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Research Article

AN IN-VIVO STUDY TO EVALUATE THE EFFICACY OF *PADMAKA* (PRUNUS CERASOIDES) AS A *VEDANASTHAPAK* IN *SUPTI* (NUMBNESS) CAUSED BY NEUROPATHY IN WISTAR RATS

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ABSTRACT

This research article attempts to address the main symptom of chemotherapy-induced neuropathy, which is 'numbness'. Neuropathic pain is a common side effect of anticancer drugs that significantly reduces a patient's quality of life. This work redefines the term "*Vedanasthapan*" by explaining the act of regaining sensation along with its analgesic properties. This study was carried out by using an animal model of rats prepared by using an anti-cancer agent cisplatin. Before inducing cisplatin, normal latency periods of all rats were noted by 'Hot plate' method. Cisplatin was given to the rats to induce numbness with the interval of a week for two weeks. Four groups of rats having six rats in each group were labelled as - 'control group' treated with daily diet only, 'vehicle group' treated with carboxymethyl cellulose, 'positive control' treated with pregabalin, and 'test drug group' treated with *'Padmaka'*. After the treatment period, all groups were tested on a hot plate to record the latency time of rats. All obtained data were processed by ANOVA test and Tukey-Kramer multiple comparison test. It showed that the means of all groups differed significantly from each other. The mean time of the positive control group was 9.06 seconds and that of the experimental drug group was 12.9 seconds, which is close to the mean time in the normal state (7.36 seconds). While the control group and the vehicle group were far apart. This means that the experimental drug *Padmaka* is also useful in reversing cisplatin-induced numbness. Further clinical studies are needed to fully understand its range of action.

INTRODUCTION

According to the Ayurvedic classics *Dravya* is the main part of any treatment. Also, *Guna* and *Karma* are bound to the *Dravya,* which denotes the importance of *Dravya*; hence *Dravyaguna Vidnyanam* has a crucial role in treatment. *Bruhatrayee, Laghutrayee* and *Nighantus* have explained *Dravyas* abundantly.

In the *Charak Samhita*, *Acharya Charak* has given fifty *Mahakashayas* in the '*Shadvirechana shatashritiya Adhyay*' of *Sutrasthan*. In each *Mahakashay*, ten drugs in each group are useful for that particular condition. '*Vedanasthapan mahakashay*' is one of those fifty *Mahakashayas* which consists of - *Shal, Katphala, Kadamba, Padmaka, Tumba, Mocha Rasa, Tumba, Mocha Rasa, Shirish, Vanjula, Elvaluka,* and *Ashok*[1]. Out of those, this dissertation focuses on the *Padmaka* i.e., *Prunus cerasoides*. '*Vedanasthapan*' word is the combination of two words, *Vedana + Sthapan*. According to Monnier Williams dictionary, one of the meanings of '*Vedana'* is 'feeling' or 'sensation'[2] and that of '*sthapan*' is 'causing to stand', 'preserving' or 'maintaining'[3]. *Amarkosha* has given- '*Samvedo Vedana'*[4]. Maximum *Dravyas* in the *Vedanasthapan Mahakashay* are having the quality to elevate *Vata Dosha*. According to classical text, *Padmaka* bears *Kashay-Tikta Rasa*, *Katu* as an *AnuRasa, Katu Vipaka* and *Sheet Veerya*[5], which are favourable for the *Vata Dosha*. So, instead of taking *Vedanasthapan* as an analgesic property; they may have capacity to generate normal sensation at the site of sensation loss. Hence this study mainly emphasized on the effect of *Padmaka* as a *Vedanasthapak* in neuropathy especially in numbness.

Peripheral neuropathy is very common and found in 10 million cases per year in India. It produces weakness, numbness, and pain from nerve damage

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usually in hands and feet. Its common cause is diabetes, but it can also result from injuries, infections and exposure to toxins. In human beings, myelin sheath provides insulation to the nerves and offers healthy transmission of impulses. If it is damaged, causes impairment in the conduction of signals in the affected nerves, thus causing numbness and other symptoms.

To analyze the concept of *Vedanasthapan,* a rat model is created by inducing neuropathy to the rats and effect of *Padmaka* on them is observed. An antineoplastic agent – Cisplatin[6] is used to develop neuropathy among all rats. To compare the effectiveness of *Padmaka*; a well-recognized drug Pregabalin is used as a standard.

Study Rationale

CIPN (Chemotherapy induced peripheral neuropathy) is a certain side effect of chemotherapeutic agents which affects the quality of life. Anticonvulsant, antidepressant, neuroprotectants, and nutraceuticals have been evaluated in clinical trials but consistent beneficial effects have not as yet been shown for any single agent (H.Starobova, 2017). But we believe that Ayurveda may come out with a better solution for this disease.

As, *Acharya Charak* has already stated a group of drugs under the *Vedanasthapan Gana;* along with analgesia, these drugs may have effect on *Supti* (numbness). Many pieces of researches have been done on the analgesic activity of *Vedanasthapak Gana* as well as *Padmaka* but unlike this study. Hence this study aims at evaluating the action of *Padmaka* as a *Vedanasthapak* and to prove its action on *Supti.*

It will further generate a new data for the understanding the concept of *Vedanasthapan* and effect of *Padmaka* on CIPN.

AIM

The study aims to evaluate the efficacy of *Padmaka* (Prunus cerasoides) as a *Vedanasthapak* in *Supti* w.s.r. neuropathy in Wistar rats.

MATERIALS AND METHODS

Preparation of Animal Model

The animal study was carried out in accordance with the recommendations and approval of the Institutional Animal Ethical Committee having approval number – SGRS/IAC/09/2020-21.

Wistar rats of either sex, 6-8 weeks old weighing 180-200 g were procured and used in this study. They were housed at standard environmental conditions (Fig.4). They were fed on standard chow and provided purified water and libitum.

Study has started with the induction of sensation loss by using drug Cisplatin intravenously for a month before study. All four groups were treated with cisplatin to induce loss of sensation. Rats of both sexes were given the chemotherapeutic agent Cisplatin 4mg/kg i.v./OD through tail vein once a week for four weeks to hamper their normal sensation. During this intervention, rats were fed with their regular diet.

30 days for induction of sensation loss with the help of chemotherapeutic agent cisplatin. After that following dosing schedule is administered:

Table 1: Group distribution

S. No.	Groups used	To be treated with	Dose	Route of Drug Administration	No. of animals
1.	Control group	Daily diet	As per decided by the animal study centre.	oral	6
2.	Vehicle group	CMC	0.5% w/v	oral	6
3.	Positive control group	Pregabalin (PGN)	5mg/kg	oral	6
4.	Test drug group	*Padmaka*	4mg/kg	oral	6
	Total				24

Experimental Design

The bark of *Padmaka* was procured from its natural habitat, authenticated at Pharmacognostical Laboratory of Alarsin, Mumbai, and made into fine powder (mesh size #85) (Fig.1, 2, 3)

Cisplatin was given by piercing the tail vein of rats (Fig.5). A suspension of test drug *Padmaka* and positive control drug Pregabalin were made with a vehicle- CMC (carboxymethyl cellulose) for oral administration to the animals (Fig.6).

Dose Fixation

The doses of the drugs were calculated by extrapolating therapeutic dose to the rat dose by calculating surface area ratio (conversion factor of 0.018 for rats) by referring to the table of 'Pagets and Barnes' (Pagets and Barnes, 1964)[7] i.e., for rats.

Human dose × 0.018 = X g/200 g of rat's body weight.

Whereas, human dose = 3gm[8]

Diet of Animals

Rats have free access to food and water during the acclimatization period and during the study. Animals of all groups have received Basal diet pellets throughout the study i.e., right from neuropathy induction to the completion of treatment. These pellets contain:

Table 2: Diet of Animals

Sr. no.	Ingredients	g/100
1	Wheat flour	22.5
2	Roasted gram flour	60
3	casein	5
4	Refined groundnut oil	4
5	Salt mixture	4
6	Vitamin mixture	0.5
	Total	96

Fig.1 Prunus cerasoides trunk

Fig.2 Peeled bark of *Padmaka*

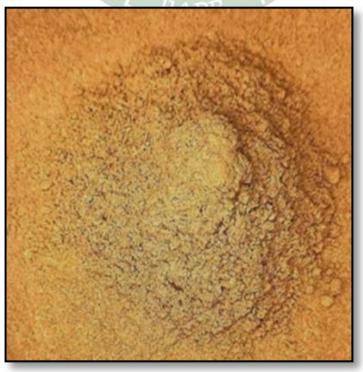

Fig.3 Powder of *Padmaka kanda* (stem)

Fig.4 - Group Formation of Rats According to the Treatment Given

Fig.5 Injection of Drug Cisplatin Through tail vein

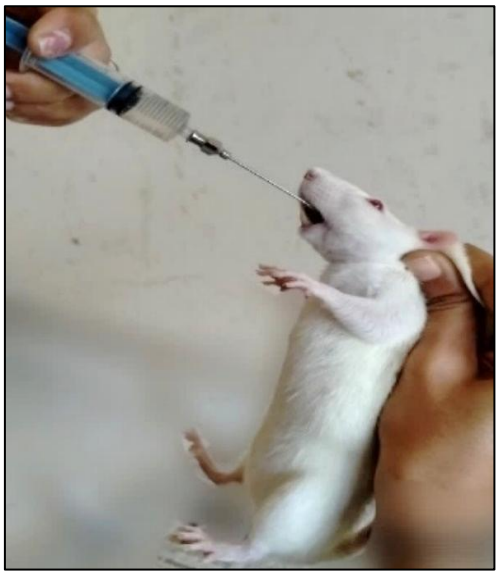

Fig.6 Oral administration of *Padmaka*

OBSERVATIONS

Assessment Parameters:

Assessment test: Hot Plate Test (Temperature: 55±1°C)

The latency to respond to a thermal stimulus applied to the paws is determined by the time (in seconds) it takes the mouse to lick or flick the hind paw or jump from the hot plate surface (IACUC standard procedure).

Table 3: Normal time of latency (sec.) of rats before treating with any medication (all four groups)

Animal	Group1 (Control)	Group 2 (Vehicle)	Group 3 (Positive Control)	Group 4 (Test drug)
1	6.9	7.2	8.4	7.9
2	7.2	8.3	7.1	8.5
3	7.1	6.9	7.6	7.4
4	6.7	7.1	6.2	6.8
5	6.8	6.5	8.1	7.5
6	7	7.9	7.3	8.2
Mean	**7.36**			

Above table covers the normal contact time of rats on hot plate when tested before administrating cisplatin and after accomplishing their acclimatization period.

Table 4: Observations on day-0

Animal	Group 1 (Control) (in sec.)	Group 2 (Vehicle) (in sec.)	Group 3 (Positive Control) (in sec.)	Group 4 (Test drug) (in sec.)
1	29.1	29.2	28.6	28.9
2	29.3	29.3	29.3	29.1
3	29.2	29.5	28.9	29.3
4	29.1	29.1	29	29
5	29.4	28.7	29.5	29.2
6	29.1	29	29.1	27.9

Above table shows the contact time of rats with hot plate after the treatment of cisplatin. Time is remarkably seen increased as compared to the table no3, which was the normal time of latency.

Table 5: Observations on day-14

Animal	Group 1 (Control) (in sec.)	Group 2 (Vehicle) (in sec.)	Group 3 (Positive Control) (in sec.)	Group 4 (Test drug) (in sec.)
1	29.2	29.2	16.5	-
2	-	-	15.8	17.8
3	29	29.1	-	18.3
4	-	28.7	14.8	17.5
5	28.3	27.3	15.1	18.7
6	28.5	-	16.3	18

Above table gives the contact time of rats on 14th day of treatment. There was no change observed in the 1st and the 2nd group but decrease contact time with hot plate was observed in the 3rd and 4th group. Mortality was also detected in the all groups, more in 1st and 2nd group.

Table 6: Observations on Day–30

Animal	Group1 (Control) (in sec.)	Group 2 (Vehicle) (in sec.)	Group 3 (Positive Control) (in sec.)	Group 4 (Test drug) (in sec.)
1	28.4	28.7	9.1	-
2			8.7	13.4
3	28.2	29	-	13.9
4		29.1	8.2	11.9
5	29.4	28.3	9.4	12.6
6	29		9.6	12.7

Above table depicts the contact times of rats with hot plate on the 30th day i.e., at the last day of the treatment. Slight change was observed in the 1st and 2nd groups. Values were considerably decreased in the 3rd and the 4th group as compared to the observations on day 0 and day 14.

RESULTS

To compare the mean values of the test group with that of control group and the positive control group, ANOVA test along with Tukey's multiple comparison test has used which will propose following hypothesis:

H_0 – Mean values of all groups are equal. Vs. H_1 – Mean value of at least one group is significantly different.

The level of significance was taken 5%. ANOVA test and Tukey's multiple comparison tests were performed by using the Instat graphpad 3 free version.

Tukey-Kramer Multiple Comparisons Test

If the value of q is greater than 4.111 then the P value is less than 0.05.

Table 7: ANOVA for observations on day- 14

Comparison	Mean Difference	q		P value
Positive Control Vs Test	-2.367	6.700	**	P<0.01
Control Vs Test	10.233	33.454	***	P<0.001
Vehicle Vs Test	10.333	33.781	***	P<0.001
Control Vs Vehicle	-0.1000	0.4004	ns	P>0.05
Control Vs Positive Control	12.600	41.191	***	P<0.001
Vehicle Vs Positive Control	19.667	41.518	***	P<0.001

** - Moderately significant, ***- Highly significant, ns- Not significant.

Table 8: Summary of data

	No of points	Mean	Standard deviation	Standard error mean	Median
Control	4	28.567	0.5354	0.2186	28.600
Vehicle	4	28.667	0.6947	0.2836	28.850
Positive control	5	15.967	0.7572	0.4372	16.300
Test	5	18.333	0.3512	0.2028	18.300

Table 9: Statistical results

ANOVA table	SS	DF	MS	F (DFn, DFd)	"P value"
Treatment (between columns)	534.4	3	178.1	F (3, 14) = 475.9	P<0.0001
Residual (within columns)	5.240	14	0.3743		
Total	539.6	17			

The P value is < 0.0001, considered extremely significant.

Variation among column means is significantly greater than expected by chance.

Considering table no.7, Mean difference between the positive control and test group is very low suggesting that test group is showing the similar activity as that of positive control group on the 14th day observation. Also, significant difference is observed between test group and control group which is suggestive of- test group is showing better activity than the control group.

Day 30th Observation

Table no.10: Multiple comparison by Tukey-Kramer test – If the value of q is greater than 4.111 then the P value is less than 0.05

Comparison	Mean Difference	q		P value
Positive Control Vs Test	3.833	12.556	*	P<0.001
Control Vs Test	15.750	59.567	***	P<0.001
Vehicle Vs Test	15.833	59.883	***	P<0.001
Control Vs Vehicle	-0.08333	0.3860	ns	P>0.05
Control Vs Positive Control	19.583	74.065	***	P<0.001
Vehicle Vs Positive Control	19.667	74.380	***	P<0.001

Moderately significant, ***- Highly significant, ns- Not significant.

Table 11: Summary of the Data

	No. of points	Mean	Standard deviation	Standard error mean	Median
Control	4	28.650	0.7036	0.2872	28.700
Vehicle	4	28.733	0.4033	0.1674	28.850
Positive control	5	9.067	0.3512	0.2028	9.100
Test	5	12.900	0.4359	0.2517	12.700

The P value is < 0.0001, considered extremely significant.

Variation among column means is significantly greater than expected by chance.

Mean from all groups are significantly different from each other as given by the one way ANOVA test and Tukey-Kramer Multiple Comparisons Test performed in Instat graph pad-free trial version.

From the obtained values, we can conclude that the latency period of rats on a hot plate from the test drug group is significantly less than the control group. It is our desirable outcome. When compared with the positive control group, the latency period of the test group was nearer to it. It suggests that the test drug is showing similar action that of the positive control group. It means, test drug *Padmaka* shows positive results in reversing the numbness caused by cisplatin but less than the positive control pregabalin.

Table 12: Statistical results

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	1276	3	425.5	F (3, 14) = 1521	P<0.0001
Residual (within columns)	3.915	14	0.2796		
Total	1280	17			

According to table no.9 and table no.12,

F-ratio 14th = 475

F-ratio 30th = 1521

F (0.05) = (3, 14) = 3.34

F-ratio calculated > F (0.05)

Hence, the null hypothesis that, there is no difference between the means of groups is rejected.

A one-way between-subjects ANOVA was run with the 'treatments given' as the independent variable and the 'latency time' of rats on the hot plate as the dependent variable. Results of ANOVA showed a significant difference between the control group and vehicle group (CMC) with the test drug *Padmaka*. No significant difference observed between the pregabalin group and *Padmaka* group.

DISCUSSION

The present study has demonstrated the *Vedanasthapak* action of *Padmaka* (Prunus cerasoides D.Don). The observed effect on the experimental animals could be due to the *Rasapanchaka* of the *Padmaka*. *Padmaka* abates *Kapha* and *Pitta Dosha* as given by all *Acharyas* in their classical text but it is not mentioned there that it aggravates *Vata Dosha*; but according to *Kashay, Tikta Rasa; Katu Vipaka* and *Sheeta Veerya* of *Padmaka* is suggestive of elevation of *Vata Dosha*. That means it may be useful in gaining normal sensation at the sensationless area. Keeping this thing in mind this study has been conducted to confirm its activity as *Vedanasthapaka* in Wistar rats.

After the approval of the animal ethical committee; selected rats were divided into 4 groups to avoid any bias in the study and to obtain a true result. Before the preparation of the study model of rats, they were tested for their normal sensation using a hot plate test, and data was recorded. Earlier mentioned dose of cisplatin was introduced to all the groups equally for a month on weekly basis. In this way, a rat model for the experiment was prepared. Before introducing test drug and control drug; they all are tested on the hot plate. Because of cisplatin, all the animals have become very weak and lost their ability to move. Weight loss was also observed in all rats. Their contact time (latency period) on hot plate has increased than the previously calculated time before the intervention of cisplatin. This was a day-0 observation. Later on, pregabalin and *Padmaka* were introduced to the groups 3 and 4 respectively for a month with a pre-decided doses schedule. Vehicle CMC was used to administer their doses. On the 14th day, again all groups are tested on hot plate and their time in seconds is noted. Contact time was found to decrease as compared to day-0 observation. The Intervention was continued till the 30th day. The third observation was taken after the completion of doses. It is observed that contact times were again decreased. Rats were seen more motile than the previous 85 observations. During the experiment, after 14th day few rats were sacrificed because of unknown reasons. Detailed investigations are required to find out the cause.

All the obtained data was processed with ANOVA test along with Tukey's multiple comparison test to know the significance of the study.

During the experiment, remarkable difference was observed between the control and *Padmaka* group according to the 14th and 30th day observations. Latency period of *Padmaka* group goes on decreasing as compared to the control group. It suggests that *Padmaka* possesses positive activity.

A slight difference was observed between the *Padmaka* and the Pregabalin group on the evaluation of the 14th and 30th day. Activity of *Padmaka* was observed less than the positive control but it was nearby to it. Moreover in previous studies analgesic and antioxidant activities of *Padmaka* are already proven (Joseph N.et al.). Taking this thing in account we can conclude that *Padmaka* as a *Vedanasthapak Dravya* is exhibiting a significant action in reversing the numbness caused by neuropathy along with analgesic effect.

Etymology of the word shows that '*Vedanasthapan*' is the combination of two words, *Vedana* + *sthapan*. According to Monnier Williams dictionary, one of the meanings of '*Vedana'* is 'feeling' or 'sensation' and that of '*Sthapan*' is 'causing to stand', 'preserving' or 'maintaining'. *Amarkosha* has also defined the term *Vedana* as –'*Samvedo vedana*' which means anything that feels is a *Vedana*.

From this study, we can conclude that '*Vedanasthapan*' term is not only limited to the analgesic effect but also possesses a broad meaning of establishment of the normal sensation at the numbed area of the body parts. Comprehensive action will be identified, if a thorough study of *Vedanasthapan Mahakashay* given by *Acharya Charaka* will be done by Ayurveda preachers. It may prove as a boon for the other diseases causing numbness and hampering the quality of life of an individual.

Pregabalin and *Padmaka* were observed equally effective having a better performance of pregabalin in the available period. Both showed significant activity as compared to the control and vehicle group. As the *Padmaka* has also shown a positive activity from first to third observation, it can be said that if more time would have been given, a better activity could have been observed.

Rats in the control and the vehicle groups were seen very less motile and weak throughout the experiment.

Reasons of mortality seen in the positive control and test group after 14th and 30th day observation are unknown; as reviewed articles regarding both have positive results. To rule out the cause, further study is needed.

This pre-clinical study is carried out based on the various references and the inherent properties of *Padmaka* have experimentally confirmed our hypothesis. So further studies are required to gain more insight into the possible mechanism of action of *Padmaka*.

CONCLUSION

- Sample of *Padmaka* (*Prunus cerasoides* D.Don) - Successfully fulfilled all pharmacopeial standards consisting of moisture content, total ash, water-soluble extractive, etc.
- After analysing the data obtained from the experimental study, it is concluded that pregabalin and *Padmaka* both are useful in gaining the strength and sensation lost by neuropathy.
- Pregabalin is more effective than *Padmaka* as observed in the study.
- To get the best results of *Padmaka*, long-term study will be beneficial.
- This study may also be useful in the population having chemotherapy with cisplatin to avoid its side effects. It requires a further clinical trial for confirmation.
- Drugs in the *Vedanasthapana* group are also useful in regaining the sensation lost by chemotherapeutic agents. This study has only experimented on the cisplatin generated neuropathy but future scope is there to study other agents too.
- There is a scope for further studies regarding this topic. Clinical trials can be conducted on patients having loss of sensation because of neuropathy.

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