



AN EXPERIMENTAL EVALUATION OF *Andrographis macrobotrys* Nees., A FOLK MEDICINAL PLANT FOR ANALGESIC ACTION IN WISTAR ALBINO RATS AND SWISS ALBINO MICE- IN VIVO STUDY

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ABSTRACT

Background and objective: In India Medicinal plants have a wide range of uses in folk medical practice. *Andrographis macrobotrys* Nees. belongs to Acanthaceae family is found in South India and Srilanka. It is in folk use by tribes in Kerala to treat muscle pain, snakebite, fever, and jaundice. The present research work aims to evaluate test drugs for their analgesic action. **Methods:** The detailed review of the test drug and disease Pain has been carried out. An animal experimental study has been carried out to assess the analgesic activity of the drug by acetic acid-induced writhing movement in Swiss albino mice and Eddy's hot plate method in Wistar albino rats. **Results and conclusion:** The test drug showed analgesic action with *Swarasa* of leaves and *Kashaya* of the whole plant. The drug was found to have both peripheral and central analgesic action.

Keywords: *Andrographis macrobotrys* Nees., Acanthaceae, folk medicinal plant, Analgesic action

INTRODUCTION

Since ancient times, human beings are utilizing plants for many purposes all around the world. From Vedic literature to recent texts of Ayurveda including Puranas, Samhitas and Nighantus, single-drug therapy is explained as a popular method of management of various diseases. Ayurveda is a research-oriented ancient medical science, Charaka explains the importance of research especially Drug research in *Vimana sthana*¹. The extinction of medicinal herbs is rapidly increasing due to various reasons, fortunately, folk herbs are densely found in India which are to be explored scientifically. Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage². Analgesics are drugs that relieve pain without causing loss of consciousness². Even if there is a wide experience in the use of non-opioid (NSAIDs) and opioid analgesics, they continue to capture attention with safety concerns and with potential risks. Ethno-medicinal studies can contribute the beginning step for the development of a new drug concept. *Andrographis macrobotrys* Nees. is one such ethnomedicinal plant in South India and Srilanka³. It is an erect, stout herb of the Acanthaceae family is used as folk medicine by tribes in Kerala for the treatment of snakebite, diarrhoea, muscle pain⁴, fever, jaundice, liver disorders and skin diseases⁵. The above drug is not explored as medicine and very little work has been carried out so far. Hence an experimental study is taken for its analgesic action through an in-vivo study.

Materials and Methods

Plant collection and authentication: The drug *Andrographis macrobotrys* Nees., the whole plant was collected from Bailur and nearby places in Udupi district, surrounding ALN Rao Ayurvedic medical college campus in Koppa and is authenticated by M. Radhakrishna Rao, Visiting Professor of Botany, ALN Rao memorial Ayurvedic College, Koppa. For the preparation of *Swarasa* plant was initially identified and cultivated in MIAMS Herbal Garden and was freshly collected.

Selection of animals for the experiment - Healthy 24 Swiss albino mice of either sex, weighing between 30-

50 gm and 24 Wistar albino rats of either sex, weighing between 200-250 gm were obtained from the Animal house SDM centre for research in Ayurveda and allied sciences, Udupi, to conduct the animal experiment.

Animal housing

The mice and rats were housed in polypropylene cages with food, water, environmental temperature, humidity and light and dark cycle. Mice and rats were with. They were acclimatized to the laboratory conditions for at least one week before using them for the experimental study. Mice and rats with pathological conditions, which are pregnant or subjected to other experiments are excluded.

Animal ethical committee clearance

The experimental study was carried out at the S.D.M center for research in Ayurveda and allied health sciences, Kuthpady, Udupi, and Karnataka. Approval no. SDMCRA/IAEC/M-DG-17 by 26/03/2018.

Acute toxicity study of test drug

An acute toxicity study was conducted to determine the LD₅₀ dose of *Andrographis macrobotrys* Nees., *Swarasa*. Drug was administered orally at different dose level in 5 different rats in 175mg/kg, 550mg/kg, 2000mg/kg and 2000mg/kg. All animals were observed at 1/2, 1, 2, 3, 4, 24 and 48 hrs dosing and thereafter daily once for mortality during the entire period of 15 days study. The maximum tolerated dose was calculated by employing OECD 425 guidelines with AOT software. The LD₅₀ value was found to be less than 2000mg/kg.

Analgesic study of test drug

Preparation of Drug used and its route of administration

Group I Water control

Group II Diclofenac sodium tablet 50 mg

Diclofenac sodium tablet weighing 50mg was finely powdered and made into a suspension by adding 10 ml distilled water and administered according to 0.1mg/100ml body weight.

Group III (Trial group I) - At SDM research centre for Ayurveda and allied sciences, Udupi- *Kashaya*(decoction) was prepared from coarse powder of the

whole plant of trial drug *Andrographis macrobotrys* Nees., adding water in the ratio 1:8 as mentioned in *Sharangadhara Samhita* for hard substances, boiled and reduced to 1/4th part of it⁶.

Group IV (Trial group II) – *Swarasa* (juice) was prepared with fresh leaves of trial drug *Andrographis macrobotrys* Nees., at SDM research centre for Ayurveda and allied sciences, Udupi

- Diclofenac and trial drugs are administered through the oral route with the help of a specific feeding syringe to mice and rats.

Chemical materials- 6% Acetic acid is used for intraperitoneal injection with the help of a 1ml syringe to induce pain.

Methodology

Analgesic action

Acetic acid-induced writhing movement test

Table 1: Grouping of animals for Acetic acid-induced writhing movement test

S. no	Grouping	Study
1.	Group I (control group)	Normal filter water
2.	Group II (Standard group)	Standard drug Diclofenac
3.	Group III (Trial group I)	<i>Kashaya</i> of <i>Andrographis macrobotrys</i> Nees., whole plant
4.	Group IV (Trial group II)	<i>Swarasa</i> of <i>Andrographis macrobotrys</i> Nees., leaves

Dose fixation Based on PAGET AND BARNES formula involving body surface area constant for **Swiss albino mice** and human dose (H.D) formula is $H.D \times 0.0026 \times 50$

Test drug: *Kashaya* of the whole plant of *Andrographis macrobotrys* Nees.,

Human dose = 96ml

Therefore, Mice dose = 12.48ml/kg body weight = 0.01248 ml/gm body weight

Test drug: *Swarasa* of leaves of *Andrographis macrobotrys* Nees.,

Human dose = 24ml

Therefore, Mice dose = 3.12ml/kg body weight = 0.00312 ml/gm body weight

Experimental Procedure:

Mice of either sex are selected, weighed, and grouped into 4 groups of 6 mice each. To Group I normal filter

water was administered to serve as control; Group II was taken as standard and administered with the standard drug Diclofenac in a single dose for 3 consecutive days; Group III was administered with *Kashaya* of *Andrographis macrobotrys* Nees., whole plant in a single dose for 8 consecutive days; Group IV was administered with *Swarasa* of *Andrographis macrobotrys* Nees., leaves in a single dose for 8 consecutive days. On the last day after administration of the drug to the respective groups after 1 hour, 0.1ml of a 0.6% solution of acetic acid is injected intraperitoneally to each mouse of drug and control groups. The mice are placed individually in the glass beakers and are observed for 30 minutes. The number of writhes is recorded for each animal. For the scoring purpose, writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

Eddy's hot plate method

Table 2: Grouping of animals for Eddy's hot plate method

Sl no	Grouping	Study
1.	Group I (control group)	Normal filter water
2.	Group II (Standard group)	Standard drug Diclofenac
3.	Group III (Trial group I)	<i>Kashaya</i> of <i>Andrographis macrobotrys</i> Nees., whole plant
4.	Group IV (Trial group II)	<i>Swarasa</i> of <i>Andrographis macrobotrys</i> Nees., leaves

Dose fixation Based on PAGET AND BARNES formula involving body surface area constant for **Wistar Albino rat** and human dose (H.D) formula is Human dose $\times 0.018 \times 5$

Test drug: Kashaya of the whole plant of *Andrographis macrobotrys* Nees.,

Human dose of *Kashaya* = 96 ml

Therefore, Rat dose = 8.64 ml/kg body weight = 0.00864 ml/gm body weight

Test drug: Swarasa of leaves of *Andrographis macrobotrys* Nees.,

Human dosage of *Swarasa* = 24 ml

Therefore, Rat dose = 2.16 ml/kg body weight = 0.00216 ml/gm body weight

Experimental procedure

Group of mice of either sex is taken weighed, and dosage is calculated. To Group I normal filter water was administered to serve as control; Group II was taken as standard and administered with the standard drug Diclofenac in a single dose for 3 consecutive days; Group

III was administered with *Kashaya* of *Andrographis macrobotrys* Nees., whole plant in a single dose for 8 consecutive days; Group IV was administered with *Swarasa* of *Andrographis macrobotrys* Nees., leaves in a single dose for 8 consecutive days. Eddy's hot plate which is commercially available consists of an electrically heated surface. The temperature is controlled at 55 to 56°C. The animals are kept on a hot plate and the time until either licking or jumping occurs is recorded by a stopwatch. Latency is recorded after 10, 20, 30, 40 and 60 min following oral administration of the respective drug to each group of rats

STATISTICAL ANALYSIS

The values noted were expressed as MEAN \pm SEM (Standard Error of Mean). The data were analysed by one-way ANOVA followed by Dunnett's multiple comparison t-test as a post hoc test. Graph pad in stat software is used for this purpose. If $p \leq 0.05$ it is considered statistically significant. Accordingly, level of significance was noted and interpreted.

RESULTS

Acetic acid-induced writhing movement

Table 3: Effect of *Andrographis macrobotrys* Nees., on latency period of writhing movement

Group	The latency period (in min)	% change
Control	0.61 \pm 0.18	
Standard	5.44 \pm 2.83	791 \uparrow
<i>Kashaya</i>	3.04 \pm 1.52	398 \uparrow
<i>Swarasa</i>	3.71 \pm 1.95	508 \uparrow

Data: MEAN \pm SEM

The data shows there was an increase in latency period of writhing movement in standard, *Kashaya* and *Swarasa* groups when compared to the control group, the

observed increase was found to be statistically non-significant.

Table 4: Effect of *Andrographis macrobotrys* Nees., on the total number of writhing

Group	Total number of writhing (in min)	% change
Control	14.6 \pm 2.61	
Standard	4.16 \pm 1.01**	71.5 \downarrow
<i>Kashaya</i>	8.7 \pm 2.50	40.4 \downarrow
<i>Swarasa</i>	4 \pm 1.52**	72.60 \downarrow

Data: MEAN \pm SEM **P<0.01

The data shows there was a decrease in the total number of writhing movements in the standard and *Swarasa* groups when compared to the control group, the observed decrease was found to be statistically very

significant. There was a decrease in the total number of writhing movements in *Kashaya* group when compared to the control group, the observed decrease was found to be statistically non-significant.

Table 5: Effect of *Andrographis macrobotrys* Nees., on writhing at peak (10-15 min)

Group	Writhing at Peak (in min)	% Change
Control	3.33 ± 0.42	
Standard	0.66 ± 0.33**	80.18↓
<i>Kashaya</i>	1.71 ± 0.83	48.64↓
<i>Swarasa</i>	0.16 ± 0.16**	95.19↓

Data: MEAN ± SEM **P<0.01

The data shows there was a decrease of writhing movement in standard and *Swarasa* groups when compared to the control group, the observed decrease was found to be statistically very significant. There was a

decrease in writhing movement in *Kashaya* group when compared to the control group, the observed decrease was found to be statistically non-significant

Eddy's hot plate method

Table 6: Effect of *Andrographis macrobotrys* Nees., on the initial response on a hot plate

Group	Initial response (in a sec)	% Change
Control	5.49 ± 0.43	
Standard	13.72 ± 2.14**	149.9 ↑
<i>Kashaya</i>	9.51 ± 1.11	73.2↑
<i>Swarasa</i>	7.5 ± 1.41	36.6↑

Data: MEAN ± SEM **P<0.01

The data shows there was an increase in time taken for an initial response on a hot plate in the standard group when compared to the control group, the observed increase was found to be statistically very significant.

There was an increase in time taken for an initial response on a hot plate in *Kashaya* and *Swarasa* groups when compared to the control group, the observed increase was found to be statistically non-significant

Table 7: Effect of *Andrographis macrobotrys* Nees., on the response at 10 min on a hot plate

Group	Response at 10min (in a sec)	% Change
Control	6.74 ± 0.79	
Standard	21.02 ± 2.89**	211.86↑
<i>Kashaya</i>	11.47± 3.46	70.17↑
<i>Swarasa</i>	12.12 ± 1.93	79.82↑

Data: MEAN ± SEM **P<0.01

The data shows there was an increase in time taken for a response on a hot plate at 10 min in the standard group when compared to the control group, the observed increase was found to be statistically very sig-

nificant. There was an increase in time taken for a response on a hot plate at 10min in *Kashaya* and *Swarasa* groups when compared to the control group, the observed increase was found to be statistically non-significant

Table 8: Effect of *Andrographis macrobotrys* Nees., on the response at 20min on a hot plate

Group	Response at 20min (in a sec)	% change
Control	9.32 ± 1.74	
Standard	12.25 ± 2.19	31.43↑
<i>Kashaya</i>	19.25 ± 2.11**	108.55↑
<i>Swarasa</i>	16.05 ± 1.91*	72.21↑

Data: MEAN ± SEM **P<0.01 *P<0.05

The data shows there was an increase in time taken for a response on a hot plate at 20 min in the standard group when compared to the control group, which was found to be statistically non-significant. There was an increase in time taken for a response on a hot plate at 20min in the *Kashaya* group when compared to the

control group, which was found to be statistically very significant. There was an increase in time taken for a response on a hot plate at 20min in *Swarasa* group when compared to the control group, which was found to be statistically significant

Table 9: Effect of *Andrographis macrobotrys* Nees., on the response at 30min on a hot plate

Group	Response at 30min (in a sec)	% change
Control	8.82 ± 0.87	
Standard	24.01 ± 7033	172.22 ↑
<i>Kashaya</i>	28.31 ± 5.71	220.97↑
<i>Swarasa</i>	16.83 ± 5.60	90.81↑

Data: MEAN ± SEM

The data shows there was an increase in time taken for a response on a hot plate at 30 min in standard, *Kashaya* and *Swarasa* groups when compared to the

control group, the observed increase was found to be statistically non-significant.

Table 10: Effect of *Andrographis macrobotrys* Nees., on the response at 40min on a hot plate

Group	Response at 40min (in a sec)	% change
Control	7.45 ± 1.05	
Standard	24.02 ± 7.11	222.41↑
<i>Kashaya</i>	34.76 ± 7.11**	366.57↑
<i>Swarasa</i>	11.89 ± 0.86	59.59↑

Data: MEAN ± SEM **P<0.01

The data shows there was an increase in time taken for a response on a hot plate at 40 min in standard and *Swarasa* groups when compared to the control group, the observed increase was found to be statistically non-significant. There was an increase in time taken for a response on a hot plate at 40min in *Kashaya* group when compared to the control group, the observed increase was found to be statistically very significant.

DISCUSSION

An experimental study was conducted at S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi. The animal experiment was conducted after getting prior permission from the Institutional Animal Ethical Committee.

Analgesic activity: Analgesic activity of test drug *Andrographis macrobotrys* Nees., is assessed by employing two methods of animal experimentation in this

study. Acetic acid-induced writhing movement in Swiss Albino mice is employed to assess analgesic effect produced by peripheral mechanism and Hot plate

method in Wistar albino rat is employed to assess analgesic effect through a central mechanism.

Acetic acid-induced writhing movement

Table 11: Concluding result on the effect of *Andrographis macrobotrys* Nees., *Kashaya* and *Swarasa* on parameters of Acetic Acid induced writhing movement:

	Standard	<i>Kashaya</i>	<i>Swarasa</i>
Latency	NSI	NSI	NSI
Total number of writhing	SD	NSD	SD
Peak (10-15 min)	SD	NSD	SD

NSI- Non-significant increase, SD- Significant decrease, NSD- Non-significant decrease

The acetic acid-induced abdominal writhing has been used as a screening tool for assessing analgesic or anti-inflammatory agents. This model is used as an antinociceptive model for opioids and is commonly employed as a visceral inflammatory model. Pain is induced by the injection of irritants into the peritoneal cavity of mice. Mice are observed for the reaction of animals which reacts with a characteristic stretching behaviour called writhing (stretch, tension to one side, extension of hind legs, contraction of abdomen or twisting of trunk). Analgesic activity of the test drug is inferred from a decrease in the frequency of writhing. Acetic acid-induced constriction is considered to be a nonselective nociceptive model, as it acts indirectly by inducing the release of endogenous mediators which stimulate the nociceptive neurons that are sensitive to nonsteroidal anti-inflammatory drugs, narcotics and other centrally active drugs. In this study, analysing the results shows, increase in latency period in the

Eddy's hot plate method

Table 12: Consolidated statement on the percentage change of pain threshold at different time intervals on the administration of *Andrographis macrobotrys* Nees, *Kashaya* and *Swarasa*

Time interval	Standard	<i>Kashaya</i>	<i>Swarasa</i>
10 min	SI	NSI	NSI
20 min	NSI	SI	SI
30 min	NSI	NSI	NSI
40 min	NSI	SI	NSI
60 min	NSI	NSI	NSI

SI- Significant increase NSI- Non-significant increase, SD- Significant decrease NSD- Non-significant decrease

standard, *Kashaya* and *Swarasa* groups when compared to the statistically non-significant control group. A decrease in the total number of writhing and the number of writhing at the peak is observed which is significant in the Standard and *swarasa* group and non-significant in *Kashaya* group. To understand and analyse obtained results of animal study, there is a need for understanding mechanism of peripheral pain mechanism and ways to produce analgesia. Analgesic action in the periphery can be probably achieved by inhibition of, enzyme cyclooxygenase and lipoxygenase, actions of prostaglandin, production of algogenic substances, at the central level of transmission of painful impulses. The acetic acid writhing movement test shows that *Andrographis macrobotrys* Nees., have analgesic action by both *Swarasa* of leaves and *Kashaya* of the whole plant. Further analysis of results shows better action in *Swarasa* group and moderate action in *Kashaya* group.

The hot plate method is employed to assess the analgesic which acts through central mechanisms by observing paw licking and jump response to assess the effect of the test drug on neurogenic pain. In the present study, the hot plate method was employed hence the analgesic activity evaluation for the central analgesic type of activity.

Careful analysis of the results indicates that, When the Standard drug administered group is compared with the control, significant elevation in pain threshold was observed at 10min but non-significant increase in pain threshold at 20, 30, 40, 60min. This shows that the standard drug has analgesic action for a lesser duration. At 20min, the pain threshold was found to be elevated significantly in both *Kashaya* and *Swarasa* groups when compared with the control group. At 40min, the Pain threshold was found to be significant elevation only in *Kashaya* group when compared to the control. Elevation of pain threshold is observed in standard and both the test drug groups when compared to control group. This indicates the presence of Central analgesic activity in both *Kashaya* of whole plant and *Swarasa* of leaves of test drug *Andrographis macrobotrys* Nees, At 10, 30 and 60 min increase in pain threshold in *Kashaya* and *Swarasa* groups was statistically non-significant which can be considered as moderate action of the drug. The probable mechanism of action of analgesic effect observed and chemical reactions responsible for the analgesia is needed to be discussed to interpret the mode of action of the test drug. Pain is interpreted and perceived in the brain. In central analgesic action, any drug which produces modulation inactivity of the opioid receptors or increase in formation and release of endogenous opiates will produce an analgesic effect. Transmission of pain impulses from site to CNS can be controlled at the Spinal level or by presynaptic inhibition of dorsal nerve ending through specific GABA_A receptors. Intracranial electrical stimulation of certain areas inhibits pain

while touch, pressure and temperature sensation remain intact. In the above procedure, Eddy's hot plate method is selective for the study on Central analgesic activity and peripheral analgesic mechanisms are not involved. Based on this, it can be inferred that test drug *Andrographis macrobotrys* Nees., have a central analgesic effect. Further analysis of results obtained from the above study gives clarity that *Kashaya* group is giving the analgesic action for a longer duration than *Swarasa* group. To understand the action of drug *Andrographis macrobotrys* Nees., in chronic pain on the central nervous system and to recognize mechanisms for early and delayed response further studies can be undertaken.

Probable mode of action

According to the observation and analysis of the present study, it is confirmed that test drug *Andrographis macrobotrys* Nees., have analgesic action. The analgesic action of *Andrographis macrobotrys* Nees. maybe due to the presence of Steroids, tannin, triterpenoid, flavonoid and Phenols. A recent study shows the presence of diterpenoid Neoandrographolide in leaves of *Andrographis macrobotrys* Nees., which may be responsible for its analgesic action. *Andrographis macrobotrys* Nees. has got *Tiktha rasa* (bitter taste), *Kashaya Anurasa* (astringent secondary taste), *Sheetha Virya* (cold potency) and *Katu Vipaka* (pungent taste after digestion) which might be responsible factors for its analgesic activity. Other than this even *Prabhava* (potency) of *Andrographis macrobotrys* Nees., can be the responsible factor for its analgesic action. *Swarasa* of *Andrographis macrobotrys* Nees., showing the best analgesic action in acetic acid-induced writhing movement and *Kashaya* showed the best action in the hot plate method. To elicit proper mechanism researchers can take up molecular level study.



Fig 1 & 2 – Plant *Andrographis macrobotrys* Nees.,

Fig no. 3 Coarse powder of *Andrographis macrobotrys* Nees.



Fig 4, 5 & 6 Writhing and hind limb extension shown by Swiss albino mice in acetic acid-induced writhing movement study.

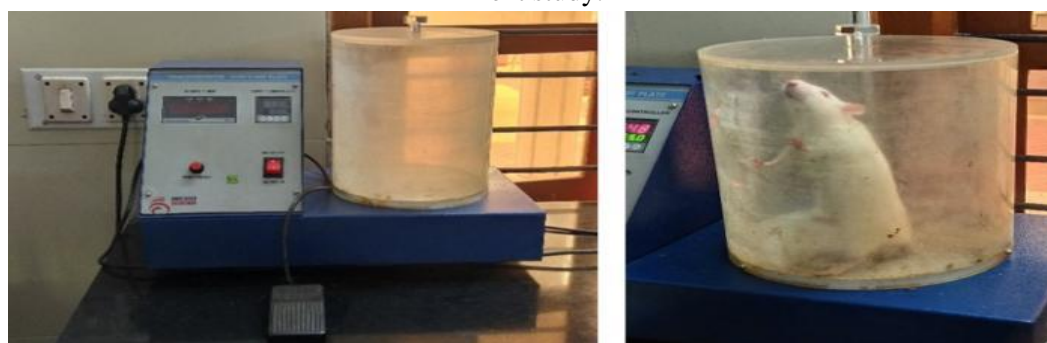


Fig 7 & 8 show Instrument Eddy's hot plate and Wistar albino rat over hot plate showing jumping and paw licking

CONCLUSION

Andrographis macrobotrys Nees. is used by tribes in the treatment of muscle pain, fever, Jaundice etc. To determine the Analgesic activity of the drug, an experimental study was carried out in 2 different models. Acetic acid-induced writhing movement is selective for the peripheral analgesic mechanisms and Eddy's

hot plate method for its action on the central nervous system. Acetic acid-induced writhing movement on Swiss Albino mice and Eddy's hot plate method on Wistar Albino rat reveals that *Andrographis macrobotrys* Nees., have analgesic action by both *Swarasa* of leaves and *Kashaya* of the whole plant. To be specific, *Swarasa* shown the best analgesic activity in

Acetic acid-induced writhing movement and *Kashaya* shown the best analgesic activity in Eddy's hot plate method. To elicit proper mechanism researchers can take up molecular level study.

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