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TOPICAL FORMULATION ASSESSMENT OF PENTOSTAM® FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS

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ABSTRACT

Background: Sodium stibogluconate, sold under the brand name Pentostam® among others, is a medication used to treat Cutaneous *leishmaniasis* (CL).It is applied by intralesional (I.L.), intravenous (I.V.), or intramuscular (I.M.) injection. This practice causes great pain and systemic side effects and results in very low patients compliance.

Objective: To develop and evaluate various Pentostam® topical formulations. To assess the pharmacokinetic properties (ADME) of Pentostam® and to evaluate the application of the formulations as an alternative therapy to the systemic use, to avoid the disadvantages of the latter.

Methods: Different formulations of Pentostam® in hydrous wool fat (HWF) ointment, polyethylene glycol (PEG), aqueous cream (AC), and gel were prepared and tested clinically against CL. The formulations were compared with other conventional medications tried by clinicians in epidemic situations. Also, SwissADME web server were utilized to evaluate the pharmacokinetic properties of the formulated preparation.

Results: The four preparations (dimethyl sulfoxide enhancing incorporated with the previous mentioned bases and Pentostam®) showed the following cure rates at the sixth week: Hydrous wool fat ointment (42.5%), Polyethylene glycol ointment (63.6%), aqueous cream (79.6%) and gel (81.6%). compliance rates percentages were (60%), (80%, 78%),(94%), (92%) respectively. Form the ADME analysis it is demonstrated that Pentostam® is a highly hydrophilic compound with -5.3 LogP value, and having a high water solubility with -0.5 LogS value. This results confirmed the drawn conclusion that the aqueous cream and the hydrogel formulations shows the best cure rate with 72% and 75% respectively. From the Log Kp (skin permeation) value was found to be -13.72 cm/s which means that this drug has high skin permeation ability, that's why when we add it to highly lipophilic bases, the drug was not able to permeate to the skin, which is clearly demonstrated with low cure rate with Hydrous wool fat ointment and Polyethylene glycol ointment

Conclusions: The 2% Pentostam® and 0.5% DMSO in aqueous cream and gel bases were found to be the most efficacious clinically in comparison to the other bases and the conventional and non-conventional regimens currently were used during the epidemic outbreak of the disease. The efficacy was assessed by the detection of the absence of L.D bodies from the lesions. The comparative studies showed that Pentostam® topical formulations were only second in efficacy to systemic Pentostam® with the obvious advantages of overcoming the drawbacks of the latter.

Keywords: Pharmacokinetic ADME, Pentostam® topical application, Pentostam® systemic application Dimethylsulfoxide

1. INTRODUCTION

Cutaneous *Leishmaniasis* (CL) is a skin disease caused in man by protozoal organisms *Leishmania Mexicana, L braziliensis, L.aethiopica, l.infantuml. major, l. Tropica and l.Donovani,* that are transmitted by the sand-fly **[1-3]**. The three latter organisms are the major causative organisms in Sudan **[4, 5]**. Mucocutaneous *leishmaniasis* can present as a complication of cutaneous *leishmaniasis* and also can

occur several months or even years after skin ulcers heal **[5, 6]**. Lesions can lead to the partial or total destruction of the mucosal membranes of the nose, mouth, and throat cavities and surrounding tissues, resulting in social stigma and disability. Unlike most forms of cutaneous *leishmaniasis*, mucocutaneous *leishmaniasis* is unlikely to heal on its own; in cases of severe mucocutaneous leishmaniasis, people who do not receive treatment could potentially die. The disease is widely distributed through the tropical and subtropical regions of the world. Around 600,000 to 1.2 million people are infected every year and it is endemic in 92 countries and 90% of all cases registered in 2018 were reported from the following countries: Afghanistan, Algeria, Brazil, Colombia, Morocco, Nicaragua, Pakistan, Peru, Sudan, Syria, Tunisia, and Yemen **[7, 8]**. The current challenge is that the effectiveness of current treatments is low and estimated to be 50% only **[1, 9-11]**.

Though the disease is self-limiting, patients urgently seek treatment due to the discomforts it causes and in fear of disfiguring scars left over after the disease has taken its course. The drug of choice in the treatment of CL is Pentostam® and it can be given by intralesional (I.L.), and sometimes intramuscular (I.M.) or intravenous (I.V.) injection **[12]**. The methods of administration of Pentostam® have severely limited the compliance of patients because of the pain associated with treatment, the trouble that the patient has to take to come to the health center to receive medication, the high cost, and importantly, the systemic side effects of Pentostam. These factors have significantly contributed to clinicians seeking other remedies to relieve pain, decrease side effects of Pentostam®, and keep the cost of treatment within acceptable limits **[13-16]**.

Most of the conventional therapies were topical and were based on the error-and-trial approach. These remedies included antibiotics, steroids, and steroids plus antibiotics and antifungals. The approaches of alternative conventional regimens in treating CL have diverted interest from the drug of choice Pentostam®. Sodium stibogluconate (**Figure 1**), sold under the brand name Pentostam® among others, is a medication used to treat *leishmaniasis* **[12]**. This includes *leishmaniasis* of the cutaneous, visceral, and mucosal types **[1-17]**. Some combination of miltefosine, paramycin, and liposomal amphotericin B, however, may be recommended due to resistance issues and it is given by injection **[18-22]**.

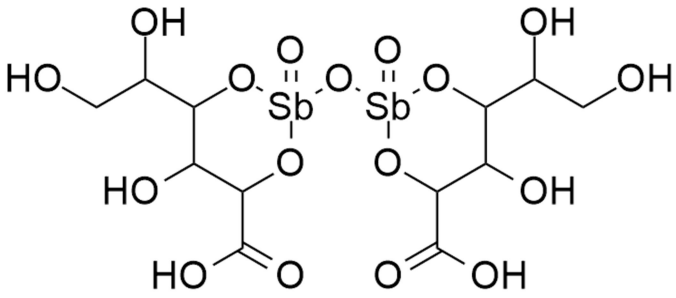


Figure 1: The Chemical Structure of Sodium Stibogluconate (Pentostam®)

Therefore, the main objective of this study is to investigate Pentostam® in topical formulations to eliminate the disadvantages of injectable forms and to limit the error- and trial-based treatment with conventional and non-conventional remedies. Also, to assess and evaluate the rate of infection and other important factors related to CL infection using questioner that was given to the patients. In the present work, topical formulations of Pentostam® were prepared in different topical bases and formulations were investigated clinically for their antileishmanial efficacy since it is well-known that the application of dermatologic medication influences the state of the skin in a variety of ways depending on the base used **[15, 23]**. Moreover, the base can exert an enormous influence on the permeability of the skin to the active ingredients. This is a fact of clinical importance in the design of topical medication **[24, 25]**. Thus, the effect of Dimethylsulfoxide (DMSO) on the absorption of Pentostam® was investigated as it is well-known to enhance the absorption of some drugs through the skin **[26, 27]**. Additionally, we were interested in performing a comparative analysis of the transdermal formulations of Pentostam® with conventional and non-conventional therapies that have been used in the treatment of CL.

2. MATERIALS AND METHODS

2.1 Study design

The study main focus was on Cutaneous Leishmaniasis Patients that confirmed infected using: positive smear, Culture, and histological testing. The trial started with 721 patients and divided as shown in the below schematic diagrams **(Diagram 1)**. The cure rate in percentage (%) was recorded for the patients at each stage. Moreover, 201 patients were excluded since they were negative in the diagnostic testing: smear, and Culture. A consent of agreement was signed by all patients at the last page of the questionnaire.

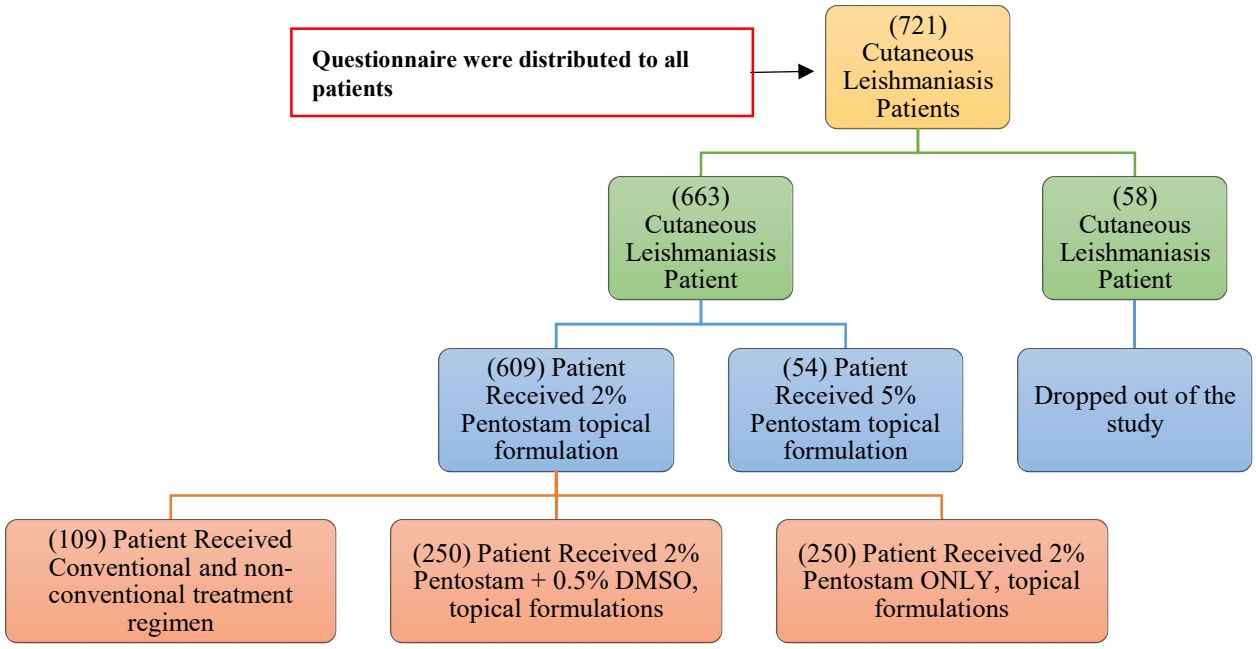


Diagram 1: The study design of Cutaneous Leishmaniasis Patients during the trial

2.2 Materials

The following materials were obtained from the Pharmacy of the Skin and Transmitted Disease Hospital, Khartoum, Sudan. Sodium stibogluconate (Pentostam®) as powder and injections (100 ml vials) (Welcome Medical Division, England). Chloramphenicol capsules (Park-Davis Medical, Hants, England).Fusidic acid 2% ointment and cream (Fucidin) (Leo Laboratories Ltd, England).Hydrocortisone 1% ointment and cream (Roussel Laboratories Ltd, England). Ketoconazole 2% cream and Ketoconazole tablets (200 mg) (Nizoral) (Janssen Pharmaceutical Ltd., England). Gentamycin 1% cream (Nicholas Laboratories, England). Chloroquine tablets and Chloroquine phosphate injection, BP, 200 mg base in 5 ml (Paris Chemicals, France). Paromycin sulfate (PR) ointment (Paromycin 12 and 15%) (Park-Davis Warner, Italy).

2.3 Methods

Selection of the patients

The selection of the patients (663) was based on specific inclusion and exclusion criteria. Patients from all age groups 0 - 70 years, males and females, with a positive demonstration of leishmania bodies from skin lesions by slit smear technique, culture, or histopathology were selected for the study. Informed consent was obtained from all patients in addition to other information related to the history of the disease. Exclusion criteria included patients suffering from chronic skin diseases, and those who were allergic to antileishmanial drugs,

especially antimonial drugs, and those with scars of healed leishmanial lesions that were clinically diagnosed as CL, but parasitologically proved to be negative, and pregnant women. Since the disease is self-limited, patients with lesions of 4 or more months duration. Patients who had previously been treated with Pentostam® by any route of administration (I.V., I.M., or I.L.) since this may play a role in enhancing healing when topical Pentostam® is used and the real response to the latter will be difficult to assess. Ethical approval of the study was obtained from the Ethical Research Committee at the Medical School. The responses were collected and analyzed with SPSS PC packages, version 17.5.

Preparation of bases used in the study

Four bases were prepared according to the methods described in the British Pharmacopoeia 1973. Preparations are O/W cream base (aqueous cream), an emulsifying ointment was W/O Cream base (hydrous wool fat, HWF, ointment), Polyethylene glycol (PEGointments), Gel base(sodium alginate 7%, glycerol 7%, methylhydroxy benzoate 0.2%, calcium gluconate 0.05% water to 100%). The drug was incorporated at 2% of Pentostam® in each base. Other preparation included 2% of Pentostam® and 0.5% DMSO.

Application of Treatments used in the study

Systemic Pentostam® application

I.M. Pentostam®: 20 mg/kg body weight with a maximum of 850 mg/day for 20 days.

I.V. Pentostam®: 20 mg/kg body weight daily for 20 days.

I.L. Pentostam®: For the treatment of acute oriental sore, the injection is infiltrated around the edges of the lesion. For simple lesions, 0.5 - 1.0 ml up to a maximum of 2 ml was injected at one time once a week for 8 weeks, while, complicated lesions needed up to 24 injections.

Topical Pentostam® application

The prepared formulations (2% Pentostam® and 2% Pentostam® + 0.5% DMSO in different bases) were applied twice daily for 20 days after washing with potassium permanganate 1/8000 solution. Other supportive medications were also allowed during the trials in case of secondary infection in the lesion accompanying the disease. Those were administered twice daily. In severely infected patients oral antibiotic use was allowed with the topical Pentostam® treatment, e.g., Erythromycin 250 mg tablet every six hours and septrin two tablets twice daily. In the case of lymphatic involvement, pain, and fever, paracetamol

and ibuprofen tablets were prescribed as necessary.

Antifungal preparations

These included a combination of topical and oral preparations. Ketoconazole 2% cream was applied twice daily for 29 days and ketoconazole tablets were taken simultaneously as 600 mg/day for the same period.

Topical antibiotics

These included tetracycline ointment 3%, chloramphenicol ointment 1%, gentamycin cream1%, and fusidic acid ointment 2%. Their application was twice daily after washing with an antiseptic solution of potassium permanganate 1/8000.

Non-conventional Medications:

Tetracycline 3% (all in the form of ointments)

Tetracycline 3% + co-trimoxazole 4% + chloroquine 2%.

Tetracycline 3% + rifampicin 1% + chloroquine 2% + metronidazole 1%.

Tetracycline 3% + chloroquine 2% + metronidazole 2% + co-trimoxazole 2%.

Centamycin 1% + co-trimoxazole 4%.

All of the above regimens were prepared in Vaseline and applied twice daily for 4 weeks.

Patient's follow-up

During treatment, the patients were examined weekly for 8 weeks and followed up for up to 6 months, which is the maximum time for healing, noticing and recording the patient's compliance, and the size and the clinical stage of the lesions. The follow-up was continued until complete healing was achieved.

2.3 Laboratory studies

During the period of the study, 721 patients were selected and 58 patients did not report back. The Leishmania parasites were isolated from the lesions of 663 patients. The causative agent was found to be *L. major* (25%), *L. tropica* (35%), and *L. donovani* (40%). The three species were found to be associated with ulcerative and nodulo-ulcerative lesions that heal slowly leaving ugly areas. In all instances with *L. major*, *L. tropica,* and *L. donovani*, the parasite was detected in stained smear; the identification technique used was the morphological method. In this method, the parasites were cultivated in Tobies medium, and on the fourth day of cultivation, when the parasites were at the logarithmic phase, the cultures were examined under the microscope. The results revealed the promastigotes in very small size (about 7.5 μm) with very short flagella. That attempt of identification technique was the only one followed because it was simple and accessible with the available facilities. The other three

techniques that can be used are parasite micro-agglutination test, isoenzyme analysis, and excreted factor serotyping (EFS). For diagnosis and confirmation of the parasites, smear test was carried out, however, when this test failed and the lesion was a clinically active culture of the parasite, or biopsy was carried out. When the culture was still negative, histological examination was done: a section of the tissue was removed from the edges of the lesion and seen under the microscope. Intradermal granulomatous infiltrate indicated the presence of the parasite (**Table 1**).

Table 1: Pre-trial Parasitological results of CL confirmation

No. of patients entering the trials	No. pf patients who dropped out	Smear	Culture	Histology
721	58	558 +ve 181 -ve	161 +ve 20 -ve	20 +ve

Laboratory tests for blood, urine, and stools were performed for all the patients. Haemogram analysis showed that 70% of the patients have HB < 60%. On contrary, 50% of the patients showed TWBC above normal. Fifty percent of the patients presented with malaria and typhoid infections. Stool general analysis showed 60% of the patients were infected with guardia flagellates and 30% with other worms. From urine analysis, pus cells, RBCs, and yeast cells were observed in some of the patients.

Determination of the optimal concentration of Pentostam®

In order to determine the best concentration of Pentostam® to be used. Pentostam® was prepared in two different concentrations: 2% + 0.5% DMSO and 5% + 0.5% DMSO, both in aqueous cream. Fifty-four (54) patients who were clinically diagnosed and parasitologically confirmed as having CL participated in the study of determining the optimum concentration of topical Pentostam®.

1) Twenty patients were treated with 2% Pentostam® + 0.5% DMSO and referred to as (2/0.5) group.
2) Nineteen patients were treated with 5% Pentostam® + 0.5% DMSO and referred to as (5/0.5) group. Both groups were treated by applying the preparations for 20 days.
3) The other 15 patients have treated with tetracycline 3% ointment and referred to as a non-conventional therapy comparative group.

3. RESULTS AND DISCUSSION

3.1 Questionnaire Results Analysis

Geographical Distribution:

Our study findings showed that Khartoum areas demonstrated the highest incidence of the infection (318 patients)

followed by the Blue Nile area (219 patients), Omdurman (159 patients), and Khartoum North (53 patients) (**Figure 2**). In areas with a high incidence of infection, it has been observed that several factors contributed to the increased rate of infection. These factors include houses, water, and soil. Most of the people in these areas live in houses made of dried mud or locally made walls covered with animal faces. This provides a suitable environment for Sandfly. Also, the individuals live in very crowded conditions with several families living in one house or tent which suggests a great possibility of person-to-person transmission of the infection. Another important factor that contributes to the infection is water. The water supplies are different in these areas in which some places the drinking and washing water is obtained from wells, street taps, and rivers (e.g. Blue Nile area). These waters are normally contaminated and contribute to increasing the rate of CL infection. Moreover, these areas have high annual rainfall which leads to floods and puddles formation. This can be pointed to as a predisposing factor to the conditions of the high rate of CL infection since they provide a suitable environment for the vector. Also, the infected Soil is crossing wide areas of mud that cannot be avoided to expose individuals to the CL infection. On contrary, areas with a low incidence of the infection (Khartoum North) exhibit less crowdedness in houses, dry environmental conditions, and less rainfall. Therefore, it can be seen that the most affected areas were along the river Nile's banks since it provides the optimum geographical and ecological conditions for the breeding of Sandflies and Nile rats.

The Influence of the Occupation and Gender

As shown in **Figure 3**, it is clear that people who do fieldwork are more exposed and showed a high incidence rate (30%) of the infection than those who work in full-time jobs (5%). Military personnel also showed a high incidence of the infection (30%) as well as housewives (23%). Among those infected patients, men exhibited a higher percentage (60%) of infection compared to women (40%) due to the fact that men venture out through hard muddy conditions in work and are exposed to a high rate of infection where the Sand-fly is present.

Age Distribution:

The age group of the participated patients was ranging from 20 to 30 years that showed the highest incidence rate (27.3%) while the elderly aged 50 to 60 years showed the least incidence rate (8.8%). This is due to more exposure of the young to the infection than

the elderly. There is no significant difference observed between age groups 10 to 20 years and 20 to 40 years (about 19%). The relatively high incident rate falls in the age group 0 to 10 years and this can be attributed to the fact that children are more exposed to their bodies, especially in sleep (**Figure 4).**

Symptoms and Self-Treatment

The majority of the patients when they first report to the clinic were suffering from pain (approximately 60%) this is due to enlarged lymph nodes that causing discomfort and pain to the patients. Moreover, throughout the time of the study, it has been observed that there is a delayed reporting of infection. Approximately 60% of the patients present to the clinic after the one-month duration of the lesion. Some waited as long as 3 months (10%) which may be due to self-medication before seeking proper treatment. Self-medication includes herbal medication (Henna and tannins), topical medications, sprinkling the lesion with ashes of some plant seeds (e.g. Dome, Mango, and date), and heat application. About 15% of the patients reported to the clinic without any medication (**Figure 5**). Self-medication could deteriorate the condition of the lesion in some cases which becomes super infected and pruritogenic.

Number of Cases Reported Over the Months of the Year

The monthly records of CL infections over the months of the year indicate that in June, July, and August (summer season), the infection reached its peak, while the number of cases declined dramatically in the winter season (**Figure 6**) suggesting there are optimum weather conditions of the breading of Sand flies and Nile rats. These data were obtained from the records of the statistics department in the Ministry of Health. According to the above-mentioned data, we speculate that there was an underestimation of the actual number of cases reported. This is because reporting was not mandatory until the epidemic was passed or at its peak, besides, many people with minor lesions did not seek medical treatment, others went to private clinics, and some acquired drugs directly from private pharmacies or friends suffering from the same disease.

3.2 Determination of the optimal concentration of Pentostam (2%, and 5%)

To determine the optimal concentration of Pentostam in the topical preparation, two formulations were prepared that contains 2% and 5% of the drug and these two formulations were tested in small group. The results of the follow-up study showed the following: In the (2/0.5) Pentostam® treated

group, 15 out of the 20 patients (75%) were cured while in the (5/0.5) Pentostam® treated group (74.2%) were cured. Therefore, based on these results, 2% Pentostam®, with and without 0.5% DMSO, was chosen as the optimal concentration for the clinical trials since there is no need to use higher concentration with no significant difference in cure rate.

3.3 Clinical results associated with Pentostam® treatment

The percentage of the cure rate of 2% Pentostam® in different bases with and without DMSO and the types of lesions are given in **Table 2**, while the patient's compliance using the different Pentostam® formulations is given in **Table 3**. The poor cure rate with HWF ointment (37%) was due to the formation of an occlusion film on the oozing and weeping lesions instead of drying, which is required in that skin condition. Poor patient compliance was due to its greasy nature and local irritation effects. The addition of DMSO increased the cure rate by 5%.

Pentostam® in PEG ointment (PEG 4000: 300 in 50% solid content concentration) showed moderate efficacy in both dry and wet superficially ulcerated lesions (~ 60%). Patients' compliance was good (76%).The addition of DMSO slightly increased the activity by approximately 2%. PEG is known to decrease the antibacterial activity of some antibacterial agents, e.g., phenol, and penicillin and bacitracin. Reduction of the antileishmanial activity of Pentostam by PEG ointment may account for the low cure rate observed in this study.

The cure rate of Pentostam® in aqueous cream was higher (72%) compared to the other formulations. Patients' compliance increased with the aqueous formulations (88%). These observations can be attributed to the formation of non-occlusive film by the aqueous cream on the surface of the lesion which enhanced the evaporation of the water and also enhanced drug absorption through the lesion. The addition of DMSO resulted in a significant increase of efficacy (by 7.6%) with an increase in patients' compliance by 2%. The absence of local irritation, the smooth texture, easiness of application, and no greasiness feeling were all factors that have contributed to the high compliance rate.

Pentostam® gel formulations were found to have nearly the same cure rate as the aqueous cream formulations (75 %) with a comparable compliance rate (90 %). The addition of DMSO resulted in a similar increase in efficacy to that of the aqueous cream (by 7% and 8%, respectively). The rapid drying property of the gel was

advantageous as far as healing of the lesions was concerned but poses storage problems. The patients' poor adherence to the "keep container tightly closed in a cool place" directions, always resulted in the gel sticking to the walls of the container. This drawback of the gel preparations gave preference for the aqueous cream preparations in treatment and use in comparative studies with other antileishmanial medications.

Table 4 gives the cure rates and patients' compliance with topical Pentostam® and some conventional and non-conventional preparations. The cure rate of topical Pentostam was only surmounted by systemic Pentostam (I.M., I.V., and I.L.), However, when considering the disadvantages of these preparations regarding the pain they cause and the systemic side effects, the Pentostam® topical preparations were to be preferred.

3.4 Pharmacokinetics ADME Results of Pentostam®

A computational study was performed to evaluate the absorption, distribution, metabolism and excreation (ADME) using SwissADME webserver. The obtained results of the pharmacokinetics ADME analysis of pentostam is summarized in the **Table 5**.

The cure rate, and the compliance rate in percentage for the prepared formulations were demonstrated in **Table 6**. Overall, the cure rate with 0.5% DMSO were found to enhance the cure rate with 5%. Therefore, it is recommended to examine and analyze the five different formulations with 2% Pentostam + 0.5% DMSO in different bases. On contrary, the Pharmacokinetics ADME analysis **[28-30]**. demonstrated that Pentostam is a highly hydrophilic compound with -5.3 LogP value, and having a high water solubility with -0.5 LogS value. This results confirm the drawn conclusion that the aqueous cream and the hydrogel formulations showed the best cure rate with 72% and 75% respectively. Moreover, the Log Kp (skin permeation) value was found to be -13.72 cm/sthat supports the fact that the drug has high skin permeation ability. Therefore, the formulation that contains a highly lipophilic bases resulted in low permeability to the skin, which was demonstrated with low cure rate with Hydrous wool fat and Polyethylene glycol ointments, whereas, incorporation of the drug in hydrophilic formulation increased the homogeneity and compatibility, thus resulted in higher cure rates.

Based on **Table 6**, it is clear that with the 2% Pentostam® and 0.5% DMSO in aqueous cream and gel bases were found to be the most efficacious clinically in comparison to the other bases and the conventional and non-

conventional regimens currently were used during the epidemic outbreak of the disease. The efficacy was assessed by the detection of the absence of L.D bodies from the lesions. The comparative studies showed that Pentostam topical formulations were only second in efficacy to systemic Pentostam with the obvious advantages of overcoming the drawbacks of the latter.

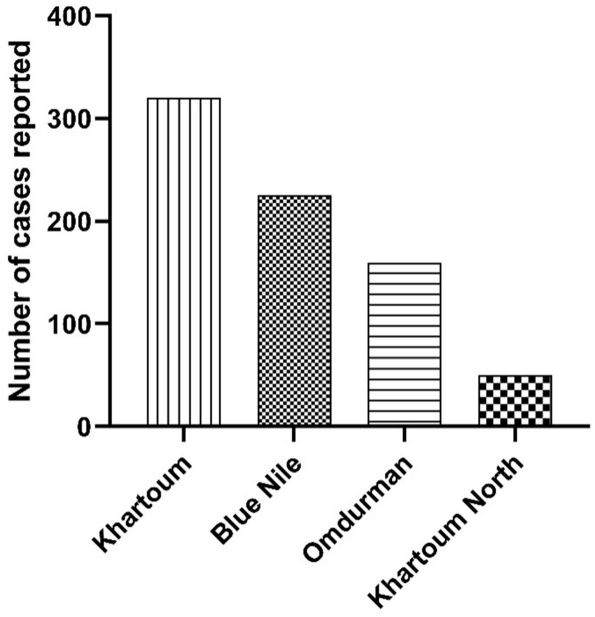


Figure 2: Distribution of Cases in Four Areas of Khartoum Province

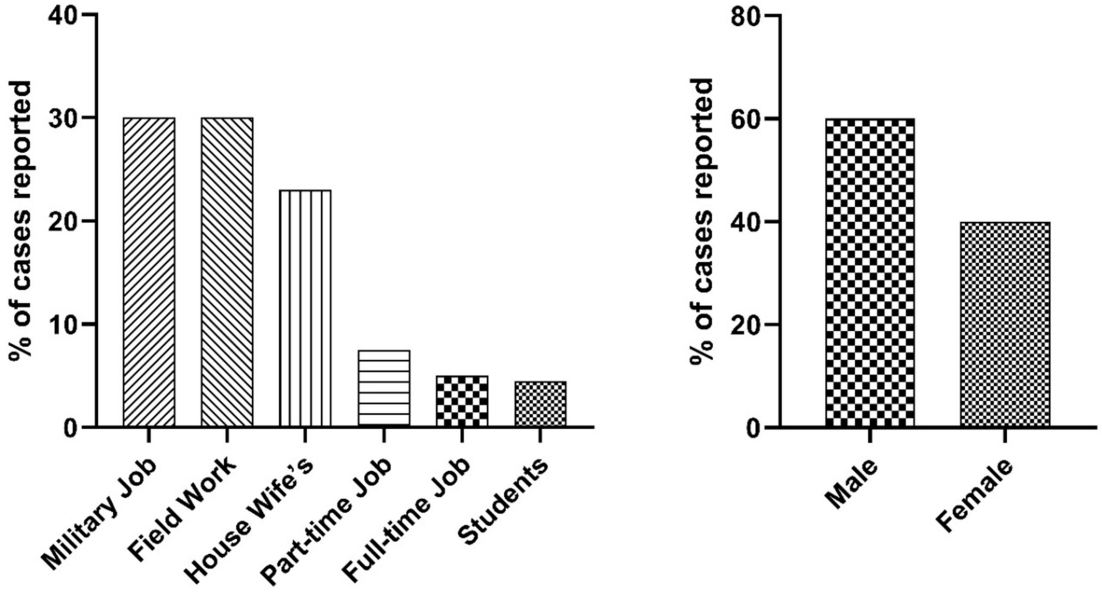


Figure 3: Occupations and Sex Distribution

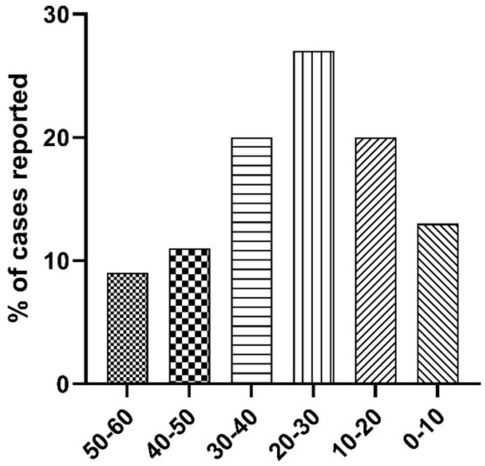


Figure 4: Distribution of Age Categories

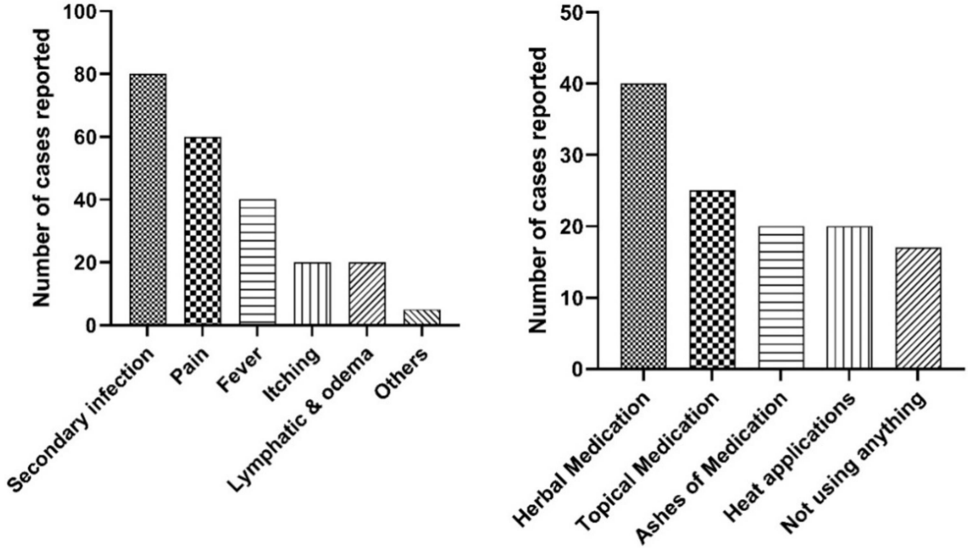


Figure 5: Percentages of Symptoms and Pre-medication Use

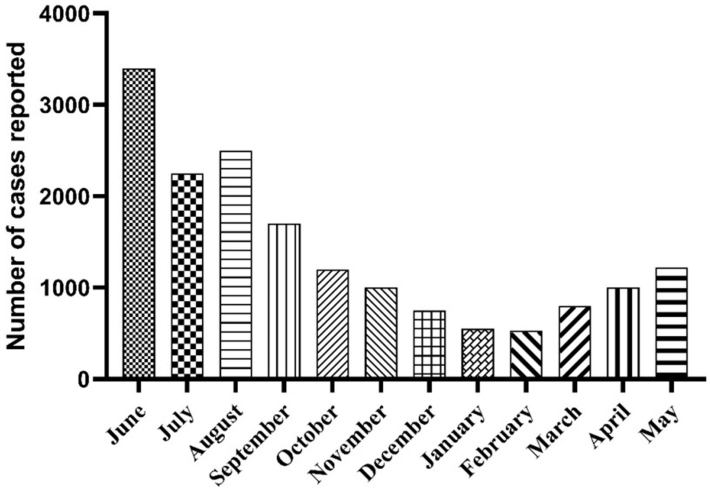


Figure 6: The number of Cases over the Months of the Year

Table 2: Different types of lesions and the percentage of the cure rate of 2% Pentostam® with and without DMSO in different bases

Formulation	Ulcerative lesion	Nodulo-ulcerative lesion	Nodular lesion	Plaque lesion	Others, fungating, warty	No. of lesions at beginning of the trial	No. of lesions showing complete healing	Percentage cure rate at the 6th week
2% Pentostam® in different bases								
HWF	46	23	15	9	12	105	40	37
PEG 4000: 300 (1/1)	36	24	15	9	10	94	58	62
PEG 4000: 400 (1/1)	38	22	13	11	8	92	56	60
Aqueous cream	55	20	17	10	8	110	80	72
Gel	44	23	19	8	9	103	77	75
2% Pentostam® + 0.5% DMSO in different bases								
HWF ointment	43	20	11	11	9	94	38	43
PEG 4000: 300 (1/1)	81	43	20	27	9	180	114	63
PEG 4000: 400 (1/1)	32	25	23	6	5	90	55	61
Aqueous cream	55	28	15	6	10	123	98	80
Gel	46	20	12	11	9	98	80	82

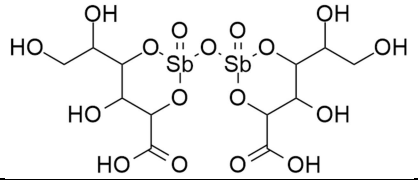
Table 3: Patients percentage compliance using different Pentostam® formulations in the clinical trials for the treatment of cutaneous leishmaniasis

Formulation	No. of patients who entered the trial	No. of patients at the end of the trial	Patients with single lesions	Patients with multiple lesions	% patient compliance
2% Pentostam® in different bases					
HWF ointment	50	28	30	20	56
PEG ointment 4000:300 (1/1)	50	38	38	18	76
PEG ointment 4000:400 (1/1)	50	40	40	19	80
Aqueous cream	50	44	21	39	88
Gel	50	45	24	26	90
2% Pentostam® + 0.5% DMSO					
HWF ointment	50	30	17	23	60
PEG ointment 4000:300 (1/1)	50	40	62	24	80
PEG ointment 4000:400 (1/1)	50	39	23	27	78
Aqueous cream	50	47	20	30	94
Gel	50	46	32	18	92

Table 4: Patients used conventional and non-conventional regimens in the treatment of cutaneous leishmaniasis

Regimen	No. of patients who entered the trial	No. of patients who completed the trial	No. of lesions at the end of the trial	No. of lesions which completely healed after 6 weeks of the start of treatment	Percentage cure rate	Patient compliance
I. Systemic Pentostam						
I.M.	9	8	32	27	84	88
I.V.	10	9	60	49	63	90
I.L.	10	8	55	45	62	80
II. Topical antibiotics						
-Tetracycline 3% ointment	10	6	30	1	7	60
Chloramphenicol 1% ointment	10	5	25	1	4	50
- Fusidic acid 1% ointment	10	7	40	3	8	70
- Gentamycin 1% cream	10	6	25	3	12	80
- Wet dressing potassium permanganate 1/8000 solution	10	5	40	1	3	50
III. Topical steroids with antibiotics						
- Hydrocortisone 1% cream	5	4	20	2	10	80
- Hydrocortisone 1% + chloramphenicol 1% ointment	2	1	12	2	17	50
- Fluocinolne + Neomycin cream	3	3	6	1	17	67
IV. Topical and oral antifungal						
- Ketoconazole 2% cream combined with oral ketoconazole	10	8	20	12	12	80
Chloroquine (I.L.)	10	6	25	12	12	60

Table 5: ADME properties for Pentostam® using Swiss ADME Webserver

Chemical Structure	Swiss ADME		
	Lipophilicity	Solubility	Log Kp (skin permeation)
	(WLOGP) -5.3	Log S (Ali) -0.5	-13.72 cm/s

Recommended range: molecular weight (MW) ≤ 600, lipophilicity log (log P) ≤ 5, aqueous solubility descriptor (log S) ≤ 0, Skin permeability coefficient Log Kp (the more negative value, the less skin permeability)

Table 6: The cure rate, and the patient compliance rate in percentage (%) for the prepared formulations

#	Formulation composition	Cure rate (%)	Patient compliance (%)
2% Pentostam in different bases			
01	HWF	37	37
02	PEG 4000: 300 (1/1)	62	62
03	PEG 4000: 400 (1/1)	60	60
04	Aqueous cream	72	72
05	Gel	75	75
2% Pentostam + 0.5% DMSO in different bases			
06	HWF ointment	43	60
07	PEG 4000: 300 (1/1)	63	80
08	PEG 4000: 400 (1/1)	61	78
09	Aqueous cream	80	94
10	Gel	82	92

5. CONCLUSION

The present study makes several noteworthy contributions to understanding the leishmaniasis and what are the factors that could significantly contribute to the disease progression. The current results showed that leishmaniasis are most common in the young men ranging from 20 to 30 years that work in field especially during summer season. Also, in most of the cases, patients suffer from pain and secondary infections that led them to self-treatment with herbs. Moreover, the formulations results demonstrated that with the 2% Pentostam® and 0.5% DMSO in aqueous cream and gel bases were found to be the most efficacious clinically in comparison to the other bases and the conventional and non-conventional regimens currently were used during the epidemic outbreak of the disease. The comparative studies showed that Pentostam®topical formulations were only second in efficacy to systemic Pentostam®.

Additionally, the cure rate with the addition of 0.5% DMSO were found to increase the cure rate by 5%. The ADME analysis demonstrated that Pentostam® is a highly hydrophilic compound and aqueous cream and the hydrogel formulations shows the best cure rate with 72% and 75% respectively. Thus, these results suggest that topical Pentostam® formulations could provide cure from leishmaniasis without systemic side effects which suggest further optimization and development of these topical formulations is warrented.

DATA AVAILABILITY STATEMENT:

The data associated with a paper is available, and under what conditions the data can be accessed.

FUNDING STATEMENT:

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

PATIENT CONSENT AND ETHICS

The study was approved by the Research Unit of Khartoum Ministry of Health and the Hospital administration. All respondents gave verbal consent prior to participating in the study. All studies involving people, medical records, and human tissues, in accordance with the Declaration of Helsinki Informed.

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