. It would be consider that Minocycline may induce pigmentation as reported by Mounton et al[8] about the mechanism of Minocycline pigmentation which may involve a reactive quinine iminium ion metabolite, iron chelation, or stimulation of melanin production. Burns et al[21] cited Minocycline association with post inflammatory hyperpigmentation in women who have undergone sclerotherapy.The diffuse muddy brown discoloration in the sun exposed areas of skin (type III reaction) induced by Minocycline-stimulated melanocytes that can lead among other things to deposits of melanin or Minocycline melanin complexes at the epidermal basal membrane.[4]

The result of group C Minocycline treated animals along with pomegranate shows that melanin pigment deposition in the epidermal layers had markedly decreased as compare to Minocycline treated group B animals. This is more obvious in the Manson's Fontana stained sections. As the melanin is formed after the conversion of tyrosine into dopa which is the rate limiting step being catalyzed by tyrosinase, therefore, inactivation of tyrosinase leads to the inhibition of melanin formation. Ellagic acid inhibits the melanin synthesis by suppressing tyrosinase enzyme activity as it penetrate into its active site and chelate firmly with copper present there. This had been proven that after the topical application of ellagic acid rich pomegranate extract, the melanin content of skin had been reduced[18]

The result is also in agreement with Kasai et al[19] who reported that the orally administered ellagic acid rich pomegranate extract provides the protective effects on the pigmentation of human skin caused by U-V irradiation.

CONCLUSION

Based on the present study it is conducted that pigmentary changes induced by Minocycline can be protected by taking pomegranate. This suggests that the

result could be considered promising enough in humans who are on Minocycline with pomegranate for long term duration. The present work is under progress for further extended studies.

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