ISSN 1996-3424

Asian Journal of Dermatology

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Asian Journal of Dermatology 4 (2): 16-28, 2012
ISSN 1996-3424 / DOI: 10.3923/ajd.2012.16.28
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Evaluation of Therapeutic Effects of Aloe Vera: Coal Tar Mixture in Psoriasis: An Immunohistochemical Study

[1]Maha A. El-Gayyar, [2]Amany A. El-Hawwary and [3]Nagwa I. Bakre
[1]Department of Dermatology, STDs and Andrology, Mansoura Faculty of Medicine, Mansoura University, 35516, Egypt
[2]Department of Histology and Cell Biology, Mansoura Faculty of Medicine, 35516, Egypt
[3]Department of Dermatology, STDs and Andrology, Mansoura University, Student Hospital, 35516, Egypt

Corresponding Author: Maha A. El-Gayyar, Department of Dermatology, STDs and Andrology, Mansoura Faculty of Medicine, Mansoura University, 35516, Egypt

ABSTRACT

Although the use of aloe vera and coal tar in psoriasis treatment is well established, their mechanism of action is poorly understood. The aim of this study was to evaluate the therapeutic effectiveness of topical mixture of aloe vera extract and 1% coal tar (AMIX) in psoriasis and evaluate its antiproliferative, apoptotic and antiangiogenic effects as possible therapeutic mechanisms. AMIX was used in 279 patients with mild to moderate plaque psoriasis and PASI scores were recorded monthly. Skin biopsies from 30 patients before and after 3 months of therapy and 10 biopsies of normal skin were immunohistochemically stained using Ki67, Bcl-2 and CD34 antibodies as markers of cell proliferation, apoptosis and neoangiosis, respectively. Results: PASI scores decreased from 8.2±1.6 to 1.3±1.1 after 12 weeks of treatment ($p<0.001$) with complete clearance in 145 patients (~52%) without relapse during follow up period. Psoriatic skin showed higher expression of Ki67 in epidermal keratinocytes, Bcl-2 in basal keratinocytes and dermal inflammatory cells and CD34 in the dermal blood vessels compared to normal skin ($p<0.001$, $p<0.01$ and $p<0.001$, respectively). After treatment, Ki67, epidermal and dermal Bcl-2 and CD34 showed decreased expression ($p<0.001$, $p<0.01$, $p>0.05$ and $p<0.001$, respectively). These changes were positively correlated with the decrease in PASI scores. In conclusion, aloe vera and coal tar (AMIX) is an effective topical treatment of psoriasis without noticeable side effects. It causes inhibition of epidermal cell proliferation, neoangiogenesis and induction of apoptosis together with its immune modulatory effects.

Key words: Antiproliferative, apoptotic, antiangiogenic, aloe vera, coal tar, psoriasis, immunohistochemical

INTRODUCTION

Psoriasis is an immune T-cell mediated inflammatory skin disease characterized by epidermal hyperplasia, dermal neoangiogenesis and greatly accelerated epidermal turnover (Krueger, 2002; Kocak *et al.*, 2003). In normal human skin, keratinocytes (Kcs) in the superficial layers of the epidermis undergo apoptosis and regulate proliferation of basal cells. On the other hand, Kc hyperproliferation is a characteristic of psoriatic lesions and could be marked by over expression of growth regulating proteins as Ki67 (Wrone-Smith *et al.*, 1995). Ki67 is expressed in all cell cycle phases reflecting the dynamics of epidermal hyperplasia. Therefore, it is considered as a marker of cell proliferation (Li *et al.*, 2007). Moreover, Kcs derived from psoriatic plaques were shown to

be resistant to apoptosis (Wrone-Smith *et al.*, 1997). Bcl-2 and its homologous proteins, are considered the most important regulators of programmed cell death and play a crucial role in the balance between cell survival and cell death (Haupt *et al.*, 2003). According to current concepts, blockage of normal apoptotic process in the epidermis is one of the factors implicated in the pathogenesis of psoriasis (Kocak *et al.*, 2003). Neoangiogenesis, including the formation of new dermal blood vessels, starts with early psoriatic changes. It increases intralesional microcirculation so as to facilitate the passage of T-lymphocytes to the skin and disappears with disease clearance. Therefore, neoangiogenesis is a key component of the psoriatic process (Heidenreich *et al.*, 2009). The CD34 protein is a transmembrane protein expressed in the endothelial linning of blood vessels. So, it can be used as a marker for neoangiogenesis (Nielsen and McNagny, 2008).

The topical coal tar has been used for the treatment of psoriasis long time ago (Slutsky *et al.*, 2010). It was reported to be an effective keratolytic agent especially when combined with other treatment modalities (Thami and Sarkar, 2002) and even as effective as calcipotriol in treatment of psoriasis (Tzaneva *et al.*, 2003) but so more cheaper.

Aloe vera plant secretes a mucilaginous gel possessing diverse putative pharmacological activities including anti-inflammatory and anti-oxidative effects. It was reported to be effective in various medicinal purposes (Shelton, 1991; Vazquez *et al.*, 1996). Syed *et al.* (1996) used aloe vera extract effectively in the treatment of psoriasis and reported that it had no toxic or objective side effects. In a previous work, we used a mixture of aloe vera extract and coal tar (AMIX) efficiently in the treatment of psoriasis and found it to be effective in its improvement. It has been found to significantly decrease CD3+, CD4+ cellular infiltrate, a finding that documents its immune modulating properties (El-Gayyar *et al.*, 2006). Choonhakarn *et al.* (2010) reported that aloe vera cream was more effective than 0.1% triamcinolone acetonide cream in reducing the clinical symptoms and signs of psoriasis.

The aim of this study was to evaluate the effectiveness of aloe vera and coal tar mixture in the treatment of psoriasis and to investigate its antiproliferative, apoptotic and antiangiogenic effects as possible therapeutic mechanisms.

MATERIALS AND METHODS

This study was carried out on 279 patients with mild to moderate chronic plaque psoriasis attending the outpatient clinics of Dermatology and Venereology Department, Mansoura University Hospital and Mansoura University Student Hospital, Egypt. All patients were subjected to thorough history taking and skin examination. Complete blood picture, renal and hepatic function tests were done for each patient and those with normal results were enrolled in the study. All patients had to discontinue any systemic or topical antipsoriatic medication for at least 8 or 4 weeks prior to therapy, respectively. Therapy was administered as twice daily application of the mixture of aloe vera extract plus 1% coal tar cream for 12 weeks. The Psoriasis Area and Severity Index scoring method (PASI) as described by Saleh *et al.* (2008) was performed at the beginning and monthly during the treatment period. The patients were followed up monthly for another 6 months. Each participant gave an informed written consent to share in the study after the procedure was explained to him.

Full-thickness 6 mm elliptical skin biopsies were obtained from lesional skin in 30 patients before treatment and from the same area after 12 weeks of treatment and 10 control biopsy specimens of normal skin after surgical procedure were used as control. Formalin fixed specimens

were prepared, then 3-5 micron thick sections were deparaffinized and used for immunohistochemical staining for detection of Ki67, Bcl-2 and CD34 proteins. The standard immunoperoxidase avidin-biotin method was used. The following primary antibodies were used: mouse monoclonal antibodies for Ki67 (Dako, Glostrup, Denmark), for Bcl-2 (from Neomarkers, Labvision, Fremont, CA, USA), for CD34 (from Dako, Glostrup, Denmark). Universal ultravision anti-polyvalent, HRO DAB detection kit was used (from Labvision, Fremont, CA, USA). Ki67 was used as a marker for proliferation. Bcl-2 was used as an antiapoptotic marker and CD34 was used as a marker of neoangiogenesis.

Positive cells (showing nuclear staining for Ki67 and cytoplasmic staining for Bcl-2) were counted under light microscope and expressed as number of positive cells per 100 counted cells (%). Grading was done as follows: (-) for negative results, (+1) for less than 10% positivity, (+2): 10-20% positivity and (+3) for >20% positivity for Ki67 and Bcl-2 expression in dermal lymphocytes. For epidermal Bcl-2 expression, grading was: (+1) for less than 10% positivity, (+2): 10-30% positivity and (+3) for >30% positivity. The visualized blood vessels detected by CD34 immune reaction were counted under light microscope at power 400 and graded as follows: (+1) for less than 5 blood vessels, (+2): 5-10 vessels and (+3) for >10 vessels. Ten fields were counted for each biopsy and the median value was considered.

The data were expressed in the form of Mean±SD. Student t-test was used to compare variables between two groups. Pearson's correlation coefficient was used to correlate variables. The $p \leq 0.05$ is considered significant. The tests were run on an IBM compatible personal computer using the Statistical Package for Social Science (SPSS) program version 16 (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinical results: This study included 279 psoriatic patients (160 females and 119 males), the mean age was 28.7±12.6 years (range: 8-50 years). The mean disease duration was 19.6±7.6 years (range: 1-30 years). In areas treated with the mixture, PASI score was significantly lowered from a mean of 8.2±1.6 at the start of treatment to 1.3±1.1 after 12 weeks ($p<0.001$) (Table 1). Nearly half of the patients (145 patients~52%) showed complete improvement without recurrence during the next 6 months of follow up. Apart from staining of clothes, no other side effects were reported during treatment with AMIX.

As for Ki67-immune staining, most cases of normal skin (90%) showed few (+1) positive basal cells in the epidermis. In psoriatic cases, there was higher expression of Ki67 in the basal and suprabasal cells in most cases compared to normal ($p<0.001$). After treatment, there was significant decrease of the number of Ki67 +ve cells compared to pretreatment cases ($p<0.001$) (Table 1, Fig. 1-4).

As for Bcl-2 immune staining, normal skin biopsies showed (+1) weakly positive staining in basal cells in 70% of cases and in dermal inflammatory cells in 30% of cases. In psoriasis, Bcl-2 expression in epidermal cells and dermal inflammatory cells was significantly higher compared to normal ($p<0.01$). After treatment, a significant decrease of Bcl-2 expression occurred in epidermal cells where <10% positive cells was found in 60% of cases ($p<0.01$) while the decrease in dermal inflammatory cells was statistically insignificant ($p>0.05$) (Table 1, Fig. 5-8).

As for CD34-immune staining, most normal skin biopsies showed few (+1) positively stained dermal vessels. In psoriatic cases, many elongated tortuous blood vessels were found compared to

Table 1: The effect of treatment on PASI and immunostaining results for Ki67, Bcl-2 and CD34 among psoriatic cases compared to normal (control) skin

	Control		Pretreatment		Posttreatment		Pretreatment vs. control		Pretreatment vs. posttreatment	
	No.	%	No.	%	No.	%	T1	P1	T2	P2
PASI(n=279)										
Range	-		5.1-10.7		0.8-4.5				T=19.4	p<0.001***
Mean±SD	-		8.2±1.6		1.3±1.1		-	-		
Ki67										
+1 (<10 %)	9.0	90	2.0	6.66	23.0	76.67				
+2 (10-20%)	1.0	10	11.0	36.67	7.0	23.33	12.6	<0.001***	9.1	<0.001***
+3 (>20%)	-	-	17.0	56.67	-	-				
Mean±SD	3.0±1.5		23.3±3.1		6.4±2.5		$r_{1a} = 0.36$	$P_{1a} = 0.023$*	$r_{1b} = 0.42$	$P_{1b} = 0.02$*
Bcl-2: Epidermis										
-ve	1.0	10	4.0	13.33	3.0	10.00	4.2	0.004**	3.6	0.007**
+1 (<10 %)	7.0	70	8.0	26.67	15.0	50.00				
+2 (10-30%)	2.0	20	14.0	46.67	11.0	36.67				
+3 (>30%)	-	-	4.0	13.33	1.0	0.33				
Mean±SD	12.8±1.92		22.8±4.9		18.0±5.3		$r_{2a} = 0.31$	$P_{2a} = 0.046$*	$r_{2b} = 0.36$	$P_{2b} = 0.043$*
Bcl-2: Dermal inflammatory cells										
-ve	7.0	70	4.0	13.33	14.0	46.67				
+ 1 (<10 %)	3.0	30	5.0	16.67	8.0	26.67				
+2 (10-20%)	-	-	10.0	33.33	6.0	20.00	3.7	0.009**	0.45	0.66
+3 (>20%)	-	-	11.0	36.67	2.0	6.67				
Mean±SD	7.3±2.5		29.0±9.5		24.0±12.2		$r_{3a} = 0.35$	$P_{3a} = 0.041$*	$r_{1b} = 0.39$	$P_{3b} = 0.033$*
CD34										
+1 (<5)	9.0	90	-	-	9.0	30.00				
+2 (5-10)	1.0	10	11.0	36.67	18.0	60.00	14.1	<0.001***	8.9	<0.001***
+3 (>10)	-	-	19.0	63.33	3.0	10.00				
Mean±SD	3.4±1.14		15.2±1.48		6.8±1.48		$r_{4a} = 0.29$	$P_{4a} = 0.048$*	$r_{4b} = 0.31$	$P_{4b} = 0.04$*

$r_{1a,b}$: Correlation between PASI and Ki67 expression before and after treatment, $r_{2a,b}$ and $r_{3a,b}$: Correlation between PASI and epidermal and dermal Bcl-2 before and after treatment, $r_{4a,b}$: Correlation between PASI and CD34 before and after treatment

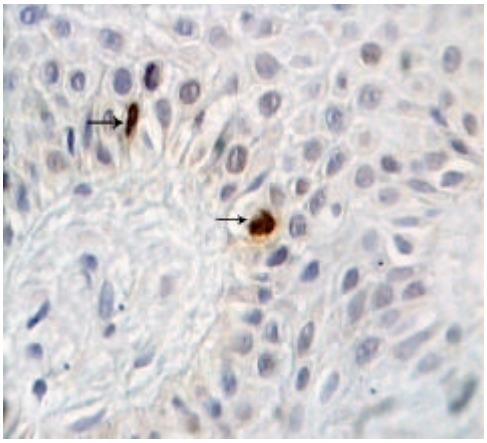

Fig. 1: Few Ki67 +ve basal keratinocytes (+1) in normal (control) skin (x400)

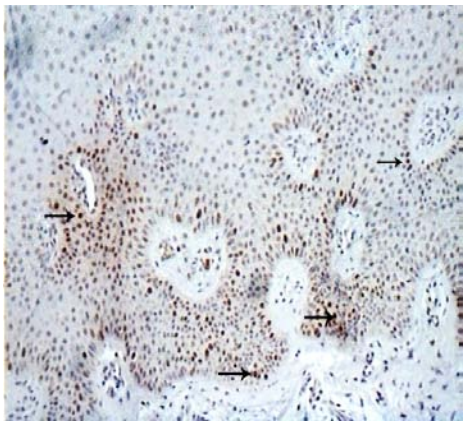

Fig. 2: Many Ki67 +ve basal and suprabasal keratinocytes (+3) in a psoriatic case before therapy (x100)

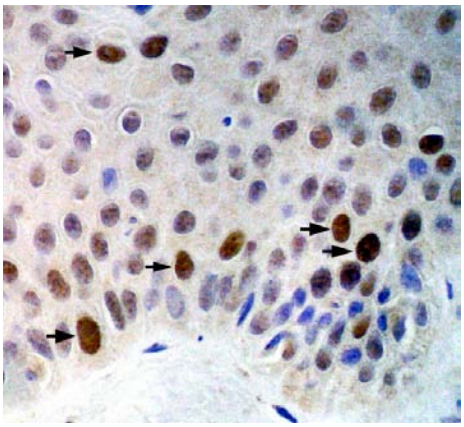

Fig. 3: Another pretreatment psoriatic case with many basal and suprabasal Ki67 +ve cells (+3) (x400)

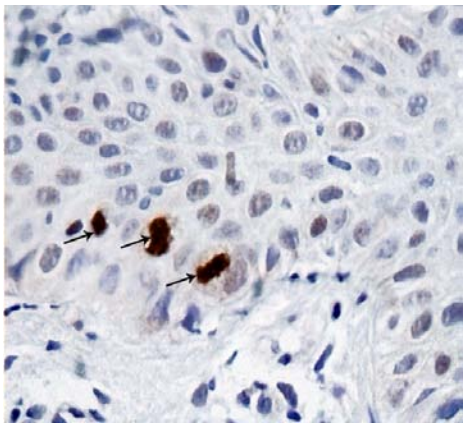

Fig. 4: Few Ki67 +ve basal cells (+1) in a psoriatic case after treatment (x400)

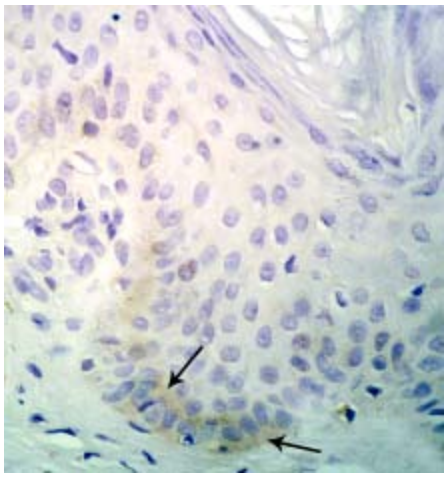

Fig. 5: Weak Bcl-2 cytoplasmic reaction (+1) in some basal cells of normal (control) skin (x400)

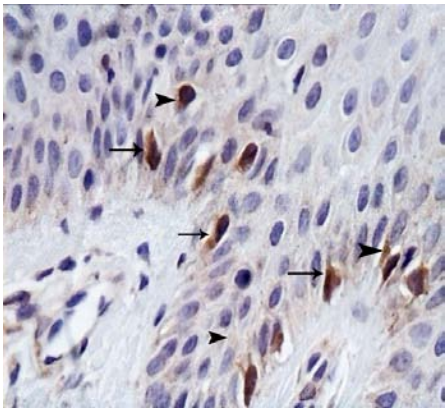

Fig. 6: Bcl-2 + ve reaction (weak: arrow heads, strong: arrows) in basal keratinocytes (+2) of a psoriatic case (x400)

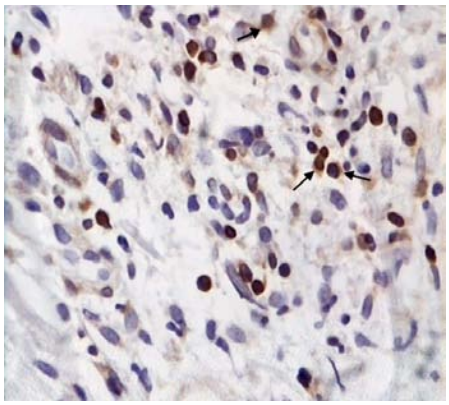

Fig. 7: Another psoriatic case with Bcl-2 +ve reaction in dermal lymphocytes (+3) (x400)

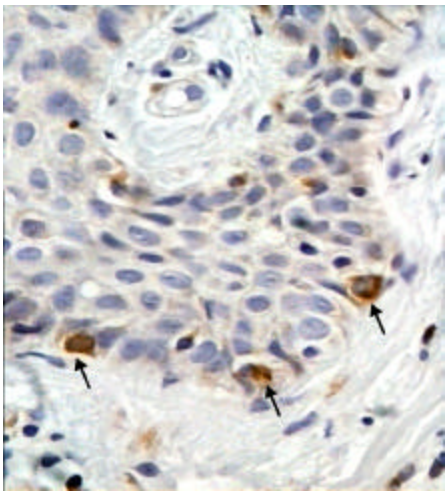

Fig. 8: Few Bcl-2 +ve basal keratinocytes (+1) in a psoriatic case after treatment (x400)

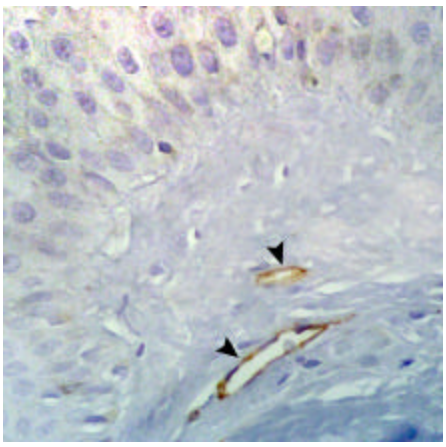

Fig. 9: Few weakly CD34 +ve dermal blood vessels (+1) in normal (control) skin (x400)

normal ($p<0.001$) and were significantly decreased after treatment ($p<0.001$) (Table 1, Fig. 9-11). The changes in Ki67, Bcl-2 and CD34 were positively correlated with PASI scores before and after treatment ($p<0.05$).

Figure 12 demonstrates the mean values ±SD of % of positive cells for Ki67, epidermal and dermal Bcl-2 with the maximum values seen in psoriasis before treatment (23.3±3.1, 22.8±4.9 and 29±9.5, respectively) and minimum values observed in the control (3±1.53±1.5, 12.8±1.92 and 7.3±2.5, respectively).

Figure 13 demonstrates the mean values ±SD of CD34 positive blood vessels with the maximum value seen in psoriasis before treatment (15.2±1.48) and minimum value detected in the control (3.4±1.14). Figure 13 also demonstrates the difference in PASI mean values before and after AMIX treatment (8.2±1.6 and 1.3±1.1, respectively).

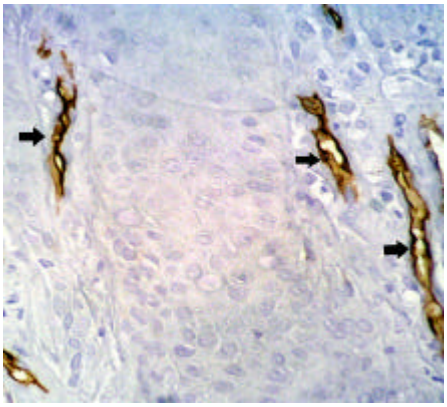

Fig. 10: Many strongly CD34 +ve elongated tortuous blood vessels in the dermal papillae (+2) of a psoriatic case before treatment (x400)

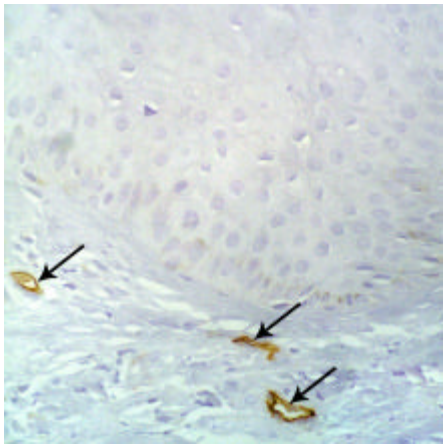

Fig. 11: Few CD34 +ve dermal blood vessels (+1) in a post treatment psoriatic case (x400)

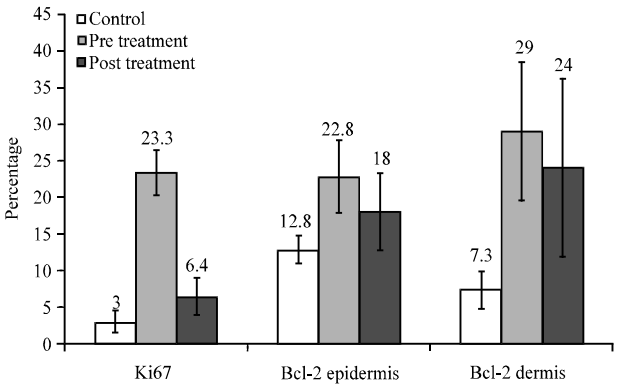

Fig. 12: Comparison of immunostaining results for Ki67, epidermal and dermal Bcl-2 in normal skin and psoriatic cases before and after treatment

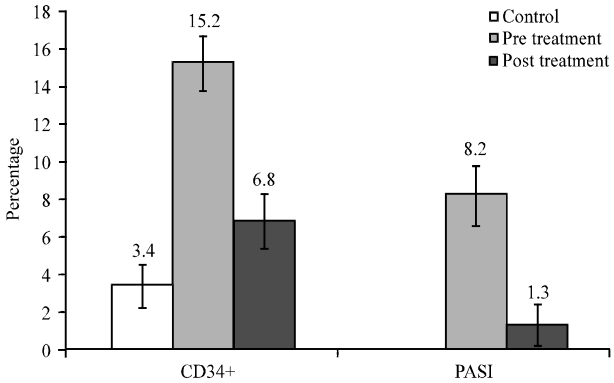

Fig. 13: Comparison of immunostaining results for CD34 in normal and psoriatic cases in addition to PASI before and after treatment

DISCUSSION

Aloe vera and coal tar, from the natural therapy field, have been tried successfully as topical treatments for psoriasis. However, their mechanisms of action has not yet been fully elucidated (El-Gayyar *et al.*, 2006). Kumar *et al.* (1997) reported improvement of psoriasis in 76.5% of their study patients using coal tar without side effects. El-Gayyar *et al.* (2006) reported good improvement of 30 psoriatic patients where PASI decreased from 7.28±3.75 to 3.88±1.17 when treated with topical coal tar for 12 weeks. Moreover, an evidence-based review of 25 studies on the efficacy of tar preparations in the treatment of psoriasis was made by Slutsky *et al.* (2010) and they concluded that the majority of these studies (21) supported the use of coal tar preparations in the treatment of psoriasis. The mechanism of action of coal tar is exactly unknown but the possibility of suppression of DNA synthesis has been proposed producing the antiproliferative action in addition to the anti-inflammatory and keratolytic effects. Many modifications have been made to tar preparations to increase their acceptability as some dislike its odour, messy application and staining of clothing (Thami and Sarkar, 2002; Paghdal and Schwartz, 2009).

Clinical improvement of psoriasis treated with aloe vera alone was reported by some researches. Syed *et al.* (1996) and Vogler and Ernst (1999) reported a cure rate of nearly 83.3% of psoriatic patients treated with topical 0.5% hydrophilic aloe vera cream compared with placebo (6.6%). Patients received the treatment over four weeks and were followed-up for 12 months without relapse. Similarly, Choonhakarn *et al.* (2010) reported marked improvement of 40 psoriatic patients treated with topical aloe vera after 8 weeks where PASI decreased from 11.6 to 3.9 compared with a decrease from 10.9 to 4.3 with 0.1% triamcinolone acetonide treatment.

El-Gayyar *et al.* (2006) reported good improvement of 30 psoriatic patients treated with topical aloe vera cream alone after 12 weeks of therapy where PASI decreased from 6.65±3.09 to 3.64±1.51. On the other hand, when we used the mixture of aloe vera extract and coal tar (AMIX) in the treatment of 180 psoriatic patients, marked improvement was found where PASI decreased from 7.31±5.13 to 1.29±1.46 with complete cure in 42.5% of patients after 12 weeks of treatment (El-Gayyar *et al.*, 2006). Moreover, immunohistochemical staining of skin biopsies of the responding cases revealed significant decrease of CD3+ and CD4+ positive cells in the cellular infiltrate ($p<0.05$), a finding that documents its immune modulating properties (El-Gayyar *et al.*, 2006). The clinical improvement of psoriasis after the treatment with (AMIX) was confirmed by the results of the current study as shown by the decrease in the PASI from 8.2±1.6 to 1.3±1.1 ($p<0.001$) after 12 weeks of treatment in addition to complete cure in about 52% of patients.

In the present study, we have investigated the antiproliferative, apoptotic and antiangiogenic effects of AMIX in psoriasis. For this purpose, we assessed the expression of Ki67, Bcl-2 and CD34 proteins using immunohistochemical techniques in normal and psoriatic skin. Ki67, as a proliferative marker, was reported to be highly expressed in psoriasis and is correlated with its clinical severity (Chang *et al.*, 2010). Bcl-2 plays a major role in the balance between cell survival and cell death. Its expression is restricted to cell populations with a long lifespan or proliferative activity, as it prevents apoptosis (Ceovic *et al.*, 2007). CD34 expression was reported to facilitate the opening of vascular lumen and cell migration (Blanchet *et al.*, 2007; Strilic *et al.*, 2009).

Our results revealed significant increased expression of Ki67 in psoriatic epidermis compared to normal ($p<0.001$). Similar results were reported by Zhang *et al.* (2009) and Lin *et al.* (2011) who concluded that the overexpression of Ki67 implies an abnormality of cell cycle regulation in psoriatic keratinocytes. The abnormality might be related to the hyperproliferation and abnormal differentiation of psoriatic keratinocytes.

After treatment with AMIX, a significant decrease of Ki67 expression was detected ($p<0.001$), a finding that confirms its expected antiproliferative effect in psoriasis. These results coincide with that reported after treatment of psoriasis with oral bexarotene, methotrexate, rambazole, an oral retinoic acid metabolism blocking agent and narrow-band UVB therapy by Smit *et al.* (2004), Yazici *et al.* (2005), Bergstrom (2007) and Yu *et al.* (2009), respectively. Smit *et al.* (2004) made a study including twenty-nine psoriatic patients who were treated with oral bexarotene in doses up to 3.0 mg/kg/day and reported a significant reduction in Ki67 expression after 12 weeks of treatment. Yazici *et al.* (2005) study included ten patients using methotrexate. Bergstrom (2007) study included six adult patients with moderate to severe plaque psoriasis used oral rambazole 1 mg daily for 8 weeks. Clinical assessment was made by SUM score, a modified PASI score of one target lesion and found it improved by 34% ($p<0.05$) and the expression of Ki67 decreased by 63% ($p<0.01$). Similarly, a decrease of Ki67 was reported after treatment of psoriasis with either topical calcipotriol or methylprednisolone aceponate (Adisen *et al.*, 2006).

Ceovic *et al.* (2007) reported similar reducing effects on Ki67 expression in two groups of patients (each included 30 patients) treated with either PUVA or topical corticosteroids. Saleh *et al.* (2008) made a study including 30 psoriatic patients to compare the effect of bath PUVA to the effect of salt water bath before UVB. Although both lines produced clinically similar effect but salt water bath before UVB sessions proved to decrease epidermal proliferation with decreased Ki67 expression to a statistically significant difference ($p<0.05$).

Bcl-2 expression in normal skin was confined mainly to the basal cell layers of the epidermis, a finding that was supported by Batinac *et al.* (2007) who provided an explanation of this pattern of expression in protecting the proliferative compartment from apoptotic stimuli. Our results demonstrated positive Bcl-2 reaction in 86.67% of psoriatic patients. Statistically, Bcl-2 showed a significant higher expression when compared to normal skin ($p<0.01$). Then a significant decrease of Bcl-2 expression was detected after treatment ($p<0.01$).

Adisen *et al.* (2006) made a study that included thirty psoriatic patients and applied either calcipotriol or methylprednisolone aceponate (MPA) ointment for 6 weeks. They reported higher Bcl-2 expression in psoriatic than normal skin ($p<0.05$) supporting our results. However, they found more increased expression of Bcl-2 following both treatments ($p<0.05$ each), a finding which is contradictory to our results after treatment with AMIX. On the other hand, Batinac *et al.* (2007) stated that Bcl-2 protein expression was significantly decreased in psoriatic skin samples, as compared to normal (3.23 vs. 6.25; $p<0.01$). They suggested that this finding could be a result of

intense proliferation, probably secondary to inflammatory stimuli in psoriasis. Kocak *et al.* (2003) suggested that the psoriatic epidermal hyperplasia may result from excessive mitogenic stimuli that might promote an increase in the proliferative cell compartment, rather than being a consequence of the loss of antiproliferative control.

The current study revealed also Bcl-2 expression in the dermal lymphocytes of 86.67% psoriatic patients. Coinciding with our results, Yildiz *et al.* (2003) observed Bcl-2 expression in lymphocytes of 71% of his study psoriatic patients, 20 out of 25 and related this to the prolonged survival of lymphocytes resulting in the relapsing and chronic course of psoriasis and suggested that Bcl-2-mediated inflammation plays a part in the pathogenesis and recurrent character of psoriasis. Also, El-Hadidi *et al.* (2008) detected Bcl-2 expression in dermal lymphocytic infiltrate in 80% of the examined psoriatic patients, 15 patients. In the current study, a decrease of Bcl-2 expression in dermal lymphocytes was detected after treatment with AMIX, however, it was statistically insignificant ($p>0.05$). This finding is supported by El-Domyati *et al.* (2007) who studied ten cases with generalized plaque psoriasis and reported a significant decrease of Bcl-2 in lymphocytes ($p = 0.01$) following topical calcipotriol therapy suggesting it to promote apoptosis of dermal lymphocytes leading to healing of psoriasis. and found that most of them showed evident decline of Bcl-2 level after 24 sessions of PUVA phototherapy.

In the present study, there was a significant increase in the number of CD34 positive dermal blood vessels of psoriatic skin compared to normal control ($p<0.001$). Similar results were detected by Li *et al.* (2006) who detected a significant overexpression of CD34 in the papillary dermal microvessels in psoriatic lesions and Simonetti *et al.* (2006) who found a significantly higher CD34 expression in psoriasis compared to control skin (19.15 ± 12.61 vs. 3.0 ± 0.23; $p<0.05$). After treatment, a significant decrease in the number of blood vessels within the treated areas was detected ($p<0.001$), a finding that reflects the antiangiogenic activity of AMIX as one of the key mechanisms in the management of psoriasis.

Going with these results, other researchers as Ceovic *et al.* (2007) studied neoangiogenesis in psoriasis before and after PUVA and local steroid therapy for five weeks and reported that both treatments decreased it markedly ($p<0.001$). They used anti-F-8 antibody as angiogenesis marker. Avramidis *et al.* (2010) reported that etanercept caused a statistically significant time-dependent reduction in the number of dermal blood vessels using endothelial nuclear factor-κB (NF-κB), angiogenic Vascular Endothelial Growth Factor (VEGF) and endothelial cell marker CD31.

CONCLUSION

The topical mixture of aloe vera extract and 1% coal tar is a safe, cheap and highly effective treatment in mild to moderate plaque psoriasis without any noticeable side effects apart from staining of clothes. It causes inhibition of epidermal cell proliferation, induction of apoptosis and amelioration of neovascularization that may be the possible mechanisms by which AMIX exerts its therapeutic effect beside its previously proved immune modulating effects. Studies of this old/new topical therapy on larger groups of patients are recommended.

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