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IMMUNOMODULATING, PROPHYLACTIC, AND THERAPEUTIC POTENTIAL OF NATURAL PHAGES IN GANGA WATER

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ABSTRACT

The present paper explores the immunomodulating, prophylactic, and therapeutic potential of Ganga water. Ganga water is reported to have 200 isolates of phages which is ten times more than Yamuna and Narmada. This diversity of phages suggests that these may act as a wide-spectrum phage cocktail. Forty-five Volunteers administered Ganga water to themselves and assessed the change in their condition on a 1-10 Likert Scale on ten morbidities. Pre-screening or pre-selection was not done. The results were sorted according to 10 morbidities and changes in the self-assessment were noted. The results indicate that Ganga water provides health benefits for a number of morbidities simultaneously and could work as a wide-spectrum phage cocktail.

Key words: Ganga Water, Phages, phage therapy, Immunomodulation, wide spectrum.

INTRODUCTION

Historically British bacteriologist Ernest Hankins observed antibacterial activity in Ganges and Yamuna waters against Vibrio cholera in 1986 [1]. At nearly the same time French microbiologist Felix D'Herelle observed that there were no germs at all even a few feet below dead bodies of people who died with dysentery and cholera floating in the Ganga. Thus, bacteriophages were first discovered in Ganga [2]. The first tests on the use of phages for treating bacterial infections were performed at the

Baylor University of Medicine in Texas in 1923 [3]. Waters *et al.* demonstrated the potential for phage therapy in the treatment of established and recalcitrant chronic respiratory tract infections [4]. Górski *et al.* have reported that the anti-bacterial action of phage offers the best perspectives for therapeutic application [5]. A bacteriophage taken from a local pond was used to treat a life-threatening bacterial infection in an 80-year-old man's chest [6]. These studies indicate that phages can act against pathogenic bacteria in the human body. Phages may interfere with eukaryotic viruses [5]. The anti-bacterial action of phages leads to an immunomodulatory effect. Phages can enhance immune responses in non-specific ways [7]. Because of their high specificity, bacteriophages may favorably manipulate the microflora of the intestinal tract. While bacteria-based probiotics introduce nonpathogenic bacteria into the GI tract, phage-based probiotics target and kill specific pathogenic bacteria in the GI tract [8]. The anti-bacterial properties of Ganga Water can strengthen the digestive system and improve the overall immunity of the person [9]. Raj Nighantu, a treatise in the field of Ayurveda, was composed by Pandit Narhari after walking from Kashmir to Kerala. It says that the water of the Ganga is "digestive, improves appetite, and reduces thirst." The water of the Yamuna is "digestive, creates vata, removes the toxins generated from pitta dosha." The water of the Narmada "removes pitta and kaf dosha and provides relief from all diseases" [10]. These statements indicate that the water of rivers is known to be used for therapeutic purposes since ancient times.

The National Environmental Engineering Research Institute, Nagpur (NEERI) has found that the waters of upper Ganga have large numbers of isolates of bacteriophages in comparison to bacteria. The ratio of isolates of bacteriophages and bacteria in different stretches of different rivers has been given in Figure 1 [11].

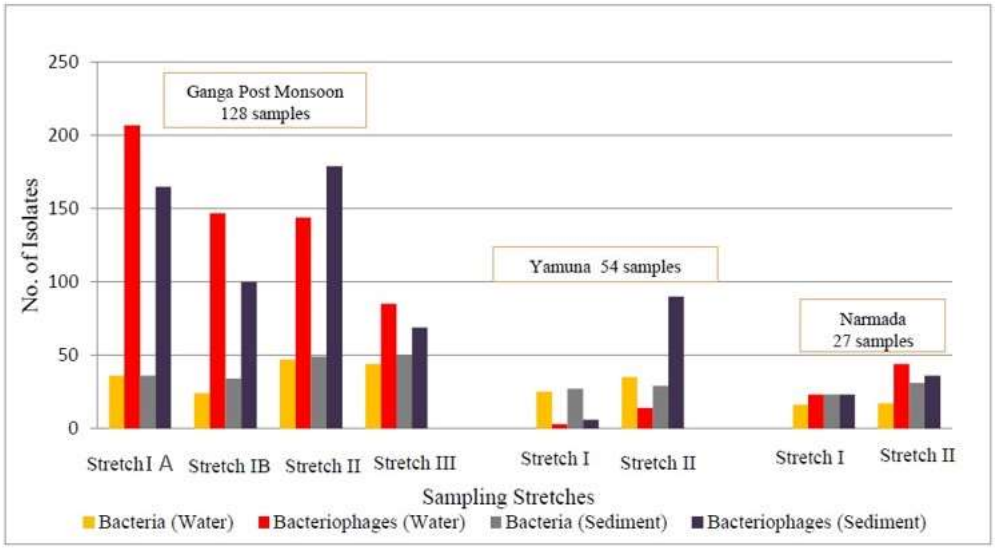

Figure 1: Isolates of Bacteria and Bacteriophages in Indian Rivers.

Stretches I, II and III refer to the upper- middle- and lower stretches of the Ganga River. Stretches IA and IB refer to two stems of the upper-Ganga River, namely, Bhagirathi and Alaknanda respectively. Stretch I and II refer to the upper- and lower stretches of the Yamuna and Narmada rivers. This study is based on water from Stretch IB of the Ganga.

The NEERI Report concludes: "The comparison of River Ganga with Rivers Yamuna and Narmada showed higher numbers of bacteriophages against pathogenic bacterial species. In River Ganga, pathogenic bacteriophages were detected 3 times more than pathogenic bacteria. In the case of opportunistic and nonpathogenic bacteria, lesser numbers of bacte-

riophages were detected compared to pathogenic bacteria. In the comparative study with control Rivers Yamuna and Narmada, pathogenic bacteriophages were found lesser in numbers than pathogenic bacterial species in both Rivers compared to River Ganga" [11]. The Institute of Microbial Technology has reported a variety of different phages having specific bactericidal activity against clinical isolates and pathogens Microcystis, Haemophilus, Synechococcus, Pseudomonas, Enterococcus, Bacillus, Rhodococcus, Caulobacter, Salmonella, Enterobacteria, and Mycobacterium [12]. These studies give us a reason to explore the use of phages in Ganga water for immunomodulating, prophylactic, and therapeutic purposes. The specific objective of this study was to generate evidence for the immunomodulating, prophylactic, and therapeutic potential of Ganga water to test with subsequent experimental studies [13].

Material and Methods

We have provided data on a number of variables in a longitudinal setting. More frequently this approach has been used for a large number of individuals [14]. We have adopted the same method for a smaller number of individuals as a pilot study. Most studies involve either direct observation by the medical practitioner or a questionnaire filled-up by a subject or by a researcher as told by the subject. We have used the latter approach. The volunteers were asked about their assessment of symptoms related to a disease over different time periods. Thus, a temporal sequence between the exposure to Ganga water and the change in symptoms was obtained [15].

Water Collection and Administration

The Ganga River is conventionally believed to be formed after the confluence of the Bhagirathi and Alaknanda rivers at Dev Prayag. Of these, the Alaknanda carries about three times the volume of water in comparison to the Bhagirathi and can be considered to be the main tributary. In turn, the main tributary of the Alaknanda is Mandakini. We used the water of the Alaknanda and Mandakini rivers at Rudra Prayag (Stretch IB) because the construction of the Srinagar Dam downstream of here would have reduced the phages as told by scientists at NEERI. Water and sediments were collected from Rudra Prayag above the confluence of Mandakini River on June 20, 2020, before the onset of the monsoons because, as per our discussions with the scientists at NEERI, the numbers of phages are less during the monsoons. Water and sediment samples were collected in pre-sterilized containers. All the containers were rinsed 3 times with the same water before collection. Collected water samples were transported to the laboratory and stored at room temperature. Subsequently, the water was filtered as mentioned below and supplied by post to the volunteers. The phages first multiply themselves by taking over the bacteria. They adsorb to the sediments when no bacteria are left in the water column. We supplied water with sediments so that the adsorbed phages are available to the volunteer. The water of the Ganga River upstream of Rudra Prayag, from where we have collected the same, has Dissolved Oxygen at 9.6 mg/liter, Biological Oxygen Demand at 1.2 mg/liter, and pH between 6.5 to 8.5 as per Central Pollution Control Board (CPCB) [16]. These levels meet the water quality standards for drinking water. CPCB has not mentioned any presence of heavy metals but certified the water to be fit for drinking except for the presence of coliforms. Hence, we assume no heavy metals were present. However, the water had coliforms at 9300 per 100 ml against the requirement of less than 50 for drinking water. We tested the water after one month of storage and found no coliforms indicating that the phages had destroyed them, and the water was fit for drinking. A membrane filter of size 0.45 µM was used to filter the samples and remove any remaining bacteria. We did not undertake any further tests to determine whether the water samples had any pathogenic bacteria remaining. Growth of algae was observed in some containers that were exposed to sunlight in mid-August 2020. The water was got tested. No coliforms were found. The water was then filtered to remove the algae and fungus. The sediment was thoroughly washed with filtered water and the washed sediment was added back to the filtered water to make sure that no coliforms had passed into the water through the sediments. The phages adsorb on to

the sediments. Hence, adding the washed sediments to the filtered water reintroduced the adsorbed phages into the water. This water was administered to volunteers orally, topically, or nasally depending upon their convenience. We informed all volunteers about the possible benefits of Ganga Water without making any claim about the therapeutic potential. We have compared the change in the Likert Scale as informed by the volunteers to assess the impact of Ganga water without prior sorting or selection. This method has been used to assess the impact of social interventions. The National Citizen Service Trust (NCS) sorted existing mixed data by certain parameters and then assessed the impact of social interventions [17]. We have followed this method. The results reported have been used to assess the effects of exposure to Ganga water. We have ensured that no other changes in the medication regimens were made by any volunteer in the course of this study. Therefore, the changes reported by a volunteer would be only due to the exposure to Ganga water even though the effect may not be comparable across volunteers. The effect due to the time lag of medication regimens cannot be ruled out [13, 14]. That is a limitation of this study.

Scoring

We have used a 1-10 Likert Scale with "1" representing the morbid situation and "10" representing the best situation [18, 19]. The outcome was assessed by the volunteer. We make no averments as regards whether a reported outcome is good, satisfactory, or unsatisfactory. Our limited purpose is to show that there is an impact that requires consideration. We realize that the scores are not comparable between cases where the case himself/herself has given them. A 7 score by one person on a 1-10 scale may not mean the same as another person's score of 7. However, we assume that a person will provide a comparable score over time. A score of "7" changing to a score of "9" may not be comparable to a score of "3" by another person changing to a score of "5." However, the change of score of "2" would be more comparable because the bias of the different beginning scores is eliminated. We hope that larger numbers of cases would eliminate this bias in future studies.

Results

1 Case Series

Eighty volunteers were enlisted by sending messages about the project on social media. Volunteers were informed that high numbers of phages in Ganga water could help cure multiple morbidities. They were requested to voluntarily administer Ganga water to themselves and record the effect on 10 morbidities: digestive, urological, neurological, metabolism, mental health, respiratory, cardiological, diabetes, dermatological, and arthritis on a scale of 1-10. Filtered water was supplied to them by post. Of these, data was available form 45 volunteers. Of these 45, data for two different time periods, often with different methods of intake, was available for nine volunteers. For example, we may have data for Case "A" between time "X" and "Y" when he/she took water orally; and between time "Y" and "Z" when he/she took water nasally. The two periods were treated as separate cases. Thus, these nine persons have provided 18 cases. Accordingly, we had data from 45 volunteers and 54 cases. Indeed, phages from one phase may confound the results of the subsequent phase. However, the alternative was to club the two methods of intake together which would prevent the study of different methods of intake hence such was not done. Every volunteer was requested to mark his/her status on each of the 10 morbidities in the beginning and after the administration of Ganga water. Thus, we had 54 x 10 = 540 observations. The volunteers were requested to take Ganga water in addition to their medical treatments by any of the four methods: nasal, oral, oral-plus-topical, and topical. They were interviewed on the phone in August-November 2020. They were requested to rank their health status regarding the 10 diseases mentioned above on a Likert Scale of 1-10 with "1" being worst, "6" being normal, and "10" being best. A volunteer may assess his/her starting present condition as, say, "3" if he/she considers himself/herself to be in lower-than-normal condition. The interview was done again after about 3 weeks after administration of Ganga Water. The volunteers were requested to again rank their status on the same 10 diseases on a scale of 1-10. A result of

"2.5" in Urological means that the volunteers taking the Ganga water by any of the three methods assessed the improvement in their urological condition by 2.5 points on a scale of 1-10. If the average score given by them, in the beginning, was 3, and the average score after administration of Ganga water was 5.5, then the improvement is assessed at 2.5 points (table no. 1). We make no claim whether a particular level of improvement is unsatisfactory, satisfactory or good.

It is found that oral administration provided the best results in 7 of 10 morbidities. It is seen that the highest improvement reported was 2.7 on the Likert Scale. The low level of improvement could be due to less time for administration or due to intermittent intake since it was undertaken without supervision. Table 1 reports the improvement irrespective of the level at the starting point. Say, a volunteer ranked his status at 7 points in the beginning in the digestive morbidity. After administration of Ganga water, he assessed his status at 9 indicating an improvement of 2 points. However, the volunteer was not "morbid" since we considered 6 or more points to indicate a "normal" situation and he/she gave 7 points in the beginning. Thus, an attempt was made to assess the impact on the morbid cases only, that is, those giving their status on that morbidity as 5 or less in the beginning. The data was sorted by the points given by the volunteers at the beginning for each morbidity. Cases, where a volunteer had given 6 or more points in the beginning, were excluded. The assessment was done of the remaining cases where the volunteer gave 5 or fewer points in the beginning. These cases give the effect only on those who may be considered "morbid." The purpose was to assess the impact of Ganga water on "morbid" cases only instead of "morbid" and "normal" cases together. These results are given in table no. 2. The numbers of morbid volunteers showing positive effects as a percentage of numbers of volunteers showing that particular morbidity is given in Column 3 to enable a quick appreciation of the percentage of volunteers reporting positive effects (table no. 2). We spoke with the volunteers reporting negative impacts. Their responses are given in the last column of the above table. On being asked, one case with three negative results bearing numbers (1), (4), and (7) said that he was not sure of the negative impact. Others reported extraneous reasons for the negative impact. It is seen that persons suffering from neurological, digestive, and dermatological problems have found benefits. A comparison of the results in Tables 1 and 2 is made in Table 3 below. This table gives us a comparative assessment of the effects on "all" cases as compared to "morbid" cases. It is seen that those suffering from a disease, that is, reporting a beginning point of "5" or less, report greater benefit except for (-)0.1 for arthritis. This suggests that Ganga water has the potential for the treatment of a number of diseases.

2 Case Study BJ

The subject BJ, male, 70 years, used the oral, topical, and nasal methods at different times as per his discretion. The results are assessed by the subject (table no. 4). This case study with one subject cannot be generalized in terms of the specific impacts observed. The subject used water from the Alaknanda and Mandakini tributaries of the Ganga. Traditionally, Ganga is formed at Devprayag, at the confluence of the Bhagirathi and Alaknanda Rivers. The Alaknanda in turn has a confluence with Mandakini upstream Devprayag at Rudraprayag. The main stem of the Ganga is Alaknanda which starts from Badrinath and goes through five confluences at Vishnuprayag, Nandaprayag, Karnaprayag, Rudraprayag, and Devprayag. The subject used water from Alaknanda which is the main tributary of the Ganga; and Mandakini which is the main tributary of the Alaknanda. Whether these results can be extrapolated to the main stem pf the Ganga requires further study. The study indicates that nasal inhalation is more effective than topical or oral intake. There was an “over doze” leading to Macular Edema and High BP. This was not anticipated when the volunteer administered Ganga water to himself. It was an unintended effect. Further, the water of different rivers may have different qualities. In this case, Mandakini water appears to be more effective than Alaknanda water.

DISCUSSION

This case series study suffers from the limitation of the placebo effect and not having a control sample. Possible cultural bias also cannot be ruled out since the volunteers may have been predisposed towards reporting curative properties of Ganga water. Another limitation of the case series study is that the volunteers continued to take other prescription medicines. Hence, it is not possible to attribute the reported change to Ganga water alone. Further, the results are dependent on the volunteer's own assessment which could be influenced by other unrelated factors. However, the results still give an indication of the effect of Ganga water. A more thorough study is required to assess whether these were any adverse events. If confirmed, there would arise a need to understand the pathogenesis of the same. The case study confirms the positive impact of Ganga water as reported in the case series study. However, it also reports a negative impact on macular edema. This underscores the need to develop a robust protocol to prevent such negative effects. The limitations of these studies notwithstanding, they indicate the need to evaluate the plausibility of the immunomodulating, prophylactic, and therapeutic effects of Ganga water and undertake subsequent experimental studies. The case series study suggests oral-plus-topical is better than nasal. More rigorous study is needed to assess the efficacy of the three methods of treatment. Additional methods to be considered would be nasal drops and adding Ganga water to bath water. The study suggests immunomodulating benefits over a large number of morbidities.

CONCLUSION

Ganga water holds the potential for immunomodulating, prophylactic, and therapeutic use against multiple morbidities simultaneously.

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Table 1: Average Results of Improvement on Likert Scale 1-10 by Morbidity by Method of Administration.

Sl. No.	Morbidity	All Methods n=56	Nasal n=15	Oral n=46	Oral + Topical n=18	Topical n=20
1	Urological	2.5	1.6	2.7	1.5	1.6
2	Diabetes	2.4	0.5	2.5	3.0	3.0
3	Neurological	2.1	2.0	2.1	2.1	2.1
4	Cardiological	2.0	1.0	2.1	1.0	1.0
5	Mental Health	1.8	0.5	2.0	2.0	2.0
6	Respiratory	1.7	0.5	1.6	2.0	2.0
7	Metabolism	1.7	1.5	1.8	1.7	1.7
8	Arthritis	1.7	*	1.7	1.5	1.5
9	Dermatological	1.6	1.0	1.3	1.8	2.0
10	Digestive	1.6	2.2	1.5	1.7	1.7

"*" no observation.

Table 2: Effects of using Ganga water on "morbid" volunteers.

	Diagnosis	Number of Observations	Numbers showing a positive effect	Average Level of positive effect on a scale of 1-10	Numbers showing no effect	Numbers showing a negative effect	Average Level of negative effect on a scale of 1-10	Reason for negative effect as per volunteer. The (-) sign indicates the negative effect of Ganga water. Responses are numbered in brackets and discussed below.
	Unit	No.	No (%)	Scale	No.	No.	Scale	Description
1	Urological	24	12 (50%)	3.3	12	0	0.0	
2	Respiratory	9	3 (33%)	3.0	6	0	0.0	
3	Mental Health	21	14 (67%)	2.6	6	1	-1.0	(1) Not sure of the negative impact.
4	Diabetes	5	2 (40%)	2.5	3	0	0.0	
5	Neurological	25	14 (56%)	2.4	8	3	-3.0	(3) Side effects of dialysis. (4) Side effect of change in medicines for Parkinson's disease. (5) Not sure of the negative effect.
6	Cardiological	11	4 (36%)	2.3	6	1	-1.0	(7) Did not do exercise. Too much use of mobile phones.
7	Metabolism	28	17 (61%)	2.2	10	1	-1.0	(2) Too much work during Diwali.
8	Digestive	22	12 (55%)	2.2	10	0	0.0	
9	Dermato-logical	13	7 (54%)	2.0	5	1	-1.0	(6) Not sure of the negative impact.
10	Arthritis	11	5 (45%)	1.6	6	0	0.0	

Table 3: Comparison of the Effect of Ganga water on "All" cases compared to "Morbid" cases (Improvement on Likert Scale 1-10).

Sl. No.	Morbidity	"All" cases (Table 3)	"Morbid" cases (Table 4)	Difference
1	Urological	2.5	3.3	+0.8
2	Diabetes	2.4	2.5	+0.1
3	Neurological	2.1	2.5	+0.4
4	Cardiological	2.0	2.3	+0.3
5	Mental Health	1.8	2.2	+0.4
6	Respiratory	1.7	3.0	+1.3
7	Metabolism	1.7	2.6	+0.9
8	Arthritis	1.7	1.6	-0.1
9	Dermatological	1.6	2.0	+0.4
10	Digestive	1.6	2.2	+0.6

Table 4: Effect of Ganga water: Case Study.

Sl	Period	Method of Intake (River)	Beneficial Effect.	Negative Effect.	Discussion
1	1.7.2020 to 10.8.2020	Oral (Alaknanda)	Skin abrasion reduced.		Dermatological benefit.
2	11.8.2020 to 17.8.2020	Nasal (Alaknanda)	Good appetite.	Hemorrhage in Macular Edema. High BP, Hyperactivity,	The same water proved to be more effective when taken nasally. There may be an "over doze" leading to Macular Edema and High BP.
3	18.8.2020 to 30.10.2020	NIL	BP is normal. Hyperactivity reduced. Good appetite.		-
4	1.11.2020	Nasal (Mandakini)		High BP, Hyperactivity.	Mandakini water may be more effective than Alaknanda water. High BP developed in a mere one day against 7 days for Alaknanda water.
5	2.11.2020 to 5.12.2020	Topical (Mandakini)	Skin abrasion less, good appetite		The topical application of Mandakini water is both beneficial and safe.