Defence Life Science Journal, Vol. 8, No. 4, October 2023, pp. 334-341, DOI: 10.14429/dlsj.8.19092


# Immunomodulatory Activity of Shilajatu (*Asphaltum punjabinum*) Processed in Different Media: An *In Vivo* Study

Rohit Singh[1*], Pramod Yadav[1] and P.K. Prajapati[2]

[1]*Department of Rasa Shastra and Bhaishajya Kalpana, All India Institute of Ayurveda, Gautampuri, New Delhi–110076, India*
[2]*Dr Sarvepalli Radhakrishnan Rajasthan Ayurved University, Jodhpur, Rajasthan–342 037, India*
[*]*E-mail: rohit13july@gmail.com*

**ABSTRACT**

Immunomodulators modify the immune response of the body to a threat by potentiating it, but conventional immunomodulatory drugs are associated with higher costs and adverse drug reactions. Therefore, the exploration of herbal immunomodulators is a need of time. Shilajatu is a compound of therapeutic importance that is described in Ayurveda as Rasayana (Immunomodulator). The current study has been planned to assess the Rasayana property (Immunomodulatory activity) of Shilajatu (*Asphaltum punjabinum*) collected from two market sources i.e. Amritsar and Nepal, processed in Water and Triphala Kwatha (decoction prepared from fruits of Terminalia chebula Retz. (Haritaki), Terminalia bellirica Gaertn. Roxb. (Vibhitaki) & Phyllanthus emblica L.(Amalaki) on cell-mediated immunity. It involved Wistar strain albino rats, weighing 180 ± 20 g of either sex, which were divided into five groups. Test animals were subcutaneously sensitized (0.5 ml/100 g body weight) with triple antigen solution on day 1 and the test drug was administered orally at the dose level of 100 mg per kg in four groups and one group was served as negative control. On the seventh day, again 0.1 ml was injected into the plantar aponeurosis, and hind paw edema was assessed at 24 and 48 hrs for cell-mediated immunity. Groups with drug intervention have shown more edema inhibitory percentage than the water control group at all intervals. Besides, Shilajatu samples processed in Triphala Kwatha have shown a better immunity-enhancing profile than water-processed samples at both the time intervals i.e., 24 and 48 hrs. Therefore, Shuddha Shilajatu can be used as a potent immunomodulator in the management of various diseases as well as enhancing the immunity of a healthy person. Moreover, processing media and supply sources of drugs should be considered for achieving desired therapeutic outcomes.

**Keywords:** Cell-mediated immunity; Immunomodulators; Rasayana; Shilajatu

## 1. INTRODUCTION

The immune system is a comprehensively organized defense system that confers protection to the human body from harmful entities or foreign bodies. It comprises various cells, proteins, and other molecules that identify and decimate pathogenic and deleterious elements. A quick response to any harmful agent is generated by the immune system through the activation of immune cells and the production of numerous chemokines, cytokines, and inflammatory mediators[1]. Immunomodulators are the agents that modulate the action of the immune system to a threat by modifying and potentiating the immune system, keeping it equipped for any threat[2]. They are the pharmaceutical[3] agents, which are generally used to replenish the immune deficit in the body.

Various conventional drugs are being used to modify or enhance the immune system, but they are not readily available and, are also not cost-effective. Moreover, associated adverse drug reactions, and the constant rise in antibiotic-resistant strains have propelled scientists to explore herbal immunomodulators to treat different diseases and infections[4].

Received : 15 May 2023, Revised : 17 August 2023
Accepted : 17 August 2023, Online published : 21 December 2023

Various studies have reported that herbal compounds enhance the innate immunity of the body against infection[5,6] and modulation of the immune system is a protective as well as effective strategy against various infectious diseases.[7] It has been reported that approximately 80% of the population in Asia and Africa rely on traditional and herbal medicine to meet their basic healthcare needs[8]. Herbal and herbo-mineral drugs mentioned in numerous texts of Ayurveda are being used in the management of various diseases since time immemorial. Over the last few decades, immunomodulation through herbal formulations for alleviating diseases has gained the considerable attention of scientists, and medical professionals around the world, and many research studies have been conducted for the same[9,10]. Plant-derived immunomodulators are also reported to be effective in preventing acquired immune deficiencies[11]. Immunomodulation is being used as a measure for the assessment of Rasayana (immunomodulatory) properties of any therapeutic preparation, and Rasayana therapy is stated for health prevention along with treatment of the diseases. It is said to provide strength to Ojus (immunity), Dhatus[12] (various body tissues), prevents Jara[13] (aging). Furthermore, it helps the individual to sustain good health. Shilajatu (*Asphaltum punjabinum*)

is one such therapeutic compound, which is described in numerous Ayurveda texts and being used as Rasayana for centuries. Various studies have been conducted to explore the immunomodulatory activity of Shilajatu. However, no work has been published to assess the effect of processing media and biogeography of the Shilajatu source on its immunomodulatory potential.

The current study has been planned to evaluate the immunomodulatory effect of Shilajatu procured from Amritsar and Nepal and processed in Water and Triphala Kwatha (decoction of fruits of Terminalia chebula Retz., *Terminalia bellirica* (Gaertn.)Roxb. and Phyllanthus emblica L.). The immunomodulatory activity of Shilajatu processed in Water and Triphala Kwatha was evaluated for cell-mediated immunity in the animal experimental model. The study involved Wistar strain albino rats of either sex weighing 180 ± 20 grams and oral administration of the interventional drug at a dose of 100 mg/kg. Immunological paw edema volume was evaluated for cell-mediated immunity[14].

## 2. MATERIALS AND METHODS

### 2.1 Study Design and Setting

The study was designed as a laboratory-based preclinical in-vivo experimental study. It was conducted at Siddhartha Institute of Pharmacy, *Dehradun, India.*

### 2.2 Test Drug

Ashuddha (Raw) Shilajatu samples were procured from raw drug markets of Nepal and Amritsar and authenticated at the Pharmacognosy lab of All India Institute of Ayurveda (AIIA), Delhi. Samples were processed with Water and Triphala Kwatha separately as per classical guidelines[15,16] in the departmental laboratory of Rasa Shastra & Bhaishajya Kalpana, AIIA, and Shuddha (Processed) samples were used as test drugs. Test preparations processed in water and Triphala Kwatha were coded as ASW, NSW, and AST, NST respectively.

### 2.3 Experimental Animals Used in the Study

The present study employed Wistar Strain albino rats (Rattus novergicus) of both sex having weight in the range of 180 ± 20 g procured from SGRR Institute of Medical & Health Sciences, Dehradun, India. They were inhabited in spacious polypropylene cages with stainless steel grills on the top, along with arrangements for food and water bottle, and clean paddy husk bedding, which was changed every morning. Male and female rats were kept in separate cages throughout the study to refrain from conception. Animals were fed with standard rat's feed, and RO water was provided ad libitum. For acclimatization, animals were kept in a standard lab environment with a 12-hour day and 12-hour night rhythm, maintained at temperature and humidity of 25 ± 3°C and 40 to 60%, respectively, for seven days before the initiation of the experiment at Siddhartha Institute of Pharmacy, Dobachi, near IT Park, Sahastradhara Road, Dehradun, India. The Institutional Animal Ethics Committee (IAEC) approved the experimental study protocol (SIP/IAEC/PCOL/3/2019), and experiments were conducted in conformity with the concerned Institutional Animal Ethics Committee. All animal care measures were adopted as recommended by the Committee for Control and Supervision of Experiments on Animals (CCSEA).

### 2.4 Dose Selection

The classical dose of Shuddha Shilajatu is 1g/day[17]. The experimental animal dose was determined by deducing the human dose to animals by referring to the standard table of Paget & Barnes[18]. The calculated and administered dose was 100 mg/Kg body weight.

### 2.5 Administration of Test Drugs

The test solutions were prepared with distilled water (10 mg/ml) and administered orally to animals in the calculated dose of 1 ml per 100 g of body weight by intubation needle (18G) sleeved to a suitably graduated syringe. The dosage volume given to an individual animal was redacted according to its most lately recorded body weight. The drugs were administered to overnight fasted animals.

### 2.6 Reason for Dosing Route

This dosing route is the same as the route of human exposure.

### 2.7 Preparation of Triple Antigen Solution:

The required solution was prepared by using Potash alum (10 %), Normal saline (0.9 %), and triple antigen (DPT) in the ratio of 1:4:1. The pH of the prepared solution was stabilized in between 5.6 - 6.8 by adding 10% sodium carbonate.

### 2.8 Procedural Protocol

The selected animals were allocated to five groups (NC, NST, NSW, ASW, AST)* comprising six animals each. The first group was given only distilled water and served as the negative control. To the second, third, fourth, and fifth groups, measured doses of NST, NSW, ASW, and AST were administered, respectively. All the animals were subcutaneously sensitized (0.5 ml/100 g body weight) by triple antigen solution i.e., potash alum (10 %) - 1 ml, normal saline/NS (0.9 %) - 4 ml, and triple antigen (DPT) - 1 ml, on the first day of drug administration. The drug was administered orally for seven continuous days. On the seventh day, sixty minutes after drug administration, the initial left hind paw volume was recorded, and triple antigen solution (0.1 ml) was injected by a 1 ml syringe into plantar aponeurosis of the same hind paw as shown in Fig. 1. The volume of thus produced immunological edema was determined by adopting volume displacement method[19] at 24 and 48 hours after the injecting triple antigen solution, using a plethysmograph as displayed in Fig. 2.

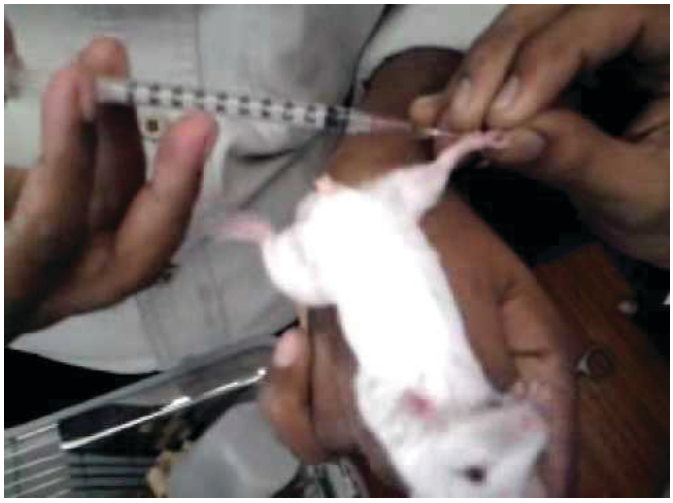

**Figure 1. Injection of triple antigen solution into the plantar aponeurosis of the left hind paw on the 7th day.**

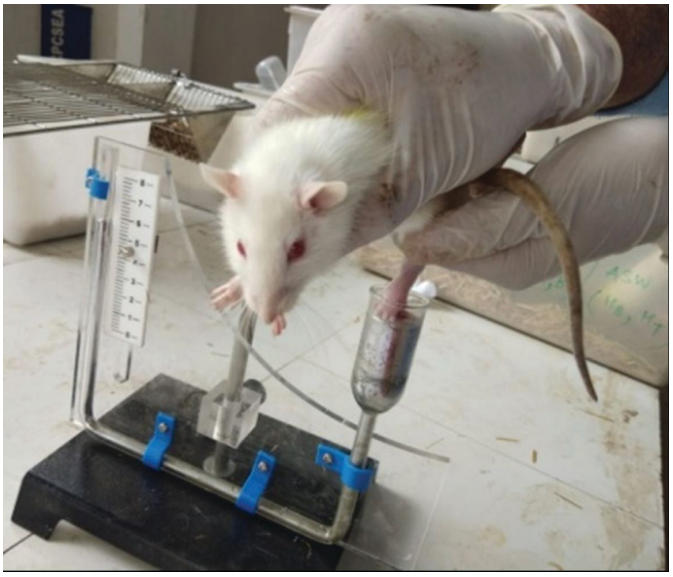

**Figure 2. Measurement of hind paw volume after 24 & 48 hours by plethysmograph.**

*NSW- Nepal Shilajatu processed in water, NST- Nepal Shilajatu processed in Triphala kwatha, ASW- Amritsar Shilajatu processed in water, AST- Amritsar Shilajatu processed in Triphala kwatha.

### 2.9 Statistical Analysis

Drug intervention groups were compared to the water (NC) group at 24 and 48 hrs individually. Difference between and within the groups was statistically calculated by applying unpaired and paired Student 't' test with level of significance at 5 % i.e. $P<0.05$. Results were represented as Mean ± SEM.

## 3. RESULTS

Initial hind paw volume and volume after 24 and 48 hours of injecting triple antigen solution in various groups has been depicted in Table 1. Actual and percentage change in mean hind paw volume of experimental animals in various groups after 24 and 48 hours of injecting triple antigen solution has been profiled in Table no. 2 & 3 respectively. Mean hind paw volume at baseline, the actual change in mean hind paw volume and percentage of edema inhibition after 24 hours in different groups has been shown in Table no. 4, while Table no. 5 represents the actual change in mean hind paw and edema inhibition percentage after 48 hours in different experimental groups.

## 4. DISCUSSION

The immune system is a systematic assembly of cells, tissues, organs, and cell products that render defence against diseases, neutralize noxious substances, and trigger phagocytic activity to ingest or injure foreign entities[20]. It comprises innate and adaptive arms of immunity. Depending on the immune mediators involved, adaptive immunity can be classified into Humoral immunity, which is mediated by secreted antibodies, and Cell-mediated immunity, which is predicated on the function of T-helper (Th) lymphocytes. Differentiation and proliferation of various immune cells are mediated by lymphocytes through the release of various cytokines like Interleukin (IL)-2,4,5, and 6 etc., which further leads to the activation of specific cell-mediated immune response[21]. This specific immune response is channelized by the interaction between T lymphocytes and macrophages[22]. T cells develop in the thymus gland, and mature T cells keep on recirculating between peripheral lymphoid tissue and blood until their encounter with the specific antigen[23]. Moreover, before their interaction with the antigen, they are designated as

**Table 1. Change in hind paw volume after 24 and 48 hours in different groups**

| Animal | Initial paw volume | Hind paw volume (ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Groups | | | | | | | | | |
| | | Water (NC) | | NSW | | NST | | ASW | | AST | |
| | | 24 hrs | 48 hrs | 24 hrs | 48 hrs | 24 hrs | 48 hrs | 24 hrs | 48 hrs | 24 hrs | 48 hrs |
| A | 5.8 | 6.2 | 6.1 | 6 | 5.85 | 6 | 5.85 | 6 | 5.95 | 5.95 | 5.9 |
| B | 5.8 | 6.2 | 6.05 | 6 | 5.8 | 5.9 | 5.8 | 5.85 | 5.8 | 5.9 | 5.9 |
| C | 5.8 | 6.25 | 6.1 | 6.05 | 5.85 | 6 | 5.85 | 5.85 | 5.85 | 6 | 5.9 |
| D | 5.8 | 6.2 | 6.1 | 6.2 | 5.9 | 5.9 | 5.8 | 6 | 5.9 | 5.9 | 5.85 |
| E | 5.8 | 6.25 | 6.1 | 6 | 5.8 | 6.1 | 5.9 | 6.1 | 5.95 | 5.85 | 5.8 |
| F | 5.8 | 6.2 | 6.05 | 6 | 5.85 | 5.9 | 5.8 | 5.85 | 5.8 | 5.85 | 5.8 |

**Table 2. Percentage increase in hind paw volume after 24 hours in different groups**

| Groups | Dose (per kg) | Initial | 24 hrs | Actual change in mean paw volume (ml) | % increase in mean paw volume |
|---|---|---|---|---|---|
| Water | | 5.800 ± 0.000 | 6.217 ± 0.0105 | 0.417 ± 0.0105A | 7.19 |
| NSW | 100 mg | 5.800 ± 0.000 | 6.042 ± 0.0327 | 0.242 ± 0.0327A | 4.18 |
| NST | 100 mg | 5.800 ± 0.000 | 5.967 ± 0.0333 | 0.167 ± 0.0333B | 2.88 |
| ASW | 100 mg | 5.800 ± 0.000 | 5.942 ± 0.0436 | 0.142 ± 0.0436C | 2.45 |
| AST | 100 mg | 5.800 ± 0.000 | 5.908 ± 0.0239 | 0.108 ± 0.0239C | 1.86 |

Mean ± SEM, A= p <0.001, B= p <0.01, C= p < 0.05 when compared to the initial value of the same group.

**Table 3. Percentage increase in hind paw volume after 48 hours in different groups**

| Groups | Dose (per kg) | Initial | 48 hrs | Actual change in mean paw volume (ml) | % increase in mean paw volume |
|---|---|---|---|---|---|
| Water | | 5.800 ± 0.000 | 6.083 ± 0.0105 | 0.283 ± 0.0105A | 4.88 |
| NSW | 100 mg | 5.800 ± 0.000 | 5.842 ± 0.0154 | 0.0417 ± 0.0154C | 0.72 |
| NST | 100 mg | 5.800 ± 0.000 | 5.833 ± 0.0167 | 0.0333 ± 0.0167 | 0.57 |
| ASW | 100 mg | 5.800 ± 0.000 | 5.875 ± 0.0281 | 0.0750 ± 0.0281C | 1.29 |
| AST | 100 mg | 5.800 ± 0.000 | 5.858 ± 0.0201 | 0.0583 ± 0.0201C | 1.00 |

Mean ± SEM, A= p <0.001, B= p <0.01, C= p < 0.05 when compared to the initial value of the same group

**Table 4. Inhibitory potential of test drugs on triple antigen-induced hind paw edema (Inhibitory percentage after 24 hours)**

| Groups | Initial | 24 hrs | Actual change in mean paw volume (ml) | % inhibition in mean paw volume |
|---|---|---|---|---|
| Water (NC) | 5.800 ± 0.000 | 6.217 ± 0.0105 | 0.417 ± 0.0105 | - |
| NSW | 5.800 ± 0.000 | 6.042 ± 0.0327 | 0.242 ± 0.0327A | 41.96 ↓ |
| NST | 5.800 ± 0.000 | 5.967 ± 0.0333 | 0.167 ± 0.0333 A | 59.95 ↓ |
| ASW | 5.800 ± 0.000 | 5.942 ± 0.0436 | 0.142 ± 0.0436 A | 65.94 ↓ |
| AST | 5.800 ± 0.000 | 5.908 ± 0.0239 | 0.108 ± 0.0239 A | 74.10 ↓ |

Data: Mean ± SEM, A P<0.001 when compared to the control group (NC).

**Table 5. Inhibitory potential of test drugs on triple antigen-induced hind paw edema (Inhibitory percentage after 48 hours)**

| Groups | Initial | 48 hrs | Actual change in mean paw volume (ml) | % inhibition in mean paw volume |
|---|---|---|---|---|
| Water (NC) | 5.800 ± 0.000 | 6.083 ± 0.0105 | 0.283 ± 0.0105 | - |
| NSW | 5.800 ± 0.000 | 5.842 ± 0.0154 | 0.0417 ± 0.0154 A | 85.26 ↓ |
| NST | 5.800 ± 0.000 | 5.833 ± 0.0167 | 0.0333 ± 0.0167 A | 88.23 ↓ |
| ASW | 5.800 ± 0.000 | 5.875 ± 0.0281 | 0.0750 ± 0.0281 A | 73.49 ↓ |
| AST | 5.800 ± 0.000 | 5.858 ± 0.0201 | 0.0583 ± 0.0201 A | 79.39 ↓ |

Data: Mean ± SEM, A P<0.001 when compared to the control group (NC).

naive T cells. To direct and participate in the adaptive immune response, the first encounter of naive T cells with the antigen is requisite because only then they become able to differentiate and proliferate into cells having the potential of rendering elimination of the antigen[24]. Therefore, in the present study, experimental animals were first sensitized subcutaneously with the triple antigen solution to make T cells able to recognize the specific antigen on their subsequent encounter. The subcutaneous route and nape of the neck have been used for the sensitization because, at this site, the antigen is absorbed slowly, drains into the lymphatic system to lymph nodes, and ultimately stimulates B and T lymphocytes[25]. In cell-mediated immunity, characteristic response peaks at 24–48 h following exposure to the antigen[26]. Based on this concept, hind paw edema was measured at 24 and 48 hours of intervals to evaluate the immunomodulation potential of Shilajatu. In the study, it has been found that animal groups with drug intervention have shown less increase in mean paw volume and more edema inhibitory

percentage than the water control group at 8, 24, and 48 hours as depicted in Fig. 3 to Fig. 5.

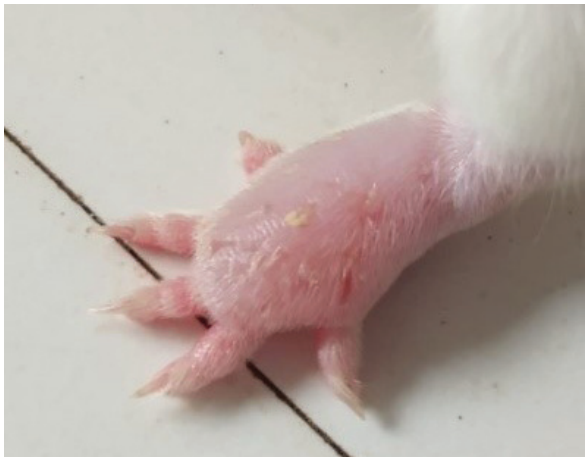

**Figure 3. Hind paw edema at 8 hours of injecting triple antigen solution in interventional groups.**

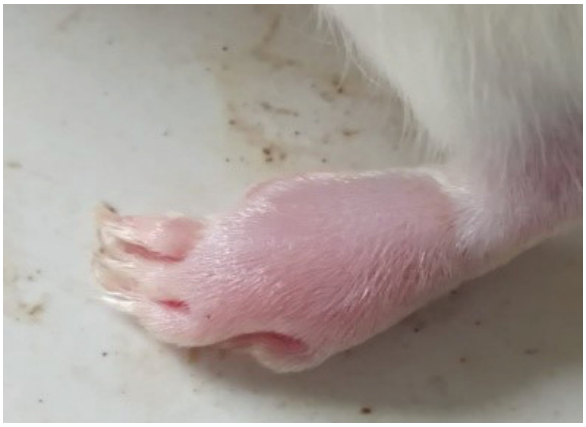

**Figure 4. Hind paw edema at 24 hours of injecting triple antigen solution in interventional groups.**

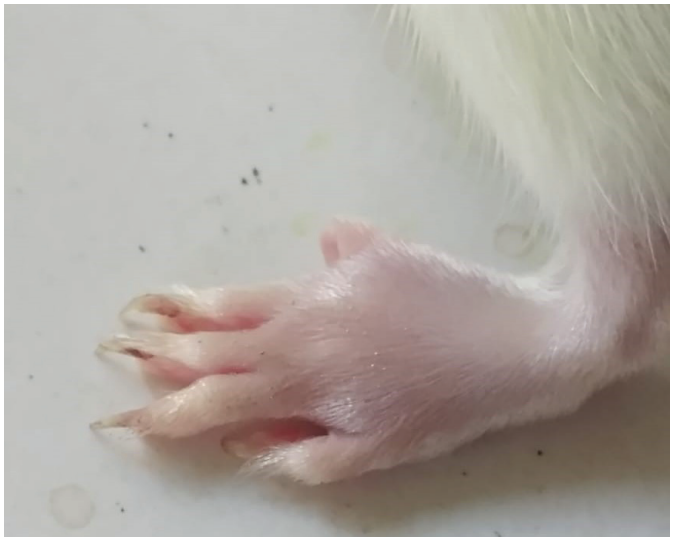

**Figure 5. Hind paw edema at 48 hours of injecting triple antigen solution in interventional groups.**

This finding suggests that Shilajatu has enhanced the immunity of the experimental animals, which further leads to the inhibition of pathological edema due to the presence of polyphenolic compounds, fulvic acid, humic acid, triterpenes, phenolic compounds, 3,4-benzo-coumarins, aromatic carboxylic acids, sterols, polyphenols, and amino acids, etc. in Shilajatu[27] and having potential antioxidant[28,29] and immunomodulatory properties[30]. One of the possible factors responsible for the anti-inflammatory and edema inhibitory action of Shilajatu is polyphenols which contribute to its probable mode of action comprising increasing the type and proliferation of immune cells, suppression of pro-inflammatory genes expression and toll-like receptor (TLR), modulation of cytokines production[31,32] inhibition of the pro-inflammatory cytokines like IL-6 and Tumor necrosis factor alpha (TNF-α),[33] along with inhibition of certain enzymes which are reported to be responsible for the generation of reactive oxygen species (ROS) like xanthine oxidase, NADPH oxidase (NOX), etc. Besides, it upregulates the production of enzymes having antioxidant potential like glutathione (GSH) peroxidase (Px), catalase, and superoxide dismutase (SOD)[34].

As per the availability of the metal cofactors in the active sites, SODs can be categorized into four distinct groups: Iron SOD (Fe-SOD), Nickel SOD, Manganese SOD (Mn-SOD), and Copper-Zinc-SOD (Cu, Zn-SOD)[35]. These trace elements are necessary for the regulation of antioxidant and immunological functions and act as cofactors of various enzymes involved in the metabolism[36]. Therefore, the considerable presence of these cofactors or trace elements in Shilajatu[37] may lead to an increase in SOD enzyme activity. Besides, It has been reported that Fulvic acid (FA) and Humic acid (HA) have increased the generation of IL-2 and the IL-2 receptor expression on lymphocytes which in turn enhanced the proliferative response of lymphocytes and leads to the enhancement of TH1 cells activity[38]. Therefore, Shilajatu, due to the presence of FA and HA, may facilitate the formation of major histocompatibility (MHC)-antigen complexes that are recognized by T cells to initiate the stimulation of immunologic activity through T lymphocytes. Some other scientific studies have reported that Shilajatu exhibits immunomodulation by significantly increasing CD4 cell count, optimizing selective Th1-type immunity, and enhancing the production of selective Th1-like cytokines[39].

The sterols present in the Shilajatu, which can up-regulate Th1 immunity could be one of the reasons for its immunomodulatory activity[40]. In a nutshell, TH1 cells stimulate the type-1 pathway (cellular immunity) to fight against viruses, antigens, and other intracellular pathogens and activate delayed-type hypersensitivity (DTH) response. CD4 and Antigen-specific cytotoxic T-lymphocytes destroy the infected cells by inducing apoptosis. Humic acid is a significant component of Shilajatu, and its treatment provides an increased number of total leukocytes leading to enhanced immunity response[41]. In-vitro, Shilajatu fractions have shown complement-fixing potential in a

dose-dependent manner[42]. The complement system performs a crucial role in innate type of immunity, facilitating the initiation of inflammatory responses leading to the destruction and elimination of pathogens[43]. These are all the possible pathways/modes of action through, which Shilajatu executes its immunomodulatory actions.

In the present study, it has been observed that hind paw edema produced was significantly less in the intervention groups than in the water (negative control) at both the time interval (Fig. 6) which indicates the immunity-enhancing activity of Shilajatu. Moreover, both samples of Amritsar Shilajatu (Water and Triphala kwatha processed) have exhibited superior immunomodulatory activity than Shilajatu samples of Nepal at the interval of 24 hours after antigen exposure while at the interval of 48 hours, both samples of Nepal Shilajatu have shown better immunomodulatory activity than Shilajatu samples of Amritsar. Succinctly, Amritsar Shilajtu has demonstrated a quick effect, whereas Nepal Shilajatu has displayed a better long-term effect. These differences are possibly due to the qualitative and quantitative variation in the elemental composition of both samples, which in turn depends on the source of origin along with climate and temperature etc. of the region of Shilajatu collection as it has been found that warm climate may alter the type and enzymatic dynamics of the soil microbial communities which generally speeds up the decaying and humification of Soil Organic Matter (SOM)[44]. It further affects the quality and therapeutic potential of Shilajatu[45].

Furthermore, the average highest temperature in India is reported to be 36.5 °C which stands out to be approximately 30% higher than Nepal (28.1 °C). The geographical position of India (distance from the equator) also adds to its warmer climate as it is closer to the equator than Nepal[46]. Similarly, variation in fulvic acid content, bioactive low molecular compound, pH, and humic constituents based on geography has also been reported[47]. Due to all these factors, samples from Amritsar (India) and Nepal have shown differences in their immunomodulatory activity which provide evidence of the impact of the source of origin on the therapeutic potential of a drug.

Shilajatu samples processed in Triphala kwatha have shown a better immunity-enhancing profile than water-processed samples of Shilajatu at both the time intervals i.e., 24 and 48 hrs. It is because Triphala is well-reported for its immunomodulatory activities[48,49] which possibly provides a synergistic immunomodulatory effect to Shilajatu. Hence, liquid media used to process Shilajatu plays a very significant role in modulating the therapeutic properties of a preparation. The comparative effect of the geography and processing media of Shilajatu on its immunomodulatory activity has been represented in Fig. 7.

## 5. CONCLUSION

The present study revealed that Pre-treatment with all test drugs, viz. NSW, NST, ASW, and AST significantly

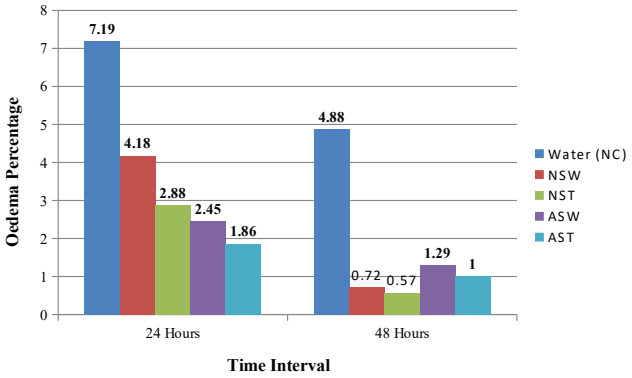


**Figure 6. Edema produced in different study groups after injecting the triple antigen solution.**

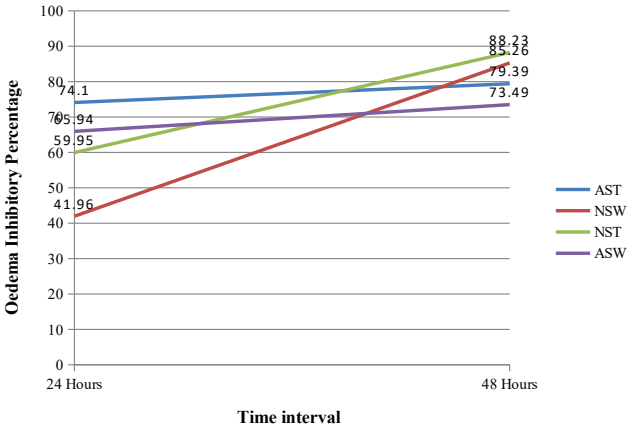


**Figure 7. Comparative edema inhibition percentage in different interventional groups.**

attenuated edema at 24 and 48 hours when compared to the control group. It shows that in the emerging threat of drug resistance, *Shuddha Shilajatu* can be used as a potent immunomodulator in the management of various diseases as well as enhancing the immunity of a healthy person. It has also been observed that processing media significantly enhanced the pharmacological action of *Shilajatu*. Thereby, different processing media can be used to achieve the desired therapeutic effect or to potentiate the effect of therapeutic preparations. Geography and climatic conditions also affect the structural composition of the *Shilajatu,* which further impacts its therapeutic ability. On this account, the source of origin of the raw drug should be considered while using it for therapeutic purposes. Incisively, the present study highlighted the immunomodulatory potential of *Shilajatu* along with the impact of processing media and origin area on it.

## REFERENCES

1. Amirghofran, Z. Herbal medicines for immunosuppression. *Iran J. Allergy Asthma Immunol*., 2012, **11**,111–119.
2. Mishra, K.; Ganju, L.; Sairam, M.; Banerjee, P. & Sawhney, R. A review of high throughput technology for the screening of natural products. *Biomed. Pharmacother*., 2008, **62**(2), 94-98.

doi.org: 10.1016/j.biopha.2007.06.012.
3. Vallance, P. & Smart, T.G. The future of pharmacology. *Br J Pharmacol.,* 2006, **147** Suppl 1(S1),304-307. doi: 10.1038/sj.bjp.0706454.
4. Hassan, J.O. & Curtiss, R. Virulent Salmonella typhimurium-induced lymphocyte depletion and immunosuppression in chickens. *Infect Immun.,*1994, **62**(5), 2027-2036. doi: 10.1128/iai.62.5.2027-2036.1994.
5. Atal, C.K.; Sharma, M.L.; Kaul, A. & Khajuria, A. Immunomodulating agents of plant origin. Preliminary screening. *J Ethnopharmacol*., 1986,**18**(2),133-141. doi: 10.1016/0378-8741(86)90025-5.
6. Dua, P.R.; Shankar, G.; Srimal, R.C. & Husian, A. Adaptogenic activity of Indian Panax pseudoginseng. *Ind J Exp Biol*., 1989, 27:631-634.
7. Hackett, C.J. Innate immune activation as a broad-spectrum biodefense strategy: prospects and research challenges. *J Allergy Clin Immunol*., 2003, **112**(4),686-694. doi: 10.1016/S0091-6749(03)02025-6.
8. Chan, M. Address at the WHO Congress on Traditional Medicine, WHO. 2008, Available online from: http://www.who.int/dg/speeches/2008/20081107/en/ (Accessed on : February 3, 2023).
9. Alamgir, M. & Uddin, S.J. Recent advances on the ethnomedicinal plants as immunomodulatory agents. *Ethnomed Source Compl Therap*., 2010, **37/661**(2), 227-244.
10. Eze, J.I. & Ndukwe, S. Effect of methanolic extract of *Mucuna pruriens* seed on the immune response of mice. *Comp Clin Path.,* 2012, **21**(6),1343-1347. doi: 10.1007/s00580-011-1294-4.
11. Jantan, I.; Ahmad, W. & Bukhari, S.N. Plant-derived immunomodulators: An insight on their preclinical evaluation and clinical trials. *Front Plant Sci*., 2015, **25**(6), 655. doi: 10.3389/fpls.2015.00655.
12. Tripathy, K. Chakrapani commentary, Charak Samhita. Chikitsasthana, chapter 1/1/8. Chaukhamba Sanskrit Sansthan, Varanasi, 2009. pp. 2224.
13. Sengupta, N.N. Jalpakalpataru and Ayurveda Dipika commentary, Charaka Samhita. Chikitsasthana, chapter 1/1/8, Chaukhamba orientalia, Varanasi, 2009. pp. 2266.
14. Bhattacharya. Manual Pre-conference, Annual conference of Indian Pharmacological society. *Work shop Res Methodol Pharmacol*., 1993.
15. Dhundhukanath. Rasendra Chintamani, Mani Prabha Sanskrit Commentary, Maniram Sharma. Chapter10/102. 2nd Ed. Shri Dhanwantari Mandir, Rajasthan, 2000.
16. Kulkarni, D.A. Vigyan bodhini Hindi commentary, Rasa Ratna Samucchaya, chapter 2/117. Meherchand Laxmandas publications, New Delhi, 2017. pp. 23.
17. Sadananda Sharma. Rasa Tarangini. Prasadini Sanskrit commentary by Haridatta Shastri and Rasa Vigyana Hindi commentary by Dharmananda Shastri, edited by Kashinath Shastri. Chapter 22/87, 11th Ed., Motilal Banarasida, New Delhi, 2000.
18. Paget, G.E. & Barnes, J.M. Evaluation of drug activities. Pharmacometrics. In: Lawranle DR, Bacharach AL, editors. vol. 1. Academic press, New York, 1969. pp. 61.
19. Bhatt, K.R.; Mehta, R.K. & Srivastava, P.N. A simple method for recording anti-inflammatory effects on rat paw oedema. *Indian J Physiol Pharmacol.,* 1977,**21**, 399-400.
20. National Cancer Institute. Immune system. Available online from: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/immune-system (Accessed on : February 6, 2023).
21. Wichmann, M.W. & Angele, M.K. Gender Issues in Host Defence. *NeuroImmune Biology*., 2010, **9**,145-158.
22. Ertel, W.; Morrison, M.H.; Ayala, A. & Chaudry, I.H. Insights into the mechanisms of defective antigen presentation following hemorrhage. *Surgery*., 1991,**110**(2), 440-447.
23. Thapa, P. & Farber, D.L. The Role of the Thymus in the Immune Response. *Thorac Surg Clin*., 2019,**29**(2):123-131. doi: 10.1016/j.thorsurg.2018.12.001.
24. Janeway, C.A.; Travers, P. & Walport, M. Immunobiology: The Immune System in Health and Disease. Chapter 8, T Cell-Mediated Immunity. 5th Ed., Garland Science. New York, 2001. Available online from: https://www.ncbi.nlm.nih.gov/books/NBK10762/ (Accessed on : February 6, 2023).
25. Greenfield, E.A. Routes of antigen injection in mice and rats. *Cold Spring Harb Protoc*. 2019, 6. doi: 10.1101/pdb.prot100230.
26. Zaveri, M.; Gohil, P. & Jain, S. Immunostimulant activity of n-butanol fraction of root bark of Oroxylum indicum. *J Immunotoxicol.*, 2006, **3**(2),83-99. doi: 10.1080/15476910600725942.
27. Kong, Y.C.; But, P.P.; Ng, K.H.; Cheng, K.F. & Cambie, R.C. Chemical studies on a nepalese panacea-shilajit (I). *Int J Res Pharm Sci.*, 1987, **25**(3), 179-182. doi: 10. 3109/ 13880208 70906 0 925.
28. Bhattacharya, S.K. & Sen, A.P. Effects of *shilajit* on biogenic free radicals. *Phytother Res.,* 1995, **9**(1), 56-59. doi: 10.1002/ptr.2650090113.
29. Singh, R.; Kaushik, S.; Yadav, P. & Prajapati, P.K. Antioxidant Profile of Shilajatu (Asphaltum punjabinum): Impact of Drava/Media and Bhumi/Geography. *Free radic antioxid.,* 2021, **11**(1), 19-23. doi: 10.5530/fra.2021.1.4.
30. Singh, R.; Kaushik, S; Yadav, P.; Ruknuddin, G. & Prajapati, P.K. Research developments in immunomodulatory and antioxidant activities of shilajatu. *Indian Drugs*., 2021, **58**(9):7-20. doi: 10.53879/id.58.09.11977.
31. Karasawa, K.; Uzuhashi, Y.; Hirota, M. & Otani, H. A Matured Fruit Extract of Date Palm Tree (*Phoenix dactylifera* L.) stimulates the Cellular Immune System in Mice. *J Agric Food Chem*., 2011, **59**, 11287–11293.

doi: 10.1021/jf2029225.
32. John, C.M.; Sandrasaigaran, P.; Tong, C.K.; Adam, A. & Ramasamy, R. Immunomodulatory Activity of Polyphenols derived from Cassia Auriculata Flowers in Aged Rats. *Cell Immunol*., 2011, **271**,474-479. doi: 10.1016/j.cellimm.2011.08.017.
33. Capiralla, H.; Vingtdeux, V.; Venkatesh, J.; Dreses-Werringloer, U. & Zhao, H. Identification of potent small-molecule inhibitors of STAT 3 with anti-inflammatory properties in RAW 264.7 macrophages. *FEBS J.,* 2012, **279**(20), 3791-3799. doi: 10.1111/j.1742-4658.2012.08739.x.
34. Yahfoufi, N.; Alsadi, N.; Jambi, M. & Matar, C. Immunomodulatory and Anti-Inflammatory role of Polyphenols. *Nutrients.*, 2018, **10**(11),1618. doi: 10.3390/nu10111618.
35. Miller, A.F.; Messerschmidt, A.; Huber, R.; Poulos, T. & Wieghart, K. Handbook of Metalloproteins. John Wiley & Sons, Chichester, John Wiley 2001; pp. 668-682.
36. Chandra, R.K. & Au, B. Spleen hemolytic plaque forming cell response and generation of cytotoxic cells in genetically obese mice (ob/ob). *Int Arch Allergy Appl Immunol*., 1980, **62**, 141-147. doi: 10.1159/000232498.
37. Ghosal, S.; Lal, J.; Singh, S.K.; Goel, R.K.; Jaiswal, A.K. & Bhattacharya, S.K. The need for formulation of Shilajit by its isolated active constituents. *Phytother Res.,* 1991, **5**:211-216. doi: 10.1002/ptr.2650050505.
38. Joone, G.K.; Dekker, J. & Jansen van Rensburg, C.E. Investigation of the immunostimulatory properties of oxihumate. *Zeitschrift für Naturforschung C*., 2003, **58**(3-4), 263-267. doi: 10.1515/znc-2003-3-421.
39. Rubab, I.; Routray, I.; Mahmood, A.; Bashir, S. & Shinkafi, T.S. Mineral pitch stimulates humoral, cellular and innate immune responses in mice. *Pharm Biol.*, 2013, **51**(8), 997-1007. doi: 10.3109/13880209.2013.774027.
40. Grimble, R.F. Nutritional modulation of immune function. Proceedings of the Nutrition Society., 2001, **60**(3),389-97. doi: 10.1079/PNS2001102.
41. Rousdy, D.W.; Rahmawati, R. & Kurniatuhadi, R. Immune Responses of Wistar Rat (Rattus novergicus) on Adduction of Humid Acid from Borneo Peat Soil. *Biosaintifika: Journal of Biology & Biology Education.,* 2016, **8**(3),400-405. doi: 10.15294/biosaintifika.v8i3.7499.
42. Schepetkin, I.A.; Xie, G.; Jutila, M.A. & Quinn, M.T. Complement-fixing activity of fulvic acid from Shilajit and other natural sources. *Phytother Res*., 2009, **23**(3), 373-384. doi: 10.1002/ptr.2635.
43. Gasque, P. Complement: a unique innate immune sensor for danger signals. *Mol Immunol.,* 2004, **41**(11),1089-1098. doi: 10.1016/j.molimm.2004.06.011.
44. Li, J.; Ziegler, S.; Lane, C.S. & Billings, S.A. Warming-enhanced preferential microbial mineralization of humified boreal forest soil organic matter: Interpretation of soil profiles along a climate transect using laboratory incubations. *J Geophys Res Biogeosci*., 2012, **117**(G2). doi: 10.1029/2011JG001769.
45. Ghosal, S. Chemistry of shilajit: an immunomodulatory Ayurvedic rasayan. *Pure Appl Chem*., 1990, **62**(7), 1285-1288. doi; 10.1351/pac199062071285.
46. UK Environmental Change Network. Factors affecting climate. Available online from: https://ecn.ac.uk/what-we-do/education/tutorials-weather-climate/climate/factors-affecting-climate#:~:text= The%20distance%20 from %20the%20equator, cooler% 20further %20 from%20the%20Equator (Accessed on : March 8, 2023).
47. Ghosal, S.; Lal, J.; Singh, S.K.; Goel, R.K. & Jaiswal, A.K. The need for formulation of Shilajit by its isolated active constituents. *Phytother Res.,* 1991, **5**(5), 211-216. doi: 10.1002/ptr.2650050505.
48. Srikumar, R.; Parthasarathy, N.J. & Devi, R.S. Immunomodulatory activity of Triphala on neutrophil functions. *Biol Pharm Bull*., 2005, **28**, 1398–1403. doi: 10.1248/bpb.28.1398.
49. Srikumar, R.; Parthasarathy, N.J.; Manikandan, S.; Muthuvel, A. & Rajamani. R. Immunomodulatory effect of Triphala during experimentally induced noise stress in albino rats. *J Health Sci.,* 2007, **53**:142-145. doi: 10.1248/jhs.53.142.

## CONTRIBUTORS

**Dr Rohit Singh**, completed his M.D. from All India Institute of Ayurveda, New Delhi in Rasa Shastra and Bhaishajya Kalpana. Currently, he is pursuing Ph.D. in Rasa Shastra and Bhaishajya Kalpana from AIIA, New Delhi.
He was involved in experimentation, data collection and manuscript writing.

**Dr Pramod Yadav**, obtained his Ph.D. in Rasa Shastra and Bhaishajya Kalpana from ITRA, Jam Nagar, Gujrat. Presently, he is working as Assistant Professor in Department of Rasa Shastra and Bhaishajya Kalpana, AIIA, New Delhi.
He has supported in providing raw material and contributed in study design, data evaluation, interpretation and structuring of manuscript.

**Professor (Vd.) Pradeep Kumar Prajapati**, is Vice Chancellor of DSRRA University, Jodhpur, Rajasthan. He is working in the area of preclinical as well as clinical trial of Rasa Aushadhi/ Herbomineral drugs.
He has contributed to conception of work and provided crucial inputs in data interpretation and discussion part of the manuscript.