STUDIES ON THERAPEUTIC EFFECTS OF MANGHOPIR HOT WATER

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Abstract

Therapeutic effect of spring water on known human pathogens revealed that the water had highest inhibitory action on micro-organisms in its fresh state, while aged spring water promoted the bacterial growth. It was observed that the high temperature of spring water was an important inhibitory factor. The chemical constituents of the spring water were not found to be of therapeutic value in the quantities present in these waters. Arsenous trioxide, one of the mineral contents of these waters had inhibitory effect on Staph. albus, strept. pyogenes, B. anthracis and Staph aureus in 0.001%.

Observations suggested that apart from the effect of temperature shock, there was a little inhibitory effect of the mineral contents to combine therapeutic properties of Manghopir spring water. Studies on the presence of bacterial viruses are suggested (JPMA 29:262, 1979).

Introduction

Hot water springs besides their geological and geographical value, have shown to have an important clinical and pathological importance.

Various hot water springs have been subjected to investigation on the therapeutic properties. Many workers in the last three decades have observed the effect of such waters in certain infections and skin diseases. Such waters have also been tested against certain micro-organisms (Yasunaga, 1953; Dostrovsky and Sagher, 1959; Condo Garcollo, 1959; Asauliuk and Kholmianskii, 1962)

Chemical analysis of hot springs to know the reasons of their therapeutic values, were conducted by a number of workers during the last two decades. Dostrovsky and Sagher (1959) reported a thermal spring containing sulphur and Randon. Presence of Randon made the water radioactive. This report did not show the presence of any other minerals. Springs containing radioactive substances have been reported by other workers (Schynoll & Chattergee, 1958). Sakanous et al. (1961) carried out the chemical study on a hot water spring in Japan. They reported the presence of chlorides, carbonates, bicarbonates and calcium alongwith Arsenic, Uranium and Randon.

Sulphur and sulphates of calcium, Magnessium and sodium have been shown in a number of reports (Yasunaga, 1953; Boerker and Staib, 1958; Condo Garcolio, 1959; Pereira, 1960). Many such reports on chemical analysis of hot springs show the presence of Arsenic compounds (Ritchia, 1961; Sakanous et al., 1961; Shah et al., 1964). Exact nature of therapeutic value and reason of this property was not described by any of those workers.

Pithwalla (1938-39) reported the therapeutic importance of hot water springs of Manghopir, Karachi. He stated that it was due to the presence of Sulfurated Hydrogen while Shah and others (1964) did not report any sulfurated hydrogen present in these water. They had shown the presence of a number of inorganic salts including sulphates and chlorides of calcium, sodium, potassium, magnesium and arsenic compounds. They did not report any bacteriological work, however, suggested that the therapeutic value of these waters might be attributed to the presence of Arsenic compounds in it.

The present work was done to investigate the nature and extent of therapeutic value of

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these waters. In this regard the effect of spring water on different common human pathogens under varying conditions was studied.

Material and Methods

At Manghopir, Karachi, number of hot springs are present. Springs located some distance away from the main spring was chosen for collection of samples. This precaution was taken to avoid contamination.

Sterilized glass containers and sterile thermos flasks were used for collection of fresh samples. Collection was always made early in the morning. The amount of each sample for analysis was about 50 ml. Samples were analysed bacteriologically 1-2 hours after the collection. The delay was due to the time taken in transporting the sample to the laboratory.

Test Organisms: Since these waters were believed to have some therapeutic effect on many skin infections, the organisms were chosen which generally are skin pathogens to man. Certain bacteria related to systemic infections were also included. Following is the list of test organisms.

Bacteria: *Staph. aureus; Staph. albus; Strept. pyogenes; B. anthracis; Salmonella typhi; Shigella dysenteraie; Cl. histolyticum.*

Fungi: *Trichophyton rubrum; Trichophyton cutaneum; Microsporum gypseum; Candida albicans.*

Standardization of Culture: Standardization of culture was performed by matching the culture suspension with the first tube of turbidometer which contained approximately 300,000,000 cells/ml and further diluted in water blanks upto 1/10,000 so that the number of organisms remained 30,000/ml. From this 1 ml was added to 9 ml of sterile spring water and saline controls separately. This gave a concentration containing 3,000 cells/ml. It was taken as the initial count, however *B. anthracis* was further diluted to get a number of 1500 cells/ml.

Exposure of test organisms to spring water: One set of each culture was kept at 47°C and the other at room temperature (25°C). 0.1 ml of suspension from each tube was drawn with the help of sterile pipettes and spreaded over the medium. Suspensions were drawn at interval of 1 minute, 10 minutes, 20 minutes and 30 minutes. Growth of test organisms on the medium made in fresh spring water was observed. For bacteria, Heart Infusion Agar (Difco) and for fungal growth Sabouraud's Dextrose Agar was used. Controls were also tested simultaneously.

Effects of various chemicals on test organisms: Different chemicals already reported in the chemical analysis of water (Shah et al, 1964) were tested against test micro-organisms. Heart Infusion Agar (Difco) and S.D.A were incorporated separately with the following: (1) Sodium sulphate 0.5%, (2) Magnesium sulphate 0.5%, (3) Arsenous trioxide 0.1%, 0.01% and 0.001% separately, (4) Mixture of chemicals containing sodium sulphate 0.5%, Magnesium sulphate 0.5% and Arsenous trioxide 0.1%, 0.01% and 0.001% separately. Controls of Heart Infusion Agar and S.D.A. were used containing no extra chemicals.

Results and Discussion

The therapeutic effect of spring water on test organisms was observed in vitro. Fresh as well as aged water used in standard initial count of 3000 cells/ml except *B. anthracis* which was used in 1500 cells/ml stocks. The results are recorded in Table I a & b and II a & b.

Media made in fresh spring water were used for observing any effect on growth of test organisms. Results are recorded in Table III and III a.

Results of effect of various chemicals on test organisms are recorded in Table IV and V.

A number of human pathogens related to skin and systemic diseases were used to observe the effect of spring water exposure in vitro. Some interesting results were observed (Table I a & b, II a & b). Test bacteria in standard dilutions were exposed to fresh, 15 days and 30 days aged spring water. All the tests were run in duplicates, one set being kept at 47°C (the mean temperature of spring water) and other at 25°C (room temperature). Varying conditions showed valuable results. Fresh water, 15 days aged and 30 days aged waters at 47°C had inhibitory effect on all the test bacteria; however the per cent inhibition was lowered in 15 days and 30 days aged waters as compared to that in fresh water test. Fresh water at 25°C showed very little inhibitory action on the test bacteria and an enhancement of growth of *Candida albicans* and *B. anthracis* was seen. 15 days and

TABLE-I a : EFFECT OF FRESH SPRING WATER AND AGED SPRING WATER ON TEST ORGANISMS AT 25°C

Name of Organisms	Initial Number/ml	1 minute*						10 minutes					
		Fresh spring water		15 days Aged spring water		30 days Aged spring water		Fresh spring water		15 days Aged spring water		30 days Aged spring water	
		Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
Staph. aureus	3000/ml	1.3% D	2.6% I	1.3% I	1.3% D	4.0% I	2.6% I	4.0% D	1.3% D	4.0% I	1.3% D	3.3% I	0.0% -
S.typhi albus	"	3.3% D	4.0% I	2.6% I	2.6% D	1.3% I	1.3% D	3.3% I	3.0% D	0.0% -	1.3% D	4.6% I	1.6% I
Strept. pyogenes	"	1.3% D	1.3% D	1.3% D	4.0% I	5.3% I	1.3% I	2.0% D	2.6% I	4.0% I	6.6% D	2.0% I	2.3% I
B.anthracis	1500/ml	1.3% I	2.6% I	2.6% I	1.3% D	4.0% I	1.3% I	1.3% D	5.3% I	0.0% -	2.6% I	0.0% -	2.6% I
S.typhi	3000/ml	4.0% I	2.6% I	4.0% I	1.3% D	5.3% I	6.6% I	10.0% D	1.0% I	1.3% I	4.0% I	6.0% I	5.6% I
Shigella dysenteriae	"	2.6% I	6.6% I	1.3% I	0.0% -	6.6% I	2.6% I	9.6% D	4.0% I	5.3% I	1.3% I	9.3% I	0.0% -
Cl.histolyticum	"	2.6% I	1.3% I	4.0% I	2.6% I	1.3% D	0.0% -	6.0% D	1.3% I	2.6% I	1.3% I	4.6% I	1.6% I
Candida albicans	"	5.3% I	2.6% I	1.3% D	2.6% I	2.6% D	4.0% I	1.3% D	1.3% D	3.3% I	0.0% I	5.3% I	4.0% D

* = Exposure time of spring water and saline control.
D = Decrease in Number of Bacteria.
I = Increase in Number of bacteria.

TABLE-I b :EFFECT OF FRESH SPRING WATER AND AGED SPRING WATER ON TEST ORGANISMS AT 25°C

Name of Organisms	Initial Number/ml	20 minutes						30 minutes					
		Fresh spring water		15 days Aged spring water		30 days Aged spring water		Fresh spring water		15 days Aged spring water		30 days Aged spring water	
		Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
Staph. aureus	3000/ml	6.6% D	2.6% D	18.0% I	1.3% I	17.6% I	1.5% I	4.6% D	0.0% D	22.0% I	1.3% I	24.6% I	0.6% I
Staph. albus	"	6.6% D	8.0% D	2.6% I	14.0% I	9.0% I	0.0% I	10.6% D	2.0% I	16.0% I	2.6% I	17.6% I	2.3% I
Strept. pyogene.	"	6.0% D	5.3% I	10.6% I	8.0% I	7.6% I	4.0% I	2.0% D	4.0% I	13.3% I	4.6% I	13.6% I	4.3% I
B. anthracis	1500/ml	8.0% I	2.7% I	8.0% I	2.6% I	6.8% I	9.0% I	10.0% I	8.0% I	14.7% I	9.3% I	15.0% I	8.6% I
S.typhi	3000/ml	13.3% D	4.0% I	9.3% -	10.6% I	16.6% I	6.0% I	13.3% D	9.0% I	30.6% I	9.3% I	38.6 I	9.0% I
Shigella dysenteriae	"	14.0% D	2.0% I	13.3% I	10.6% I	21.3% I	3.3% I	22.6% D	8.0% I	34.6% I	9.3% I	35.3% I	8.6% I
Cl.histolyticum	"	10.0% D	5.0% I	9.3% I	1.3% I	8.0% I	4.6% I	8.0% D	8.6% I	12.3% I	8.6% I	15.0% I	9.3% I
Candida albicans	"	5.3% -	3.3% I	12.0% I	6.6% I	9.0% I	1.7% D	2.0% I	3.3% I	17.3% I	1.3% I	18.0% I	2.0% I

D = Decrease in Number of Bacteria.
I = Increase in Number of Bacteria.

30 days aged water at 25°C exhibited marked increase in the growth of all the test organisms. Yasunaga (1953), while working with spring water in Japan found that the water had enhancing properties on bacterial growth specially on *Salmonella typhi*. He also stated that longer the period of aging of water from its collection, the heavier the growth of *S. typhi*. Almost similar results were observed during the present studies, and all the test organisms showed more growth when exposed in 30 days aged water than that on exposure to 15 days aged

water. Aged sea water stored in glass bottles or in flasks in the dark for few weeks was recommended for use in preparation of media for study of marine micro-biology (Khatija 1969). It might be due to decomposition of organic matter present in it, further the Biomass already present in it served as a nutrient. Parallel possibilities are likely for the spring water aging phenomenon.

TABLE-II a : EFFECT OF FRESH SPRING WATER AND AGED SPRING WATER ON TEST ORGANISMS AT 47°C

Name of Organisms	Initial Number/ml	5 minutes*						10 minutes					
		Fresh spring water		15 days Aged spring water		30 days Aged spring water		Fresh spring water		15 days Aged spring water		30 days Aged spring water	
		Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
Staph. aureus	3000/ml	3.0% D	1.3% I	2.6% D	0.0% D	4.0% D	1.3% D	10.6% D	28.0% D	11.3% D	13.3% D	9.3% D	12.3% D
Staph. albus	"	3.6% D	2.6% I	4.0% D	1.3% I	3.3% D	1.3% I	24.0% D	14.6% D	17.3% D	8.6% D	9.6% D	15.0% D
Strept. pyogenes	"	5.3% D	4.0% D	5.3% D	3.6% D	6.0% D	1.0% D	20.3% D	20.0% D	22.0% D	10.6% D	5.6% D	13.3% D
B.anthracis	1500/ml	1.6% D	1.4% D	1.3% D	0.6% I	1.3% I	0.6% I	10.6% D	4.0% D	6.0% D	4.6% D	2.6% D	3.0% D
S.typhi	3000/ml	8.0% D	6.0% D	6.6% D	3.3% D	5.3% D	1.6% I	22.0% D	29.3% D	16.0% D	21.3% D	12.3% D	12.6% D
Shigella dysenteriae	"	6.6% D	8.0% D	5.3% D	6.6% D	4.3% I	4.0% I	8.0% D	9.3% D	12.6% D	11.3% D	15.0% D	12.3% D
Cl.histolyticum	"	4.0% D	2.6% D	4.0% D	1.3% D	1.3% D	4.3% D	16.0% D	14.0% D	7.1% D	6.0% D	1.3% D	6.6% D
Candida albicans	"	9.3% D	8.0% D	8.0% D	4.0% D	6.6% D	4.0% D	12.0% D	10.6% D	6.0% D	7.3% D	3.3% D	14.3% D

* - Exposure time of spring water and saline control.
D - Decrease in number of Bacteria
I - Increase in number of Bacteria.

TABLE-II b : EFFECT OF FRESH SPRING WATER AND AGED SPRING WATER ON TEST ORGANISMS AT 47°C

Name of Organisms	Initial Number	20 minutes*						30 minutes					
		Fresh spring water		15 days Aged spring water		30 days Aged spring water		Fresh spring water		15 days Aged spring water		30 days Aged spring water	
		Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
Staph. aureus	3000/ml	28.0% D	32.6% D	32.0% D	29.3% D	21.3% D	30.6% D	59.3% D	50.0% D	40.0% D	47.3% D	38.0% D	48.6% D
Staph. albus	"	34.6% D	20.0% D	28.0% D	14.0% D	26.6% D	26.0 D	51.3% D	37.3% D	41.3% D	38.6% D	39.3% D	38.3% D
Strept. pyogenes	"	38.0% D	35.3% D	26.0% D	22.3% D	27.0% D	32.3% D	56.0% D	42.0% D	37.3% D	40.0% D	33.3% D	41.6% D
B.anthracis	1500/ml	16.0% D	6.6% D	12.3% D	21.3% D	10.6% D	11.3% D	27.3% D	18.6% D	20.0% D	19.0% D	20.0% D	17.6% D
S.typhi	3000/ml	54.6% D	52.6% D	38.0% D	32.0% D	17.0% D	38.0% D	65.3% D	50.6% D	51.3% D	57.3% D	41.3% D	58.0% D
Shigella dysenteriae	"	28.0% D	22.6% D	33.3% D	30.6% D	27.0% D	23.6% D	45.3% D	40.0% D	41.0% D	39.6% D	40.6% D	40.3% D
Cl.histolyticum	"	23.3% D	18.0% D	18.6% D	21.3% D	11.6% D	20.3% D	33.3% D	26.0% D	22.0% D	26.6% D	31.0% D	26.3% D
Candida albicans	"	18.6% D	22.6% D	15.3% D	24.0% D	9.0% D	22.6% D	28.6% D	33.3% D	27.3% D	32.6% D	20.6% D	32.3% D

* - Exposure time of spring water and saline control
D - Decrease in number of Bacteria
I - Increase in number of Bacteria.

TABLE-III : GROWTH OF TEST ORGANISMS ON SPRING WATER MEDIA

Name of Organisms	Initial count/ml	Test medium	Control
Staph. aureus	3000/ml	3.6% D	1.6% I
Staph. albus	"	2.2% D	3.4% I
Strept. pyogenes	"	5.5% D	2.0% D
B. anthracis	1500/ml	3.4% I	2.3% I
Salm. typhi	3000/ml	6.3% I	3.8% I
Sh. dysenteriae	"	8.6% I	4.3% I
Cl. histolyticum	"	9.6% D	3.7% D
Candida albicaus	"	6.1% I	

D - Decrease in growth of test organisms.
I - Increase in growth of test organisms.

TABLE-III a :GROWTH OF TEST DERMATOPHYTES ON SPRING WATER MEDIUM

Name of organisms	Test medium mean	Control mean	Remark
Trichophyton rubrum	3.1 Cms.	3.2 Cms.	3.1% D
Trichophyton cutaneum	2.8 Cms.	2.6 Cms.	7.7% D
Microsporum gypseum	3.7 Cms.	3.9 Cms.	5.1% D

D - Decrease in growth of test organisms.

TABLE-IV :EFFECTS OF VARIOUS CHEMICALS ON TEST ORGANISMS

Name of Organisms	Initial No./ml	Control	$MgSO_4$ 0.5%	Na_2SO_4 0.5%	AS_2O_3 alone 0.1%	0.01%	0.001%	AS_2O_3 in mixture 0.1%	0.01%	0.001%
Staph. aureus	3000/ml	4.1% I	1.4% D	6.4% I	100% D	100% D	99.1% D	100% D	100% D	96.2% D
Staph. albus	"	3.0% I	5.7% I	2.0% I	100% D	100% D	100% D	100% D	100% D	100% D
Strept. pyogenes	"	7.0% I	1.6% D	9.7% I	100% D	100% D	100% D	100% D	100% D	99.2% D
B. anthracis	1500/ml	3.4% I	2.3% D	9.2% I	100% D	100% D	100% D	100% D	100% D	99.2% D
Salm. typhi.	3000/ml	3.9% I	Inm% I	Inm% I	100% D	100% D	93.6% D	100% D	100% D	91.7% D
Sh. dysenteriae	"	3.6% I	Inm% I	Inm% I	100% D	100% D	91.9% D	100% D	100% D	36.9% D
Cl. histolyticum	"	5.1% I	4.9% I	3.3% I	100% D	100% D	86.9% D	100% D	100% D	78.5% D
Candida albicans	"	1.3% I	3.5% D	12.3% D	100% D	100% D	69.6% D	100% D	100% D	53.5% D

D - Decrease in growth of test organisms
I - Increase in growth of test organisms
Inm - Immeasurable.

TABLE-V : EFFECT OF VARIOUS CHEMICALS ON DERMATOPHYTIC FUNGI

Name of Fungi	Control	Na_2SO_4		$MgSO_4$		As_2O_3 alone						As_2O_3 in mixture					
		0.5%	Remark	0.5%	Remark	0.1%	Remark	0.01%	Remark	0.001%	Remark	0.1%	Remark	0.01%	Remark	0.001%	Remark
Trichophyton rubrum	3.2	3.3	3.1% I	3.2	No Change	2.8	12.5% D	3.0	6.2% D	3.0	6.2% D	2.9	9.3% D	3.1	3.1% D	3.1	3.1% D
Trichophyton cutaneus	2.8	2.7	3.6% D	2.9	1.8% I	2.6	7.1% D	2.7	3.6% D	2.7	3.6% D	2.5	10.7% D	2.7	3.6% D	2.8%	No change
Microsporum gypseus	3.1	3.2	3.2% I	3.1	No change	2.8	9.6% D	2.9	6.4% D	3.0	3.2% D	2.8	9.7% D	3.1	No Change	3.1	No change

* = Measurement of fungal colouring in Ums.
D = Decrease in growth
I = Increase in growth.

The growth of test organisms on spring water medium and control medium was also observed. It was noted that no significant change occurred in the growth of any test organisms. The inhibition of many bacteria to a minor degree was observed suggesting that there was very little inhibitory effect of chemicals present in the water under the test conditions.

Certain chemicals reported in the water (Shah et al., 1964) were tested against the test organisms. Sodium sulphate 0.5% and Magnessium sulphate 0.5% showed varying effects on various organisms growth except the *Candida albicans* which was decreased by 12.5% in sodium sulphate and *Staph aureus, Strept. pyogenes. B. anthracis* and *Candida albicans* were inhibited to a degree ranging from 1.4% to 3.5% in Magnessium sulphate. Boerker and Staib (1958) while working on water containing sodium sulphate and Magnessium sulphate, reported that *E. coli* was markedly increased by the treatment with water and an inhibition of *Proteus species* was observed. In the present work it was noted that medium containing sodium sulphate and Magnessium sulphate markedly enhanced the growth of *Salmonella typhi* and *Sh. dysenteriae*. *Candida albicans* showed little decrease in Sodium sulphate and Magnessium sulphate. Other bacteria showed no significant change. Dermatophytic fungi showed little fluctuations from 3.2% increase to 3.6% decrease, showing that the chemicals had no appreciable effect on dermatophytes.

Arsenous trioxide in the concentration of 0.1%, 0.01% and 0.001% and in mixture with sodium sulphate 0.5% and Magnessium sulphate 0.5% showed valuable results. In the concentration of 0.1% and 0.01%, it was inhibitory to all the test bacteria and *Candida albicans*. However 0.001% of Arsenous trioxide alone and in mixture showed varying results. 99.1% inhibition of *Staph. aureus* was observed in 0.001% Arsenous trioxide. The same concentration showed inhibition of 69.6% — 93.6% for *S. typhi, Sh. dysenteriae, Cl. histolyticum*, and Candida albicans. A total inhibition of *Staph. albus, Strept. pyogenes* and *B. anthracis* was observed. The same concentration in mixture showed a decreased percentage of inhibition and the only bacterium inhibited completely was *Staph. albus*. The lowered inhibition of test organisms by Arsenous trioxide in mixture suggest the enhancing effect of sodium sulphate and Magnessium sulphate. Varying degrees of inhibition of test dermatophytes were observed by varying percentages of Arsenous trioxide alone as well as in mixture. The inhibition to some extent in Arsenous trioxide alone and in the mixture suggests that the presence of sodium sulphate and Magnessium sulphate had no appreciable effect on the growth of dermatophytes.

The concentration of Arsenous trioxide used in the present work was considerably higher than that already reported to be present in the water, i.e. 0.003 p.m. (Shah et al., 1964). It seems that the therapeutic role of different chemicals in spring water including arsenic

compound was negligible. The therapeutic effect of spring water as observed was mostly due to the temperature of water as the saline control also, showed inhibitory action on all the test organisms though to a lesser degree than that by spring water at 47°C, suggesting that there was, but a very little therapeutic effect of chemicals present in the spring water. The role of such chemicals on skin is so far uncertain. Cure of non-infectious diseases including Psoriasis and Neurodermatitis was a matter of pathological interest. Arrighi (1960) treated Psoriasis with Arsenics. The actual therapeutic value of the spring water is not known, but it has been observed that cases of Psoriasis and Neurodermatitis responded to the regular exposure to spring water.

It seemed likely that a combined effect of temperature shock and chemicals present in the water might be responsible for its therapeutic value. In this connection role of Bacteriophages may not be neglected. Though, no plaques were observed during this study it might be possible that Bacteriophages might play an important role in the therapeutic values of hot springs. However, further studies on this aspect are suggested.

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