



A STUDY ON ANALGESIC EFFECT OF *Syzygium aromaticum* IN ALBINO RATS USING INTRAPERITONEAL INJECTION

SENTHIL KUMAR ^a AND SOMASUNDARAM ^{a*}

^a Department of Pharmacology, Sri Lakshmi Narayana Institute of Medical Sciences Affiliated to Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

Editor(s):

- (1) Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
(2) Dr. Sirigireddy Sivajothi, Sri Venkateswara Veterinary University, India.

Reviewers:

- (1) Khalid Chillab Kridie Al-Salhi, University of Basrah, Iraq.
(2) Mohammadreza Mohammadabadi, University of Kerman, Iran.

Received: 09 October 2021

Accepted: 18 December 2021

Published: 20 December 2021

Original Research Article

ABSTRACT

The current study's goal was to assess the effect of clove bud powder on analgesia, which had never been done before. In the rat formalin test, the normal saline through control group received normal saline (3 ml/kg). The second, and followed by third and fourth groups traditional ketorolac (15 mg/kg intra-peritoneal injection), *syzygium aromaticum* powder 100 mg/kg and 200 mg/kg, respectively. The results found for the control showed a biphasic pain response with an instant and short burst of activity lasting around the first 5 min, followed by a prolonged phases of activity starting at minute 11, peaking between 10 to 30 min. Tiny nociceptive performance was observed during a 5-min erratic between the 6-10 minutes. Our results revealed that the nociception seen in phase 1 is a result of direct nerve stimulation by the formalin. Further, it has been demonstrated that the nociception produced in phase 2 of the formalin test is a result of chemical insult resulting in tissue damage.

Keywords: Analgesic, *Syzygium Aromaticum*, Albino Rats, Intraperitoneal Injection

1. INTRODUCTION

Pain is an unpleasant sensation, and a very common phenomenon. There is no doubt that pain acts as a warning signal against disturbances either in the body or in the external environment of an individual. The principal objective of the treatment of pain is to remove or abolish the cause of pain. But it is not always possible to do so; hence, analgesics are used

for the symptomatic treatment of pain. Opioids are the most potent and commonly used group of analgesic drugs e.g. Morphine and Pethidine. But their analgesic action is associated with a greater degree of adverse drug reactions, most of which are dose dependent.

Increased pain in response to noxious stimulation following peripheral tissue injury depends on an

*Corresponding author: Email: s_omasundaramganesan8@gmail.com;

increase in the sensitivity of primary afferent nociceptors at the site of injury (peripheral sensitization) [1] and on an increase in the excitability of neurons in the CNS (central sensitization) [2]. Central sensitization is triggered by inputs from nociceptive afferents and is associated with a reduced threshold of dorsal horn neurons to noxious stimulation [3], an expansion of the receptive fields of dorsal horn neurons [4] a summation of slow postsynaptic potentials resulting in a cumulative depolarization and a prolonged after discharge or "windup" of dorsal horn neurons [5], and an increased excitability of the flexion reflex in response to peripheral stimulation [6].

The use of plant products is increasing in many segments of the population. According to an estimate, 80% of the world's population relies upon plants for their medication. Most of the synthetic drugs used at present for analgesic and anti-nociceptive effect have many side and toxic effects [7]. Plants still represent a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [8]. Many medicines of plant origin with analgesic and anti-nociceptive activity had been used since long time without any adverse effect. North East India is considered as one of the "hotspots" for biodiversity in India, since out of the 1500 species of medicinal plants available in India, almost 350 species belong to Assam and many of these traditionally used plants have not yet been studied scientifically which can be developed as a potential drug after scientific validation. Cloves have been cultivated for at least 2,000 years. They are an important part of the spice trade and are highly valued for their medicinal properties [9].

The clove (*Syzygium aromaticum*, sometimes included in the genus *Eugenia*) comes from the Myrtaceae family, which is a pink flower bud of the clove tree that turns brown when dried. Cloves have a warm, sweet, and aromatic flavour and an oily compound that is vital to their medicinal and nutritional properties. The Clove is indigenous to the Moluccas volcanic islands of Indonesia previously known as the Spice Islands [10]. The aim of the current study was to assess the effect of clove bud powder on analgesia.

2. MATERIALS AND METHODS

2.1 Collection of Material Plant Material

Clove buds (*Syzygium aromaticum*) was Purchased from the local market in Chennai and identified by

The Director, National institute of herbal science, West Tambaram, Chennai.

2.2 Experimental Animals

Albino rats of Wistar strain of either sex weighing 200gm were used for the study. Animals were procured from King Institute of Preventive Medicine, Guindy, Chennai and maintained in the Central Animal House, Bharath Institute of Higher Education and Research, Chennai, India. The animals were individually housed under controlled infection and hygienic circumstances. They all received a standard pellet diet and water ad libitum.

2.3 Preparation of *Syzygium aromaticum* Powder

Clove buds were obtained from the local market was shade dried and powdered using mechanical mixer. The clove bud powder was mixed with normal saline and administered orally to albino rats. All the other chemicals like formalin, Pethidine, Ketorolac were of analytical grade and were procured from local commercial companies.

2.4 Selection of Doses

For the assessment of analgesic activity, two dose levels were chosen i.e, 100mg/kg and 200mg/kg respectively [11].

2.5 Screening Methods for Analgesic Activity Formalin Test

Thirty mm after supervision of drugs the right posterior paw is inserted with 0.05 ml of 5% formalin intravenously using a 27 gauge ½-inch needle. The animals are continuously observed from all angles from transparent cage, for 30 min. The time spent licking and biting is monitored continuously and recorded as seconds per 5 minute from minute 1 to minute 30. The performances of the animals are verified by stop watch physically and data entered into a computer [12]. It is regularly been initiate that two disparate phases of licking and freezing occur, phase 2 a prolonged response starting at crudely minute 10 and ending at about minute 30. Among segments 1 and 2, there is a recurrent dated from minute 6 to minute 10 phase 1 a little but immediate response eternal the first 5 min after the hind stroke is inoculated; where little nociceptive behavior is pragmatic. Phase 1 is a done inspiration of the nerve by the formalin and phase 2 is a stimulating reaction-induced pain. It is supposed that the two contrasting points represent two qualitatively different types of pain.

2.6 Study Design

Number of groups: 8.

Number of animals in each group: 6 Total number of animals: 48.

Experimental Design: Formalin Method.

Group 1: Control (Normal saline 0.3 ml, i.p)

Group 2: Ketorolac (15 mg/kg, i.p)

Group 3: *Syzygium aromaticum* (100 mg/kg, oral)

Group 4: *Syzygium aromaticum*

(200mg/kg, oral) Compare Control group, *Syzygium aromaticum* 100 mg/kg and 200mg/kg group and Ketorolac group to see for the peripheral action of *Syzygium aromaticum*. The control group, consisting

of six animals (N= 6). The experimental group of *Syzygium aromaticum* is divided into two subgroups: 100 and 200 mg/kg. Each subgroup of *syzygium aromaticum* consisted of six animals (N = 6) and ketorolac group consisted of 6 animals (N=6). In which *Syzygium aromaticum* is administered orally and ketorolac administered via intra-peritoneal route, 30 minute before administration of formalin [13].

3. RESULTS AND DISCUSSION

This study was carried out with an attempt to evaluate the analgesic activity of *syzygium aromaticum* in Rats. Data collected from the study are tabulated in Table 1. The reaction time of formalin test in different phases of time interval is presented. The VI rat shows high reaction time in 0 to 5 mins.

Table 1. Results of formalin test with different concentrations

Rat	I	II	III	IV	V	VI
Formalin test with normal saline - Reaction time (sec)						
Phase I						
0-5 min	53	55	66	60	89	93
Phase II						
10-15 min	37	33	30	16	25	22
15-20 min	35	35	38	58	63	48
20-25 min	25	36	39	45	59	47
25-30 min	38	38	26	45	48	50
Formalin test with ketorolac 15mg/kg						
Reaction time(sec)						
Phase I						
0-5 min	55	62	64	55	68	62
Phase II						
10-15 min	0	0	6	0	8	0
15-20 min	14	15	14	15	17	19
20-25 min	12	22	16	18	15	22
25-30 min	19	15	22	17	19	15
Rat	1	2	3	4	5	6
Formalin test with syzygium aromaticum 100mg/kg						
Reaction time(sec)						
Phase I						
0-5 min	41	38	33	34	29	42
Phase II						
10-15 min	0	5	0	0	12	0
15-20 min	12	17	11	14	16	13
20-25 min	17	16	14	19	11	12
25-30 min	11	14	18	14	20	15
Formalin test with syzygium aromaticum 200mg/kg						
Reaction time (sec)						
Phase I						
0-5 min	32	29	38	33	31	28
Phase II						
10-15 min	0	3	10	2	6	0
15-20 min	11	20	14	14	19	16
20-25 min	12	15	13	21	10	14
25-30 min	17	19	15	13	12	14

Table 2. Results of Student's t-test for comparison between control, ketorolac & *Syzygium aromaticum* (100 mg/kg and 200 mg/kg)

Groups	Time in Minutes									
	0 TO 5 MIN		10 TO 15 MIN		15 TO 20 MIN		20 TO 25 MIN		25 TO 30 MIN	
	t	p	t	p	t	p	t	p	t	p
Control vs ketorolac	1.124	0.287	7.147	24.833	6.051	0.000	4.912	0.001	6.106	0.000
Control vs syz.aromaticum 100mg/kg	4.48	0.001	6.537	0.000	6.384	0.000	5.575	0.000	6.667	0.000
Control vs syz.aromaticum 200mg/kg	5.167	0.000	6.736	0.000	5.906	0.000	5.618	0.000	6.881	0.000
ketorolac vs syz.aromaticum 100 mg/kg	8.454	0.000	-0.200	0.846	1.478	0.170	1.299	0.233	1.458	0.175
ketorolac vs syz.aromaticum 200mg/kg	11.445	0.000	-0.535	0.605	0.000	1.000	1.489	0.167	1.844	0.095

* Significantly ($p < 0.01$)

Comparing the control group and ketorolac group there was no significant change in phase I whereas there was a significant variance ($p < 0.01$) in all time intervals in phase II except between 10 - 15 minutes (Tables 1 - 2). Comparing the control group and *syzygium aromaticum* 100 mg/kg and *syzygium aromaticum* 200 mg/kg, there was a significantly ($p < 0.01$) in all time intervals in both phase I and phase II. Comparing ketorolac group and *syzygium aromaticum* 100 mg/kg and *syzygium aromaticum* 200mg/kg, there was a significantly ($p < 0.01$) in phase I and, there was no significantly ($p < 0.01$) in phase II except for time interval 10 - 15 min when compared with *syzygium aromaticum* 200mg/kg. The present study was conducted to study the analgesic effect of *syzygium aromaticum* on albino rats. To see the effectiveness of *syzygium* it is compared with a standard drug ketorolac, and pethidine. *Syzygium aromaticum* is found abundantly in India and also in tropical and sub-tropical countries throughout the world. Every part of the plant has some medicinal properties that has been used traditionally since ancient times.

4. CONCLUSION

The current study was the analgesic effect of *syzygium aromaticum* powder on albino rats". The clove bud powder was studied to evaluate its effect on analgesia, as it is not been evaluated till now. From this study it was found that *syzygium aromaticum* powder significantly induces analgesia in albino rat models. After receiving literature available in support of analgesic action of *syzygium aromaticum*, it was found that *syzygium aromaticum* has beneficial effect in inducing analgesia. From this

study, it was found that in nociception state the clove bud shows significant decrease in.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

ACKNOWLEDGEMENTS

The encouragement and support from Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India is gratefully acknowledged for providing the laboratory facilities to carry out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhu F. A review on the application of herbal medicines in the disease control of aquatic animals. *Aquaculture*. 2020;526:735422.
2. Arnaudo E. Psychogenic pain as imaginary pain. *Rivista internazionale di Filosofia e Psicologia*. 2021;12:190-199.
3. Coderre TJ, Melzack R. Increased pain sensitivity following heat injury involves a central mechanism. *Behavior Brain Research*. 1985;15:259- 262.
4. Cook AJ, Woolf CJ, Wall PD, McMahon SB. Dynamic receptive field plasticity in rat spinal dorsal horn following C primary afferent

- input. *Nature*. 1987;325:51-53.
5. Cordell WH, Keene KK, Giles BK, Jones JB, Jones JH, Brizendine EJ. The high prevalence of pain in emergency medical care. *American Journal of Emergency Medicine*. 2002;20:165-169.
6. Craig AD. Interoception: The sense of the physiological condition of the body. *Current Opinion in Neurobiology*. 2003;13:500-505.
7. Chunchuwar MD, Rathi B, Wanjari A, Rathi R. Pharmaceutical Standardization and Drug Dosage Modification of Laghu Sudarshan Churna with Comparative Assessment of its Antipyretic and Analgesic Activities in Albino Rats. *JPRI*. 2021;33(45B):130-7.
8. Craig AD, Krout K, Andrew D. Quantitative response characteristics of thermoreceptive and nociceptive lamina I spinothalamic neurons in the cat. *Journal of Neurophysiology*. 2001;86:1459-1480.
9. Craig AD. Pain mechanisms: labeled lines versus convergence in central processing. *Annual Reviews in Neuroscience*. 2003;26:130.
10. Shirzaei M, Neshat F. Effect of Areca Nut on Oral Health: A Review. *Journal of Research in Dental and Maxillofacial Sciences*. 2020;5:1-6.
11. De M, De AK, Banerjee AB. Antimicrobial screening of some Indian Spices. *Phytotherapy Research*. 1999;13:616-618.
12. Deans SG, Ritchie G. Antibacterial properties of plant essential oils. *International Journal of Food Microbiology*. 1987;5:165-180.
13. Colasanti A, Critchley HD. Primordial Emotions, Neural Substrates, and Sentience: Affective Neuroscience Relevant to Psychiatric Practice. *Journal of Consciousness Studies*. 2021;28:54-73.