PIJAR
Paryeshana International Journal of Ayuredic Reserach
www.pijar.org
ISSN:2456:4354

AN EXPERIMENTAL STUDY ON ANALGESIC ACTIVITY OF BHANDIRA (Clerodendrum infortunatum L)

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DOI: https://doi.org/10.47071/pijar.2020.v05i05.003

ABSTRACT: From Vedic period to till now majority of the preparations used in Ayurveda is based on medicinal plants. But still a lot of medicinal plants are not explored for its medicinal properties. Traditional healers and folklore people have more knowledge of locally available plants and natural resources.The drug Bhandira (Clerodendruminfortunatum Linn.) is a softly tomentose perennial shrub. It is classified under the family Lamiaceae. Bhandiraislaghu, tikta, ushna, Kaphavatashamaka and has krimighna action. It is indicated in Kushta, Amavata, Jwara, andKrimi. The decoction of the root is traditionally used for joint pain by folklore practitioners around Alappuzha. Need of the hour is to explore the traditional uses of the plant, hence the study was under taken to evaluate drug Bhandira (Clerodendruminfortunatum L.) experimentally for its analgesic activity.In experimental study 18 Wistar albino rats of either sex with average weight of 150 – 200g were selected randomly for the study and arranged into 3 groups of six each, ie Control, Standard and Trial group. Combiflam syrup was given to Standard group and Bhandirakwatha was given to trial group. Pain threshold of each group was assessed at 0th minute (Basal reaction time), 15th, 30th, 60th,120th and 180th minute using Eddy's hot plate method. The statistical data of the results of three groups were analyzed and compared.The result were analyzed using Anova test and found highly statistically significant. The action of Trial drug Bhandira was slower to onset but the effect of the drug lasted longer than the Standard drug.

Key words: Analgesic,Bhandira (ClerodendruminfortunatumL.), Pain.

INTRODUCTION

The drug Bhandira (Clerodendrum infortunatum Linn.) is a softly tomentose[1] perennial shrub attaining a height of around 1m and possessing pinkish white or light purple coloured flowers[2] It was classified under the family Verbenaceae. But recently it is categorized into the family Lamiaceae[3].Bhandira was first mentioned by Acharya P V Sharma in his book DravyagunaVijnana. He mentions Bhandira has laghu, tikta, ushna, Kaphavatashamaka and has krimighna action. It is indicated in Kushta, Amavata, Jwara, and Krimi[4]. The decoction of the root is traditionally used for joint pain by folklore practitioners around Alappuzha.

Vernacular names[5]

English - Hill glory bower, Kannada- Thaggi-gida, Malayalam- Peruku, Peringilam, Perivelam, Hindi – Bhant, Ghato, Tamil – Perukilai, Sanskrit – Bhandira, Telugu – Gurrappukatilyaku, Bengali – Bhant, Nepali – Rajbeli, Manipuri – Kuthapmanp

Morphology of Clerodendruminfortunatum L[6] **-** A large shrub, tomentose. Stem and branches quadrangular. Leaves: opposite, up to 25x20cm, ovate or suboricular, shortly acuminate at apex, cordate at base, denticulate or serrate, pubescent above, pubescent or tomentose beneath; petiole up to 12 cm long. Flower: large terminal panicles. Calyx: deeply 5 lobbed, much enlarged in fruit; lobes ovate-acuminate. Corolla: white; tube 2.5cm long; lobes lanceolate, subequal. Drupes**:** bluish black when ripe, enclosed in accrescent calyx.

Distribution and Habitat[7] **-** Degraded forest areas, moist evergreen forests and plain lands. Found all over India, Srilanka, Myanmar, Andaman & Nicobar Islands, Thailand, Malaysia, and Bangladesh.

OBJECTIVES

Phytochemical study on Bhandira (Clerodendruminfortunatum L) root by

- Systemic extraction and chemical analysis of the extract
- Ash analysis

Experimental Study on Bhandira (Clerodendruminfortunatum L) root by

- Analgesic activity by Eddy's hot plate method.

MATERIALS AND METHODS

Collection of sample:The botanically identified and authenticated samples of ClerodendruminfortunatumLinn roots

were collected from Alappuzha district in Kerala.

Place of work:Phytochemical study was carried out in P G Department of DravyagunaVijnana, Alvas Ayurveda Medical College. Moodbidri. Experimental study was conducted at Animal house facility at Alvas Ayurveda Medical College, Moodbidri.

Preliminary phytochemical study[8]

Various test were conducted to know the presence of Proteins, Carbohydrates, Tannins, Saponins, Flavanoids, Steroids, Alkaloids, Triterpenoides, Starch, Resins, Phenolics, Elagic acid.

Ash Analysis[9]: The air dried powdered drug was taken in a crucible and heated in an electric Bunsen burner to make the ash. Then it was diluted with distilled water, boiled and filtered. The solution was tested to know the presence of Carbonates, Fluorides, Chlorides, Sulphates, Chromate, Phosphate, Potassium, Sodium, Aluminium, and Calcium.

Experimental study : Aims and objectives: For the scientific evaluation of the analgesic property of the drug Bhandira (C.infortunatumLinn.) an animal experimental study is needed. Hence the present study had been undertaken to study the action of Bhandira root in selected Wistar albino rats by Hot plate method using Analgesiometer, developed by Eddy and Leimbach (1953). The study was carried out at Animal house at Alvas Ayurveda Medical College, Moodbidri.

Materials and Methods:

Materials required:BhandiraKwatha, Standard drug – Combiflam syrup, Analgesiometer, Insulin syringe, Infant feeding tube, Weighing machine, Gloves.

Collection and preservation of drugs:Botanically identified Bhandira was collected and root was separated. Then the root was dried and made in to small pieces to increase the surface area for drying.

Preparation of trial drug:The kwatha was prepared in Bhaishajya lab in Alvas Ayurveda Medical College. The Bhandira dried root was made into Yavakutachurna. 1 part of drug was taken in a vessel and 16 part of water was added and heated over Mandagni and reduced to 1/8 quantity and filtered with clean cloth according to Sharangdhara reference.

Animal selection:18 healthy Wistar albino rats were selected and divided into three groups.

Inclusion criteria:

- Healthy albino rats of either sex
- Albino rats weighing between 150-200gm.

Exclusion criteria:

- Albino rats which are infected.
- Albino rats which are pregnant.
- Albino rats under other experiments.

Examination of the animal prior to the experiment: All the Wistar albino rats were subjected to general check-up for weight. Weight of each animal was checked by using spring balance. Heart rate was counted as number of beats/minute by feeling the heart rate by thumb. Each rat was identified by the colouring in body by picric acid. The cages were labelled with name of the group and drug.

Grouping:18 healthy Wistar albino rats were divided into 3 groups, ie; Control, Trial, and Standard group for the experimental study. Each group consists of 6 albino rats. (Table no:1**)**

TABLE 1 Grouping

SI NO	NO. OF ANIMALS	NAME OF THE GROUPS	ROUTE OF ADMINISTRATION	MEDICINE	DOSE
1	6	Control	Oral	Water	Normal
2	6	Standard	Oral	Combiflam syrup	.2ml
3	6	Trial	Oral	*Bhandirakwatha*	1.7ml

Dose fixing: The trial drug BhandiraKwatha and Combiflam syrup was administered orally. The dose was fixed using the table of Paget and Barnes, 1964. Rat dose = Human dose x 0.018x5/kg body weight.

For Bhandirakwatha: 96 x 0.018 = 1.7ml/200g body weight.

For Combiflam syrup: 15 x 0.018 = .27/200g body weight.

Mode of administration of drug: The fixed dose of the Bhandirakwatha and Combiflam syrup was taken in an insulin syringe and pushed directly in to the stomach of the rats after inserting the infant tube into the oesophagus carefully.

Analgesiometer: The temperature of the hot plate was maintained at 55^{o}C. The

temperature can be adjusted with the help of front panel control mark (set up and down). It is provide with acrylic box with lifting lid fitted over the hot plate for placing the rat on the hot plate.

Procedure: 18 healthy Wistar albino rats of either sex was selected randomly and grouped into three groups having 6 rats. Rats were kept in separate cages. They were numbered for their individual identification.

The Basal Reaction Time of each animal was noted using stop watch after placing the rats on the hot plate on which the temperature was maintained at 55^0C. The rats were removed from hot plate by taking of the lid immediately when the paw licking or jump response was observed. These observations was taken for each animal and the mean value was noted. This reading was taken as Basal Reaction Time.

The trial and standard drug were given orally and the reaction time were noted at 15, 30, 60, 120, 180 minutes.

RESULTS

Phytochemical study

1. Preliminary phytochemistry :The different phytochemical components present in C.infortunatum Linn Carbohydrates, Flavonoids, Steroids, Resins, Phenolics, Tannins and Triterpenoids. (Table no.2)

Table no. 2Phytochemical components present in various extracts:

Components	Observation	C.infortunatumLinn.
Proteins a)Biuret's test b)Millon's test	 No red colour No white precipitate	Absent
Carbohydrates a)Benedict's test b)Fehling's test c)Molisch'test	 A coloured precipitate A red precipitate A red violet ring	Present
Tannins	Green colour	Gallo tannins present
Saponins	No honey comb structure	Absent
Flavonoids a)Shinoda test	 Pink or reddish brown colour	Present
Steroids a) Leibermann-Burch b)Salkowski reaction	 Greenish colour Red colour is produced	Present
Alkaloids a)Dragendroff's test b)Mayer's test	 No red precipitate No white/yellow precipitate	Absent

Triterpenoides a) Leibermann-Burch	Violet coloured ring	Present
Starch	No colour change	Absent
Resins	Turbidity present	Present
Elagic acid	No red colour	Absent
Phenolics	Bluish black colour is formed	Present

2. Ash analysis: Ash analysis of C.infortunatum Linn showed the presence of Fluoride, Phosphate and Sodium. (Table no.3)

Table no 3 Ash Analysis

Results of Ash analy components	**C.infortunatum Linn**
Carbonate	Absent
Fluoride	Present
Chloride	Absent
Sulphate	Absent
Chromate	Absent
Phosphate	Present
Potassium	Absent
Sodium	Present
Aluminium	Absent
Calcium	Absent

Experimental Study

Basal reaction time observed in each groupand their mean values were taken (Table no 4) and the pain threshold of each animal in Trial group (Table no 5), Standard group (Table no 6) and Control group (Table no 7) was observed at 15min, 30min, 60min, 120min, 180min.

Test type -The study design has numerical data of reaction time of 18 albino rats which were equally divided into 3 groups, taken from normally distributed population.So the size of individual group was similar and groups were independent. Hence ANOVA test was conducted to compare the effect of Bhandira (ClerodendruminfortunatumLinn.) root for the analgesic activity.

Table no 4 Mean Basal Reaction Time

MEAN in TRIAL	MEAN in STND	MEAN in CNTRL
6.041	6.52	6.041
5.826	6.006	5.826
5.98	5.926	5.98
6.01	5.933	6.01
5.87	6.046	5.87
5.946	6.073	5.946

Table no: 5 Showing the Pain threshold observed at different intervals (Trial group)

SI NO	15MIN	30MIN	60MIN	120MIN	180MIN
1	6.13	6.16	8.04	8.01	7.98
2	5.71	5.78	7.93	7.9	6.75
3	6.18	6.25	8.13	8.1	8.01
4	6.21	5.91	7.81	7.18	6.98
5	6.01	6.01	8.17	7.75	6.99
6	5.86	5.78	7.98	7.35	7.01

Table no:6 Showing Pain threshold observed at various intervals (Standard group)

SI NO	15MIN	30MIN	60MIN	120MIN	180MIN
1	6.78	8.78	8.997	7.01	6.45
2	6.32	8.51	8.78	7.98	6.03
3	6.01	8.07	8.37	7.23	6.001
4	5.98	8.03	8.45	7.17	6.1
5	6.12	8.17	8.31	7.03	6.23
6	6.08	8.31	8.56	7.56	6.56

Table no: 7 Showing Pain threshold observed at various intervals (Control group)

SI NO	15MIN	30MIN	60MIN	120MIN	180MIN
1	6.1	6.07	6.01	5.98	5.91
2	5.87	5.78	5.78	5.75	5.7
3	5.91	5.61	5.98	5.75	5.65
4	6.03	6.07	6.01	5.83	6.98
5	5.98	6.01	5.87	5.37	5.78
6	5.91	5.98	5.98	5.86	5.97

Statistical analysis

The data were statistically analyzed with repeated measures of ANOVA test and multiple comparison procedures with Holm-Sidak testby SigmaStat software.In the present study 3 groups of Albino rats were used. The result was assessed statistically in different aspects for the better understanding such as comparison within group, between groups etc. Each group pain thresholdis assessed at 0 minute (Basal reaction time), 15 minutes, 30 minutes, 60 minutes, 120 minutes and 180 minutes.

a) Comparison of treatment effects between the groups at 15 minutes:On comparing the data of the treatment effect after 15 minutes, there is not a statistically significant difference in the effect of drug in showing analgesic action among the three groups at the interval of 15 minutes.(Table no 8)

Table no: 8 Repeated measure of Annova test at 15min

Source variation	**Df**	**SS**	**MS**	**F value**	**P value**
Between Groups	2	0.00272	0.00136	0.122	0.886
Residual	15	0.167	0.0111		
Total	17	0.169			

b) On comparing the treatment effect after 30 minutes, 60 minutes, 120 minutes, and 180 minutes,there is a statistically significant difference in the effect of drug in showing analgesic action among the three groups at all these intervals. (Table no 9,10,11,12)

Table no:9 Repeated measure of Annova test at 30min

Source variation	Df	SS	MS	F value	P value
Between Groups	2	18.077	9.039	617.747	<0.001
Residual	15	0.219	0.0146		
Total	17	18.297			

Table no:10 Repeated measure of Annova test at 60min

Source variation	Df	SS	MS	F value	P value
Between Groups	2	21.673	10.836	277.857	<0.001
Residual	15	0.585	0.0390		
Total	17	22.258			

Table no: 11 Repeated measure of Annova test at 120min

Source variation	Df	SS	MS	F value	P value
Between Groups	2	10.184	5.092	25.973	<0.001
Residual	21	4.117	0.196		
Total	23	14.302			

Table no: 12Repeated measure of Annova test at 180min

Source variation	Df	SS	MS	F value	P value
Between Groups	2	4.220	2.110	16.157	<0.001
Residual	15	1.959	0.131		
Total	17	6.179			

Multiple comparisons between the groups at 15 min:While comparing with the pain threshold time observed at time interval of 15 minutes shows that there is no significant difference in the treatment effect in the Standard group than Control group.On comparison between Control and Trial group that there is no significant difference in the treatment effect. On comparison

between Standard and Trial group there is no significant difference in the treatment effect. (Table no 13)

Table no: 13 Multiple comparisons between the groups after 15 min:

Comparison	Difference of means	"t" Value	"p" Value	Result
Control-Standard	0.166	1.938	0.081	Not Significant
Control-Trial	0.0500	0.567	0.583	Not Significant
Standard -Trial	0.198	1.347	0.208	Not Significant

Multiple comparisons between the groups at 30 min.:While comparing with the pain threshold time observed at time interval of 30 minutes in Control and standard there is significant difference in the treatment effect. On comparison between Control and Trial group there is no much significant difference in the treatment effect. On comparison between Standard and Trial group there is no significant difference in the treatment effect in the Standard group when compared to Trial group.(Table no 14)

Table no: 14 Multiple comparisons between the groups after 30 min:

Comparison	Difference of means	"t" Value	"p" Value	Result
Control-Standard	2.113	30.264		Significant
Control-Trial	0.0245	0.351	0.731	Not Significant
Standard -Trial	2.138	30.614		Significant

Multiple comparisons between the groups at 60 min:While comparing with the pain threshold time observed at time interval of 60 minutes in Control and standard groups there is highly significant difference in the treatment effect. On comparison between Control and Trial group there is highly

significant difference in the treatment effect. On comparison between Standard and Trial group there is significant difference in the treatment effect.(Table no 15)

Table no: 15Multiple comparisons between the groups after 60 min:

Comparison	Difference of means	"t" Value	"p" Value	Result
Control-Standard	2.547	22.342	<0.001	Highly Significant
Control-Trial	2.016	17.684	<0.001	Highly Significant
Standard -Trial	0.531	4.657	<0.05	Significant

Multiple comparisons between the groups at 120 min:While comparing with the pain threshold time observed at time interval of 120 minutes in Control and standard groups there is significant difference in the treatment effect. On comparison between Control and Trial group there is significant difference in the treatment effect. On comparison between Standard and Trial group there is no significant difference in the treatment effect. (Table no 16)

Table no: 16Multiple comparisons between the groups after 120 min:

Comparison	Difference of means	"t" Value	"p" Value	Result
Control-Standard	1.464	6.611	<0.05	Significant
Control-Trial	1.575	6.161	<0.05	Significant
Standard -Trial	0.111	0.503	0.620	Not Significant

Multiple comparisons between the groups at 180 min.:While comparing with the pain threshold time observed at time interval of 180 minutes in Control and standard groups there is no significant difference in the treatment

effect. On comparison between Control and Trial group there is significant difference in the treatment effect. On comparison between Standard and Trial group there is significant difference in the treatment effect.(Table no 17)

Table no: 17Multiple comparisons between the groups after 180 min:

Comparison	Difference of means	"t" Value	"p" Value	Result
Control-Standard	0.404	1.935	0.0721	Not Significant
Control-Trial	0.764	3.662	<0.05	Significant
Standard -Trial	1.168	5.96	<0.05	Significant

DISCUSSION

The drug Bhandira (Clerodendruminfortunatum Linn.) is a softly tomentose perennial shrub attaining a height of around 1m and possessing pinkish white or light purple coloured flowers. Pain is considered as a main pathological condition seen in so many diseases.The International Association for the study of Paindefines pain as "An unpleasant sensory and emotional experience arising from actual or potential tissue damage or described in terms of such damage".Pain is classified into Chronic pain and Acute pain.The decoction of Bhandira root is traditionally used for joint pain by the folklore practitioners around Alappuzha district, Kerala.

The preliminary phytochemical study revealed the presence of Carbohydrates, Flavonoids, Phenolics, Steroids, Resins, Tannins and Triterpenoids in root. The analgesic activity may be attributed to reported phyto-constituents present in the drug like Carbohydrates, Flavonoids, Phenolics, Steroids and Resins. Herb containing flavonoids performed many effects by blocking the cyclooxygenase enzyme. The chemical constitute iridoid and flavonoid in extracts of herb is responsible for analgesic activity. (Zafar A et al 2010). The Flavonoids, Phenolic compounds have been known to exhibit antipyretic, analgesic as well as antioxidant properties. (Narayanan et al 2001)

Experimental study:The aim of the present study was to evaluate the Analgesic property of the drugBhandira

(C.infortunatum L) given internally in the form of Kashaya

Overall effects of the treatment in individual groups were assessed statistically. It founds that the test is statistically highly significant with P value <0.0001 in all the groups. It shows that at every reading there was a significant difference were observed. Overall effects of the treatment between the groups were analysed with Anova test. It is observed that the results are not statistically significant at 15 min, ie it shows there is no statistically significant effect of treatment in increasing the pain threshold at 15 min in between groups. The test found statistically significant at 30 minute, 60 minute, 120 minute and 180 minutes.

Multiple comparisons of the treatment effects between groups at different intervals were analysed with Holm-Sidak test. It is found that the test is not statistically significant in all groups when assessed at 15 min. this indicated that both the standard and trial drug did not produce any significant action at 15 minutes. In multiple comparisons with Control to Standard group, showed significant difference at 30 minutes, highly significant at 60 minutes, significant at 120 minutes and not significant at 180 minutes. This data indicates that the effect of the standard drug is faster but the effect didn't last up to 180 minutes. In multiple comparison with Control to Trial group, it didn't show any statistical significance at 30 minutes, showed highly significant at 60 minutes, significant at 120 minutes and 180 minutes. The data can be interpreted as the action of the trial drug starts slower, but its effect lasted longer than the Standard drug. In multiple comparison with Trial to Standard, it showed significant statistical difference at 30, 60 and 180 minutes. But it didn't show any statistical difference at 120 minute. Data can be interpreted as at 30 and 60 minutes Standard drug shows sudden increase in values where trial drug showed gradual increase only. At 120 minutes the effect of the Standard drug started to reduce a bit and the value of Trial drug kept gradually increasing. The values of both trial and standard become almost same so there was no statistical difference. At 180 minutes the effect of the standard drug kept decreasing while the effect of trial drug kept gradually increasing. So there was

statistically significant difference at 180 minutes.

CONCLUSION

The drug Bhandira is well known for its folklore use among various folklore practitioners and also mentioned in some of contemporary Ayurvedic texts. The preliminary phytochemical study of the root extract of Bhandira (C.infortunatumL.) shows the presence of Carbohydrates, Flavonoids, Tannins, Triterpenoids, Steroids, Resins and Phenolics. The percentage of hot water extract was higher than all other extracts.Ash analysis of the drug Bhandira (C.infortunatum L.) showed the presence of Fluoride, Phosphate and Sodium.Experimental evaluation of Bhandira (C.infortunatum L.) on Wister albino rats has shown significant analgesic activity.The trial drug Bhandira (C.infortunatum L.) was found to be significantly effective and having sustained effect during the study.The outcome of the study suggests that Bhandira (C.infortunatum L.) is effective as an analgesic drug.

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Published BY:
Shri Prasanna Vitthala Education and Charitable Trust (Reg)

Source of Support: NIL
Conflict of Interest : None declared