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TOWARD AN APPROACH FOR CUTANEOUS LEISHMANIA TREATMENT

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Abstract
Introduction: Most drugs being used for cutaneous leishmania treatment are still non well effective, extremely expensive, risky with side effects, more invasive and relapses still occur. The purpose of the study is to achieve more understanding of cutaneous leishmania disease and through that to try an other form of treating substance that can provide better results with less damages to the tissues.
Methods: Seven patients infected with cutaneous leishmania were chosen for DAB-1 application. Clinical as well as microscopic study and follow-up with documenting photos for the lesions, indicating the starting point for the cases before treatment initiation, and the disease development after DAB-1 application was accomplished.
Results: Before treatment, the lesions size was between 1.8-7 cm. Cases were inflamed and ulcerated. After 8 days of treatment, inflammation shrank. After 16 days, the lesions and the ulcers decreased into almost half their size. 24 days post treatment, inflammation began disappearing and epithelial islands continued to grow inside the ulcers filling a considerable part of them. By the end of day 32, ulcers were covered with a continuous layer of epithelium, and heal is achieved after two to three months of treatment.
Conclusion: The study proved that DAB-1 is capable of healing leishmania in 6-8 weeks after application and is compared favorably to the other traditionally used drugs. DAB-1 could be a breakthrough in cutaneous leishmania treatment.

Key words: cutaneous Leishmaniasis; Leishmaniasis; epithelium regeneration

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Introduction

Many studies have indicated that the drug of choice for treating cutaneous leishmania is Sodium antimony gluconate (Pentostam) [1]. It is applied intramuscularly in case of multiple lesions. In a single lesion, it is usually injected into the ulcer margin [2]. The healing process of the lesion usually takes between 14 to 16 weeks and in some cases even more. The heal is usually completed with a scar formation. If present in a delicate location like in the face, the lesion can cause a bad defect and deformation for the patient. Although it is the drug of choice, Pentostam induces side effects [3]. Even with its application, Pentostam cannot induce a complete heal with a perfect epithelization. The final result in the lesion in-position is a permanent scar formation. Follow-up studies in southwestern Europe, using pentavalent antimonials, show a positive response in 83% of cases. However, 52% of patients relapse within a period of one month to three years [4].
Other drugs being used for cutaneous leishmania treatment are paromomycin, Amphotericin B, Fluconazole and Pentamidine, but relapses still occur with a risk of side effect and the drugs remain extremely expensive.
DAB-1 on the other hand, is a topical ointment, which is applied topically to the ulcer lesion. It is produced from natural substances and obviously, has no side effects.
The purpose of this study is to measure the DAB-1 effect on cutaneous leishmania by tracking both the clinical and the cytomorphologic effect, and checking the healing process over a period of two to three months.

Material and Methods

Seven patients infected with cutaneous leishmania and presented with clear lesions were chosen for DAB-1 application. Before applying the ointment, couple of microscopic slides were prepared from each lesion stained with Wright stain and tested for all the suspected pathological features including the parasite form found in the intra or extra cellular space. Clinical as well as microscopic photos for the lesions beginning with the starting point for the cases before treatment initiation were taken for documentation.
Each lesion was cleaned with normal saline and dried with gauze. The ointment is added to a sterile gauze and applied directly over the lesion. The ointment dressing was changed once every day for 24 consecutive days. After that and for the next 24 days, the ointment dressing was changed once every three days. Every three days couple of photos for each lesion were taken to mark the clinical signs of the healing process.

At 8, 16, 24, 32, 45, 60 days interval, other couple microscopic smears were prepared from each lesion, stained with Wright stain and tested to identify the cytomophologic development of the cure process. Documentary microscopic photos were taken for the cytomorphologic features for more detailed study and analysis.

Results (Tabl. I-X)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Lesion size	7 cm	3 cm	5 cm	3 cm	1.7 cm	4 cm	1.8 cm
Inflamed	+++	++	+++	+/-	+/-	++	-
Ulcerated	+++	-	+++	++	-	++	-

Table I. The clinical features present for each lesion before the ointment application

Note: (+++) Highly inflamed, or ulcer> 2cm in diameter. (++) Moderately inflamed or ulcer 1-2 cm in diameter. (+/-) indicates mild inflammation. (-) No inflammation or nodular type with no ulcer

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Lesion size	6 cm	2 cm	4 cm	2 cm	1.3 cm	3 cm	1.5 cm
Inflamed	++	+	++	+/-	+/-	+	-
Ulcerated	+++	-	++	++	-	++	-

Table II. The clinical features development for each lesion after 8 days of the ointment application

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Lesion size	4 cm	2 cm	1.5 cm	2 cm	1 cm	2 cm	1 cm
Inflamed	+	+	+	-	-	+	-
Ulcerated	++	-	++	+	-	++	-

Table III. The clinical features development for each lesion after 16 days of the ointment application

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Lesion size	3 cm	1 cm	1 cm	1 cm	0.8 cm	1 cm	0.5 cm
Inflamed	+	-	+	-	-	+	-
Ulcerated	+	-	+	-	-	+	-

Table IV. The clinical features development for each lesion after 24 days of the ointment application

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Lesion size	3 cm	1 cm	1 cm	1 cm	0.6 cm	1 cm	0 cm
Inflamed	-	-	-	-	-	-	-
Ulcerated	-	-	-	-	-	-	-

Table V. The clinical features development for each lesion after 32 days of the ointment application

Cytomorphologic figures appearance	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Phagocytes with intracellular amastigotes	-	++	++	-	++	+	-
Extracellular amastigotes	-	++	+++	-	+	++	-
Promastigotes	+++	+	+	++	++	++	++
Lymphocytes	++	++	+++	++	+	++	+
Neutrophils	++	-	+	+	-	++	-

Table VI. The cytomorphologic features present in the slides for each lesion before the ointment application

Note: (+++) > 5 cells or leishmania configurations seen per HPF. (++) 3-5 cells or leishmania configurations seen per HPF. (+) 1-3 cells or configurations in average seen per HPF. (-) No cells or leishmania elements seen

Cytomorphologic figures appearance	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Phagocytes with intracellular amastigotes	-	-	-	-	+	-	-
Extracellular amastigotes	-	-	-	-	+	-	-
Promastigotes	+++	++	+++	+	+++	++	+
Lymphocytes	+	++	+	+	+	+	+
Neutrophils	++	++	+	++	+	++	+

Table VII. The cytomorphologic features present in the slides for each lesion after 8 days of the ointment application

Cytomorphologic figures appearance	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Phagocytes with intracellular amastigotes	-	-	-	-	-	-	-
Extracellular amastigotes	-	-	-	-	-	-	-
Promastigotes	+	+	+	+	++	+	-
Lymphocytes	+	-	+	+	+	+	-
Neutrophils	+++	++	++	+	++	+	-

Table VIII. The cytomorphologic features present in the slides for each lesion after 16 days of the ointment application

Cytomorphologic figures appearance	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Phagocytes with intracellular amastigotes	-	-	-	-	-	-	-
Extracellular amastigotes	-	-	-	-	-	-	-
Promastigotes	+	+	+	-	+	+	-
Lymphocytes	+	+	+	-	+	+	-
Neutrophils	+	+	-	-	+	+	-

Table IX. The cytomorphologic features present in the slides for each lesion after 24 days of the ointment application

Cytomorphologic figures appearance	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Phagocytes with intracellular amastigotes	-	-	-	-	-	-	-
Extracellular amastigotes	-	-	-	-	-	-	-
Promastigotes	+	+	-	-	-	+	-
Lymphocytes	+	-	+	-	-	-	-
Neutrophils	+	+	-	-	-	+	-

Table X. The cytomorphologic features present in the slides for each lesion after 32 days of the ointment application

Tables I-V show the clinical features present for each lesion before and after DAB-1 application on time bases.

It is clear from table 1 that the size of the lesions is between 1.8 and 7cm when cases were referred. All the cases except one were clinically inflamed. Four of the cases were ulcerated and the other three were not ulcerated (Fig. 1a, 1b).

After 8 days of treatment with DAB-1, the first sign of healing process to show in table II is the decrease in the size of the lesion characterized by the inflammatory reaction in a range between 16-33%. The inflammation also showed a clear reduction in its virulence while a decrease in the ulcer size appeared in only one case with the ulcer decreased about 0.4 cm in its size (Fig. 2a).

After 16 days of treatment and according to Table III each lesion was reduced in size to about half.

The inflammation virulence was further decreased in almost every case and the inflammation disappeared from two additional cases. All ulcers were reduced in size to less than 2 cm in diameter.

Isolated islands of epithelial cells started to show up inside the ulcers (Fig. 3a).

By the end of day 24 of treatment, as seen in Table IV all the cases were reduced to less than half their original size. The ulcers and the inflammation totally disappeared from 4 of the cases. Mild inflammation and small ulcers with less than 1 cm in diameter were seen in the three left cases. The epithelial islands continued to grow inside the ulcers filling a considerable part of them (Fig. 4a).

By the end of day 32 of treatment, looking back to Table V, a relief in the inflammatory reaction was noticed and the ulcers disappeared from all the studied cases. Ulcers were covered with a continuous layer of epithelium. The size of the lesions continued to be the same as was the case in day 24 but with the disappearance of the clinical disease signs (Fig. 5a). On day 45, the lesion area continued in process toward a normal looking skin (Fig. 6). And on day 60, the lesion looked almost close to normal with a remote inflammation (Fig. 7). After 75 days of treatment initiation, the skin started to appear normal (Fig. 8), and after three months completion, the skin in the area turned into normal looking (Fig. 9).

Tables VI-X show the cytomorphologic features present for each lesion before and after DAB-1 application. In Table VI we notice the cytomorphologic features before DAB-1 application. In four of the cases, the intracellular and extracellular amastigotes were present together with the infected macrophages in the smear. Promastigote- like figures and lymphocytes appeared in all the seven studied cases at different concentration, indicating the chronic nature of the disease. Neutrophils were present in 4/7 cases (Fig. 1c).

After 8 days of treatment as appears in Table VII, a disappearance of the amastigotes and their infected macrophages from the smears appeared in 6/7 cases, while the promastigote like forms continued to appear in all the cases. Under the microscope, a decrease in lymphocytes count and elevation in the neutrophils count appeared in each case. The presence of such increase number of neutrophils is a sign of the disease conversion from a chronic to an acute inflammatory reaction (Fig. 2b, 2c).

By day 16 as shown in Table VIII, there was a total disappearance of the infected macrophages together with the amastigotes from all the cases. Neutrophils continued to appear with a little less count in all cases but one, where a complete disappearance of all the disease microscopic cytomorphology was noticed. Promastigote like forms appeared in 6/7 of the cases but in less concentration. Lymphocytes were present in less count. Larger area of immigrating epithelium was noticed (Fig. 3b, 3c).

In day 24 (Tabl. VIII): A less concentration of all the microscopic disease figures including promastigote like forms, Lymphocytes and neutrophils was noticed with a total disappearance of all the microscopic signs in two of the cases. More epithelial cells were present (Fig. 4b, 4c).

In day 32 very few promastigote like forms were still present in three of the cases and few lymphocytes and neutrophils were seen in the smear. Overall, other smears appeared almost like normal blood smears with few migrating e pithelium (Fig. 5b, 5c).

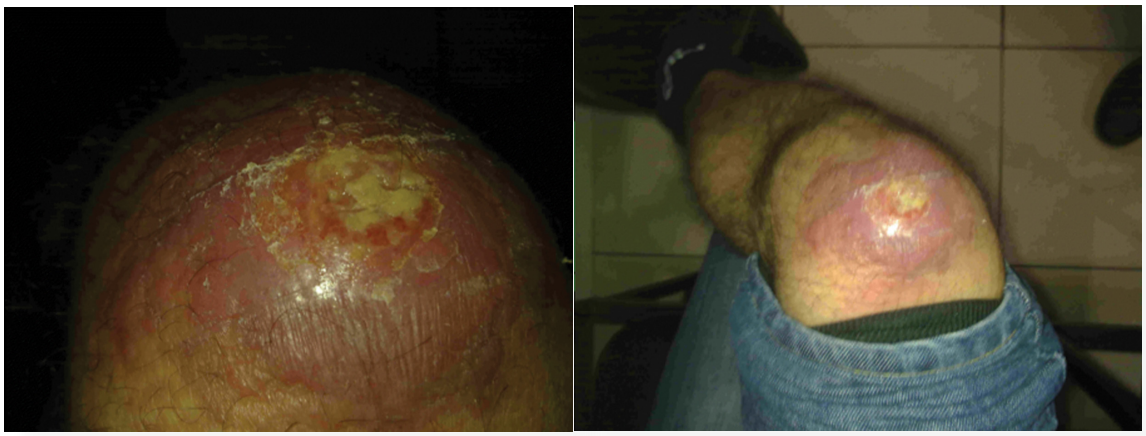

Figure 1a. A closer shot. 1b. A distant -shot. The infected lesion before DAB-1 application

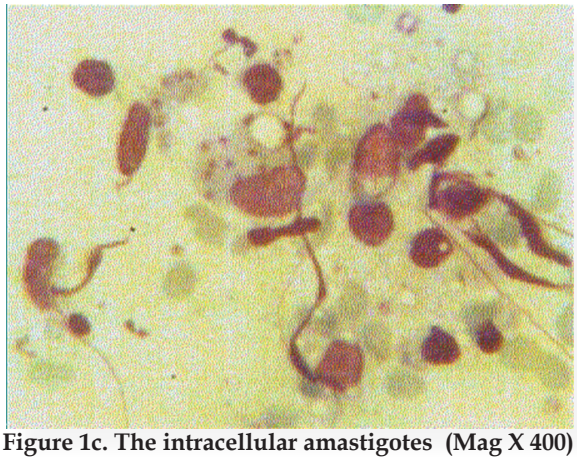

Figure 1c. The intracellular amastigotes (Mag X 400)

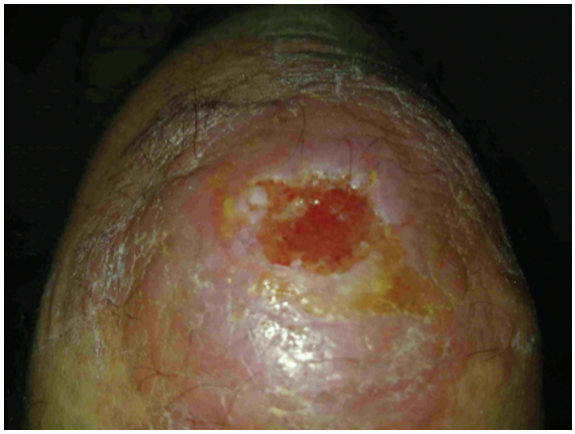

Figure 2a. A closer shot. The infected lesion 8 days after DAB-1 application

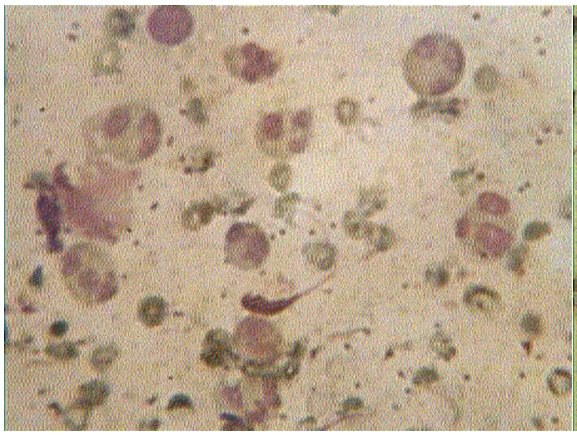

Figure 2b. Note the promastigote and the neutrophils. (Mag. X 400)

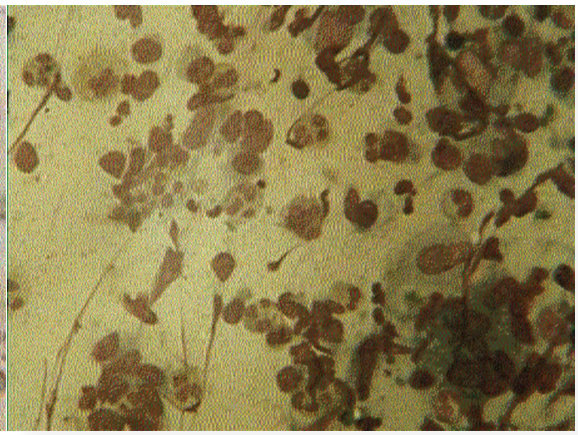

Figure 2c. Note the promastigotes with the cellular elements. Including neutrophils and lymphocytes (Mag. X 200)

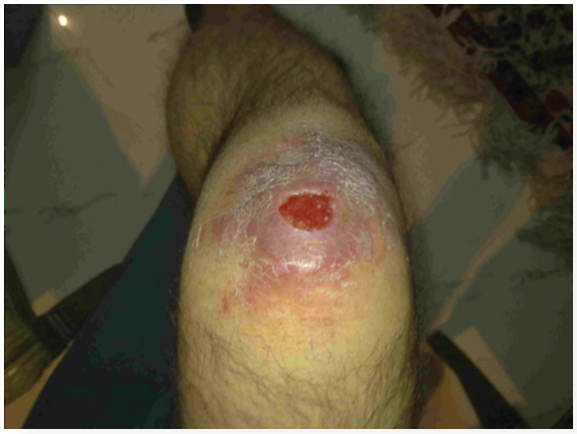

Figure 3a. A distant -shot. The innfected lesion 16 days after DAB-1 application

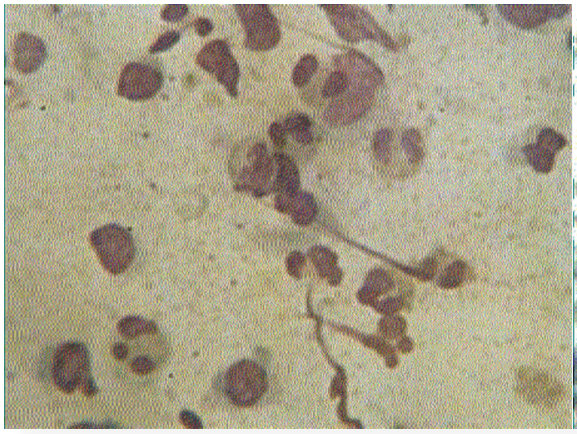

Figure 3b. Note the elevated neutrophils number (Mag. X 400)

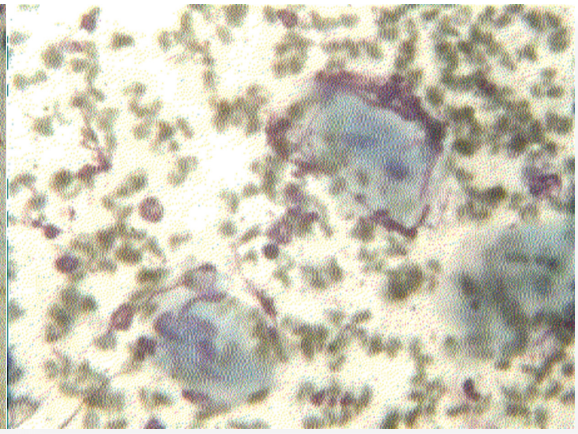

Figure 3c. Note the epithelial cells invading the tissue. (Mag. X 100)

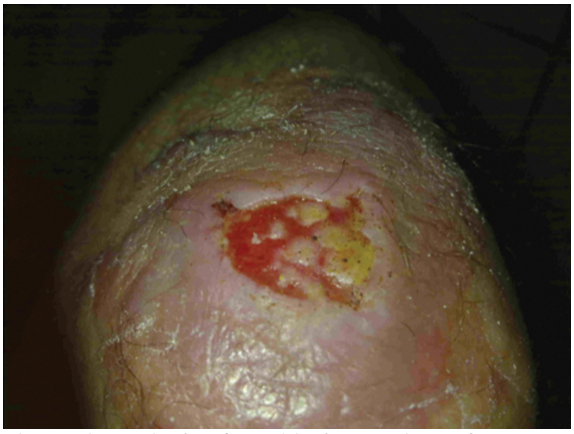

Figure 4a. A closer shot. The innfected lesion 24 days after DAB-1 application

Figure 4b. Note the promastigote appearance. (Mag. X 400)

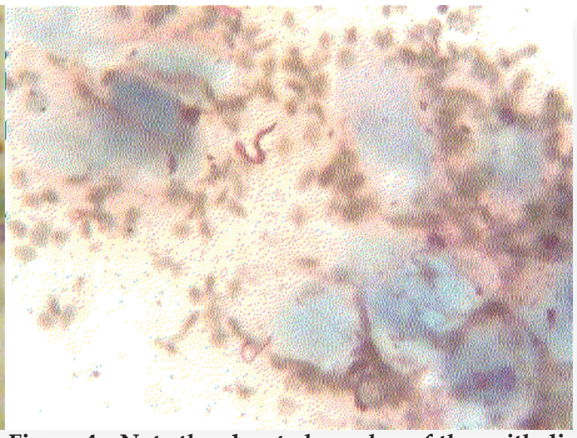

Figure 4c. Note the elevated number of the epithelial cells. (Mag. X 100)

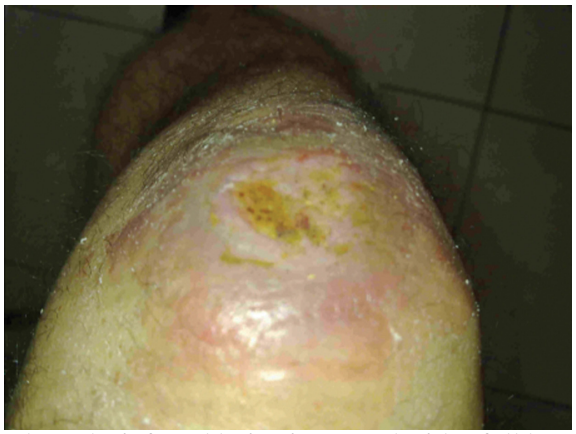

Figure 5a. A closer shot. The infected lesion is covered with a full layer of epithelium. The innfected lesion 32 days after DAB-1 application

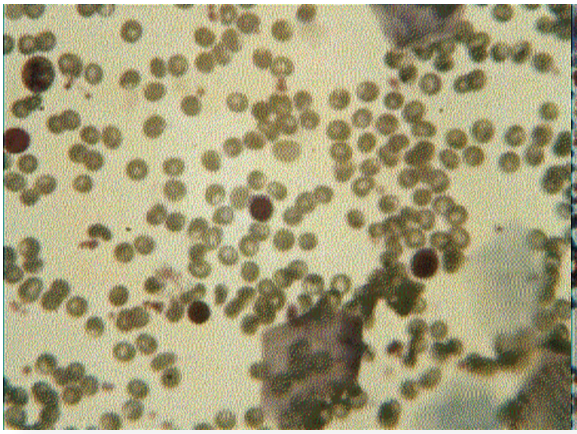

Figure 5b. Note the normal looking blood smear. (Mag. X 200)

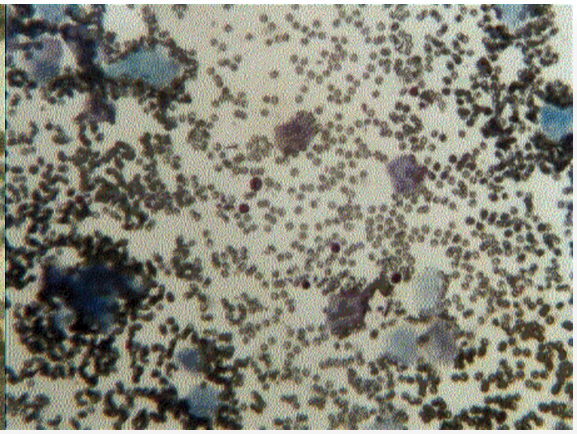

Figure 5c. See the epithelial cells within normal smear. (Mag. X 100)

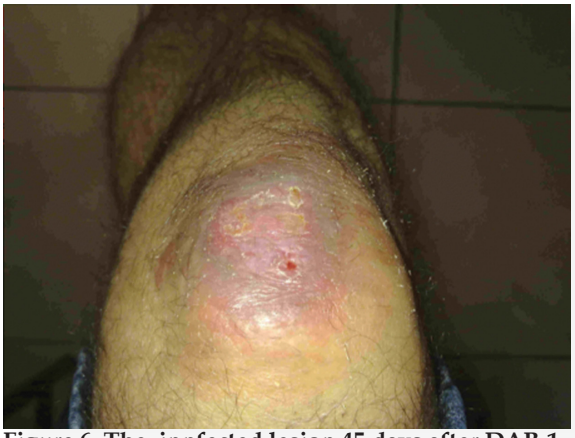

Figure 6. The innfected lesion 45 days after DAB-1 application

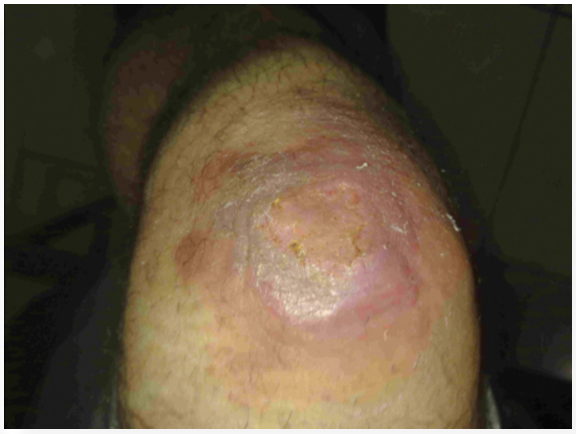

Figure 7. The innfected lesion 60 days after DAB-1 application

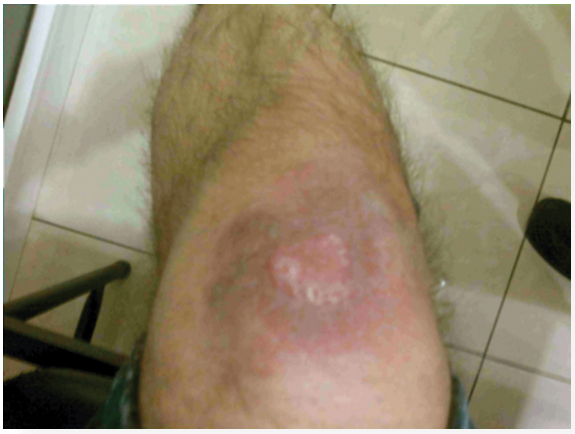

Figure 8. The innfected lesion 75 days after DAB-1 application

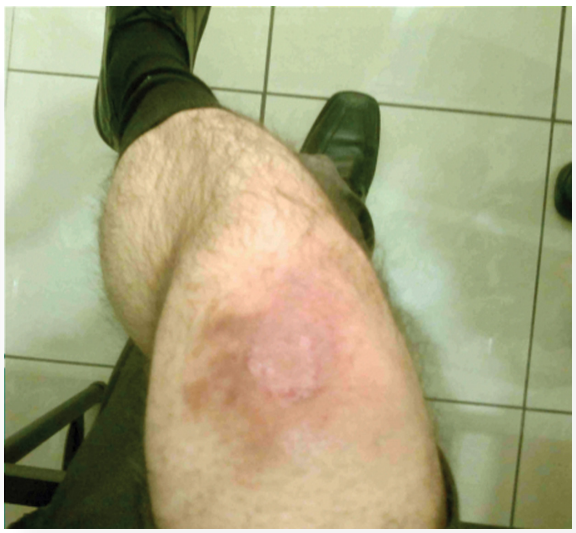

Figure 9. The innfected lesion 90 days after DAB-1 application

Discussion

One of the important things to be illustrated in this study is the total disappearance of the amastigotes form both in the intra and the extracellular reign together with their engulfing phagocytes. It is interesting to see the lesion still clinically hyper active and inflamed with the disappearance of such important disease figures. Other studies have observed this same phenomenon [5]. One answer for that dilemma is that the amastigote form is not necessarily the only influential factor during the whole process of the disease course, or the amastigote might have been active at one stage and then disappeared from the lesion. That contradicts the previous basic understanding of the disease process. It is clear from all previous data and literature presented, that the amastigote form is the main acting player and causing organism in cutaneous leishmania disease process.

According to Hepburn NC who summarizes the general previous understanding of the aspects of the disease: (In all forms of leishmaniasis the presence of amastigotes within the cells of the mononuclear phagocytic system remains the hall mark of the disease, though they sometimes may be difficult to detect) [6]. Indeed, the true fact is that the amastigotes at certain time of the disease are impossible to detect. The disappearance of such giant cells infected with the amastigote form while the disease process both clinically and cytomorphologically is still in action, indicates that such phagocytes, giant cells, macrophages and monocytes, at specific point of the disease process become resistant to be infected with the amastigotes. Literature tells (the amastigote forms multiply by binary fission, within the macrophage until the host cell is packed with the parasites and ruptures, liberating the amastigotes into circulation-then the free amastigotes invade fresh cells, thus repeating the cycle) [7]. In contrary with the previous concept, those amastigotes released into the extracellular fluid at a specific stage, become unable any more, to invade the adoptive phagocytes or replicate within those macrophages.

This inability, is explained by an immune interaction between the host immune system and the leishmania parasite determinants [8] which causes a development of resistance in the phagocytes against the parasites. This is in the disease course, where we notice the disappearance of the phagocytes with the intracellular amastigotes from the smear.

Tables VII -X confirm the disappearance of the amastigotes from the smear at that point.

As noticed in this study, DAB-1 ointment worked favorably on hastening the disappearance of the amastigotes from the lesion when compared to the disease in its natural process.

According to the traditional definition: (Leishmania are intracellular parasites that infect the mononuclear phagocytes.

Leishmania are obligatory intracellular parasites) [7]. This definition means that extracellular amastigotes cannot survive the harsh extracellular environment. Literature did not explain the way the expelled amastigotes disappear after being released from the infected macrophage. As we see clinically, the disease in that stage is deeply active and still not yet healing (Tabl. VII-IX). It is clear then that, the extra cellular amastigotes must have been able to find a way to survive in the extra cellular fluid [9] and to grow from such an inactive form (the amastigote form) which is an ova like form into the active form which is the flagellate a (promastigote like form) [10]. The promastigote form then that replaces the amastigote is the one that is developed and becomes the active form penetrating the subcutaneous tissues and causing the real signs and symptoms of the disease at that stage.

Coming to the pathology of the disease, according to Hepburn: (Over the following months, there is a gradual decrease in the number of amastigotes and macrophages, leaving a granulomatous infiltrate consisting of lymphocytes, epithelioid cells and multinucleate giant cells. At this stage it may be difficult or even impossible to detect organisms "pointing to the amastigote form" in H&E, or Giemsa, stained sections) [6]. As shown in Tables II, III, IV, VI-IX, the lesion at that stage is still clinically deeply inflamed with large ulcer and exudates. In fact, Those clinical and pathological signs still represent an active form of the disease process. Here we notice clearly, the appearance of many cytomorphologic forms that have been developed through the process of amastigote transformation. Those forms are Promastigote and fiber forming promastigote like forms, candle flame forms, the spherical and polygon forms [11]. All those forms are active forms of the parasite inside the tissue, and at that point, they are the ones causing the pathological inflammatory signs (Fig. 1c, 2b, 2c, 3b, 3c, 4b). After the amastigotes are released from the infected macrophages to the extracellular space, the fetus erupting from the amastigote will develop in a series of steps starting from a candle flame shape into a mature promastigote like form [12]. Those active forms of the parasite will never turn back or transform in side the host vertebrate into the amastigote form again. By that the amastigote forms disappear from the smear and what is left is destroyed by the function of neutrophils [13] (Tabl. VII-IX). Once the parasite is in its active form in the body, which is the promastigote form, and without drugs interference, the immune system represented by the lymphocytes will further react to control the flagellate type of the parasite by introducing T type lymphocytes with a protruding tail that is capable to form a base for a nest- like; that will trap those flagellate type of parasites and terminate the disease with a permanent scar formation [11]. That comes in agreement with the statement written by Hepburn: (There is, however, considerable variation: some lesions do not ulcerate, others develop sporotrichoid nodular lymphangitis. Most lesions heal over months or years, leaving an atrophic scar) [6].

In most cases of treatment using the different types of drugs and injections like Sodium antimony gluconate (Pentostam) or Pentamidine, the disease will heal leaving a permanent scar behind it. Relapses still occur and the drugs remain extremely expensive. That is not the case with DAB-1 product. As seen from the follow-up smears, DAB-1 has a stimulatory effect on the epithelium. This study shows that from day 16 of DAB-1 application (Fig. 3a, 3b, 3c), the epithelial cells become active and regenerate moving from the edges forward into the ulcer center, forming isolated islands. In day 24, the islands gather and start covering the whole ulcer and by day 32, one whole layer of epithelium covers the full ulcer (Fig. 4a, 4b, 4c). DAB-1 and according to the cytomorphologic figures seems to be active in killing the parasite. It is obvious that the parasite count was decreasing from the time of DAB-1 application. Not only that, but DAB-1 seems to have a chemotactic effect on neutrophils.

As we notice from Tables VII-VIII, the neutrophils increased in number in the lesion after 8 days of DAB-1 application and continued to increase by time. The fiber forming promastigotes with their fibers are left to the action of those neutrophils to degenerate and get red of. By the end of the disease process, the connective tissues with the upper layer of the epithelium will rejuvenate forming a normal cutaneous tissue with no scar left (Fig. 4c, 5a, 5b, 5c). Follow up of one case up to 2 years after treatment with DAB-1, declares no relapses observed .

Conclusions

DAB-1 is capable of healing leishmania in 6-8 weeks after application and is compared favorably to any other used drugs where the healing process may take between 14 to 16 weeks and in some cases even more.

DAB-1 has the power to stimulate the epithelium regeneration, migration and multiplication. it is more effective than the other applied drugs in generating normal tissues for cosmetic reasons.

DAB-1 has a strong anti-parasite effect on leishmania. This can be confirmed by the parasite disappearance from the smears within time.

DAB-1 seems to have a stimulatory effect on neutrophils function, as they act on the sporotrichoid nodular fibers produced by the parasite reducing the effect of the scar formation.

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