ISSN 2456-3110
Vol 4 · Issue 6
Nov-Dec 2019

Journal of Ayurveda and Integrated Medical Sciences

www.jaims.in

JAIMS

An International Journal for Researches in Ayurveda and Allied Sciences

Charaka Publications

Indexed

Journal of
Ayurveda and Integrated Medical Sciences
ISSN 2456-3110
ORIGINAL ARTICLE Nov-Dec 2019

Immunomodulatory activity of *Mashaparni* - *Teramnus labialis* Spreng

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ABSTRACT

Background: Mashaparni is a plant belonging to the family Fabaceae and used in ayurvedic system of medicine and is being used in diet by various people of India and it also forms a part of traditional Indian cuisine. *Teramnus labialis* Spreng as accepted botanical source of Mashaparni. Mashaparni is widely used as Balya drug as mentioned by Charaka acharya in Balyamahakashaya gana. Hence this study is aimed to evaluate the Immunomodulatory activity of Mashaparni. **Methodology**: Experimental studies to determine the Immunomodulatory activity of Mashaparni *Teramnus labialis* Spreng by antigen induced rat paw oedema in Wister albino rats and the drug in the Aqueous & Ethyl extract form respectively. The studies were carried out on 4 groups i.e. Control group, Standard group, Aqueous extract and Ethyl extract form. Standard drug taken for the study is Guduchi *Tinospora cordifolia* in Ethyl extract form. Immunomodulatory activity determined by Delayed Type Hypersensitivity method with the help of Digital Plethysmograph method for duration of 15 days. The rat paw volume was the assessment parameter and the results were recorded at 2^{nd}, 4^{th}, 6^{th}, & 24^{th} hours after induction of antigen. **Results:** The Aqueous extract of Mashaparni shows a significant Immunomodulatory action compared to Ethyl extract of Mashaparni.

Key words: *Mashaparni, Guduchi, Extract, Antigen, Digital Plethysmograph.*

INTRODUCTION

Ayurveda is a traditional system of medicine, which plays a crucial role in current health care and modulation of immune response to treat the diseases.[1] Plants are one of the main sources of medicine since immemorial time. Ayurveda, the science of life emphasizes on use of plants as medicine and says that each and every dravyas in this universe possesses some medicinal property.[2] The Balya medicinal plants in Ayurveda are reported to have qualities such as the capability to develop health and strength. Mashaparni is one of the drugs which is mentioned in the Balyagana,[3] also there are many activities of Mashaparni mentioned in Samhita as Balya, Shukrajanana, Jeevaniya, Bhrumaniya etc. As per Charaka there are three types of Bala in which Yuktikrutabala is gained by Ahara, Vihara and Yogas (Acquired immunity).[4] As there are minimal scientific details available in the literature on the Immunomodulatory activity of Mashaparni *Teramnus labialis* Spreng (whole plant in the form of extract).

Therefore, a sincere attempt is made to find out the Immunomodulatory activity of Mashaparni *Teramnus labialis* Spreng through an experimental evaluation using Wistar albinorats on DTH (Delayed type hyper sensitivity) method.

Synonyms[3-6]: Rushyaprokta, Mahasaha, Kamboji, Hayapucchi, Pandulomashaparni.

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Submission Date: 08/11/2019 Accepted Date: 19/12/2019

Access this article online

Quick Response Code

Website: www.jaims.in

DOI: 10.21760/jaims.4.6.3

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Vernacular names[7]

Table 1: Vernacular names of Mashaparni

Language	Name
Sanskrit	Mashaparni
English	Vogel – Tephrosis
Hindi	Mashaparni, Mashavan, VanaUrada, Jangliudad, Banurdi, Banudad, Mashoni, Mashani.
Marathi	Ran udida
Bengali	Mashance, Bankalai, Mashani
Kannada	Kadu uddu

Rasapanchaka[8]

Rasa: Madhur,Tikta

Guna: Ruksha

Viry: Sheetavirya

Vipaka: Madhur

Doshagnata:Vata hara and Kaphavardhak.

MATERIALS AND METHODS

Botanical name: *Teramnus labialis* Spreng

Family: Fabaceae

Collection and Identification of Plant Material

Collection of the Trial Drugs: Plant material of Mashaparni was collected from herbal garden of Sri Sri College of Ayurvedic Science and Research and surrounding natural habitat Bangalore.The genuinity of the whole plant was confirmed by Botanist, Department of Dravyaguna, Sri Sri College of Ayurvedic science and Research Bangalore.

Standard Drug Material: The standard drug taken for the study is Guduchi (*Tinospora cordifolia)*. The Guduchi stems was collected from the herbal garden of Sri Sri College of Ayurvedic Science and Research Campus.

Macroscopic Evaluation: The morphology of the whole plant of Mashaparni was studied with the help of available literature, and was observed for the following features - morphology of leaf, root, stem.

Microscopic evaluation: The cross section of root, stem, and leaf of Mashaparni was done and observed under compound microscope and captured using camera microscope.

Preparation of Extract: The extraction was carried out by Soxhlet apparatus.[9]

Physicochemical Evaluation: Determination of Foreign Matter, Asha value, Loss on drying, Acid Insoluble Ash, Alcohol Soluble Extractive Value, Water-Soluble Extractive, Ph.[9]

Phytochemical Evaluation: The extract was screened for various constituents (alkaloids, saponification, tannins, flavonoids, Proteins, Glycosides, Triterpenoids, Carbohydrate, Steroids).[9]

Physicochemical Evaluation, Phytochemical Evolution was carried out in Dravyaguna Laboratory of Sri Sri College of Ayurvedic Science and Research, Bangalore.

Thin Layer Chromatography:[9] was carried out in Sri Sri Tattava, Bangalore.

Acute toxicity study

Acute toxicity of the mashaparni extract were performed according to the OECD Guidelines 423

METHODOLOGY

Place of Work

The Experimental study was carried out in Department of Pharmacology, Acharya & Reddy College of Pharmacy, Bengaluru.

Ethical Clearance

Ethical clearance approval was obtained from Institutional Animal Ethics committee (IAEC) with reference no: IAEC/ABMRCP/2018-2019/7.

Dose fixing

Acute oral toxicity studies revealed that there was no toxicity and no mortality of the rates upto 5000mg / kg body weight with aqueous and Ethanol extract of

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Mashaparni. Hence the dose of the present study is taken as, 1/10th, of the limit test dose.

Mashaparni Aqueous extract - 500 mg/kg body weight

Mashaparni Ethanol extract - 500 mg/kg body weight

Standard drug dose calculation

The dose of the standard drug Guduchi was taken as 100mg /kg body weight.[10]

Immunomodulatory activity[11]

Study Design

Experimental evaluation of Immunomodulatory activity of Mashaparni *Teramnus labialis.* Spreng is done by Delayed type hypersensitivity responses and measured by Digital Plethysmograph method.

Inclusion criteria

- Healthy Wister strain albino rats of either sex
- Albino rats weighing 150- 200 gms

Exclusion criteria

- Albino rats which are pregnant.
- Albino rats showing the signs of infection during the course of study.
- Albino rats those who are under other experiments.
- Albino rats younger than 45 days.

Preparation of antigen

Fresh blood of the sheep was collected from the local slaughter house in a sterile bottle. Blood was kept in refrigerator and processed for the preparation of SRBCS batch by centrifuging at 2000 rpm for 15 mins and the supernent layer was collected in a sterile bottle and used for the further study.[11]

Treatment protocol and immunization[11]

Treatment Protocol

- Total 16 numbers of Albino Rats of weight ranging from 150- 200g were taken for thestudy.
- The animals were divided into four groups, comprising of 4 rats in each group.
- The animals were marked priorly on head, body, tail, and no marking
- All the rats were fed with normal water and food throughout the study.
- Group one (G1) control animals received normal water and food only for 7 days per orally and on 7th day SRBC antigen injection in sub-plantar region of right hind paw.
- Group two (G2) animals received Ethanol extract of Guduchi as a standard drug at a dose of 100mg/kg per orally for 7 days and on 7th day 0.1 ml of SRBC antigen injection in sub plantar region of right hind paw.
- Group three (G3) animals received Aqueous extract of Mashaparni (*Teremnus labialis*) test drug at dose of 500mg/kg for per orally for 7days and on 7th day 0.1 ml of SRBC antigen injection in sub-plantar region of right hind paw.
- Group four (G4) animals received Ethanol extract of Mashaparni (*Teremnus labialis*) test drug at a dose of 500mg/kg per orally for 7days and on 7th day 0.1 ml of SRBC antigen injection in sub-plantar region of right hind paw.
- In delayed type of hypersensitivity prior sensitization is important. After sensitization, all the animals were observed every day for the behavioral changes for 7 days.

Delayed Type of Hypersensitivity Reaction

- On the 14th day of the study again all the animals were injected with 0.1ml of SRBC insub-plantar region of the right hind paw.
- The thickness of the right hind paw of the rat induced with SRBC was measured using Digital Plethysmometer
- Hind paw reaction was accessed at the interval of 2nd, 4th, 6th and 24th hours after the injection of SRBC.
- The hind paw reaction was expressed as the difference in thickness between the 2nd, 4th, 6th & 24th hours in all the four groups.

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Statistical Analysis

The results were presented as a Mean ± Standard Error of the Mean. The difference among the data was statistically analysed using one-way ANOVA followed by turkey test to determine the level of significance using prism. Graph pad version5 (Graph pad software. Inc). Difference between the data were considered significant at P<0.05.

OBSERVATION AND RESULTS

Phytochemical Analysis

Table 2: Phytochemical Analysis

Constituents	Tests	Aqueous Extract of Mashaparni	Alcoholic Extract of Mashaparni
Alkaloids	Dragendroff's	-	+
	Wagner's	+	+
Flavonoids	Led Acetate test	+	-
Saponins	Foam test	+	+
Triterpenoids	Salkowski test	-	-
Tannins	Gelatin test	-	-
Proteins	Biuret test	-	-
Carbohydrates	Molisch's test	-	-
Steroids	Salkowski test	+	-
Starch	Iodine test	-	-
Fixed Oil	Stain test	-	-
Glycoside	Lieberman's test	+	-
Phenols	Ferric chloride test	+	-

TLC Result

Table 3: Thin Layer Chromatography Result

Stationary phase	Silica gel 60F 254
Mobile phase	Toluene: Ethyl acetate
Detection	366nm
Standard concentration	Toluene: Ethyl acetate (9:1)
Sample concentration	2 g

Under UV 366nm the Rf value obtained are 0.82, 0.26, 0.57 (Green), 0.43, 0.22 (Violet), 0.74, 0.53 (Purple), 0.64 (Sky-blue).

EXPERIMENTAL STUDY

Statistical Analysis

- The group which received aqueous extract of Mashaparni at dose of 500 mg/kg body weightshowed mean percentage increase in right hind paw thickness and it was highestafter 2 hrof injection of SRBC antigen. There was a reduction in the increase of right hind pawthickness after 6 hr post-antigen injection, and this later reduced at 24 hr.
- However, it was not true for the group that received 500 mg/kg body weight of ethanolextract of Mashaparni that showed a significantly steady increase in right hind paw thicknessup to 6 hr post-antigen injection.
- The group that received Guduchi at dose of 100mg/kg body weight as a standard drugshowed a steady increase in right hind paw thickness after 4 hr and 6 hr of post antigeninjection.
- Hence there was a statistically significant decrease in percentage of right hind paw thickness(p _ 0.5) in the group that received 500 mg/kg body weight of aqueous extract of Mashaparniup to 24 hr when compared with control and ethanol group.

Table 4: Effect of *Teramnus labialis* Spreng on Delayed Type Hypersensitivity Reaction.

Group	2 Hour	4 Hour	6 Hour	24 Hour
Control	1.86± 0.18	1.76±0. 18	1.73±0. 11	1.41±0. 29
Tinospora cordifolia (100 mg/kg, p.o.)	1.62± 0.21	1.56±0. 22	1.54±0. 1	1.23±0. 15
Aq. Extract of *Teramnus*	1.46±	1.39±0.	1.22±0.	1.26±0.

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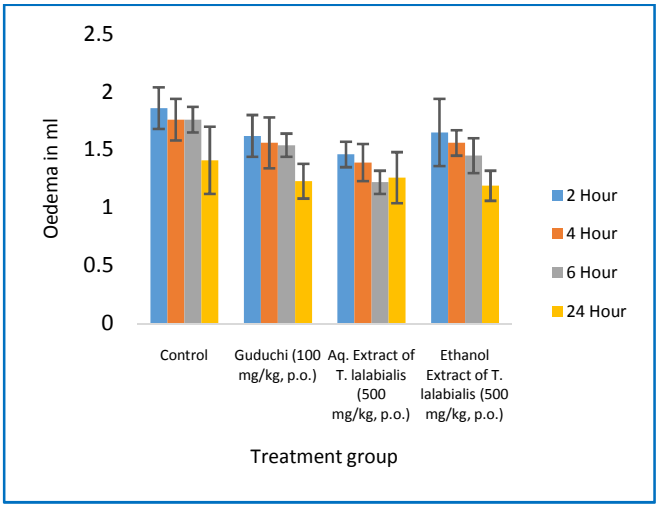
labialis (500 mg/kg, p.o.)	0.14	16*	1*	22*
Ethanol Extract of *Teramnus labialis* (500 mg/kg, p.o.)	1.65± 0.12	1.56±0. 11	1.45±0. 15*	1.19±0. 13*

All the values were expressed in Mean ± SD (n=4). The statistical analysis was carried out using one-way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett test. **P<0.5,* when compared to control group.

Fig. 1: Graphical Presentation of Delayed Type Hypersensitivity Reaction.



DISCUSSION

Physicochemical Study

Physicochemical parameters of the drug Mashaparni obtained was within the permissible limit of Ayurvedic pharmacopeia of Indian medicinal plants. This infers the proper collection of theplant material for the research work.[9]

Phytochemical Study[12]

The aqueous extract of Mashaparni showed the presence of Glycosides, Alkaloids, Flavonoids, Saponins, Tannins & Phenolic compounds, and Steroids, whereas the Ethanol extract of Mashaparni showed the presence of only Alkaloids and Saponins. This could be because of phytochemical constituent of Mashaparni are more soluble inaqueous media than in ethanol media.

Discussion on Results

The group that received 500 mg/kg body weight of ethanol extract of Mashaparni showed a significantly steady increase in right hind paw thickness up to 6hr postantigen injection. The group that received 100 mg/kg Guduchi as a standard drug showed a steady increase in righthind paw thickness is observed after 4hr and 6hr. There was a statistically significant decrease in percentage right hind paw thickness (p0.5) in the group that received 500 mg/kg bodyweight of aqueous extract of Mashaparni.

Probable Mode of Action of Drug

According to Charaka, Prakruta kapha is bala. The usage of dravyas having madhura rasa, sheeta veerya, snigdha guna is going to enhance kapha. So, the former qualities are also going to increase Bala.[13] According to Vaghbhata, Ojas is also considered as Bala.[14],[15] As per the *Ashraya-Ashryee Bhavas Shleshma* takes ashraya in Rasa, Mamsa, Meda, Majjadhatu. The state of these dhatus are directly dependent on the status of kapha. As Mashaparni is having madhura rasa, sheeta virya and snigdha guna it increases prakruti kapha which in turn is going to increase Rasa, Mamsa, Meda, Majja adi dhatus and may bring about enhancement in strength andimmunity of an individual. The Panchabhoutik composition of Kapha is Prithvi and Aap mahabhoota. Similarly, the madhura rasa is predominant of prithvi and Aap mahabhoota. By samanya siddhanta (samanyam vriddhikaranam), it can be inferred that the Madhura rasa present in the Mashaparni is responsible for the increase in the Prakruta kapha i.e., Bala. Presence of Some of the phytoconstituents like saponins-2.5%, flavones-5.4% andpolyphenol-2.6% of *Teramnus labialis* can be attributed to the immunomodulatory actionof the drug.[12] Hence all these factors of the drug attributes to the enhancement of Bala.

CONCLUSION

The term Bala mentioned in Ayurvedic classics can be attributed to physical strength and for immunity of an individual.[2] *Teramnus labialis* is botanical source of

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Mashaparni and synonyms Mahasaha, Hayapucchi can be attributed for its morphological identification. Phytochemical studies confirmed the presence of Phyto-constituents like Alkaloids, Flavonoids, Saponins, Tannins, Phenolic compounds and Steroids in aqueous extractand Alkaloids and Saponins in Ethanol extract. Acute toxicity studies revealed there is no toxicity and death of the rats with Aqueous and Ethanolic extracts of Mashaparni up to 5000mg/kg body weight. Aqueous extract of *Teramnus labialis* showed more reduction in paw oedema of rats compared to its ethanolic extract in antigen induced Delayed type of hypersensitivity experimental model. Mashaparni *Teramnus labialis* is found to have statistically significant Immunomodulatory action in its Aqueous extract form.

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How to cite this article: Dr. Shweta, Dr. Seema Pradeep, Dr. Shiva Manjunath M.P. Immunomodulatory activity of Mashaparni - Teramnus labialis Spreng. J Ayurveda Integr Med Sci 2019;6:13-18.
http://dx.doi.org/10.21760/jaims.4.6.3

Source of Support: Nil, **Conflict of Interest:** None declared.

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