

Effectiveness of low dose physostigmine for dose reduction of morphine in pain management**Rahul P. Bhavasar¹, Suyog S. Chopade^{1*}, Yogita D. Sulaxane², Devendra R. Chaudhari¹, Bapurao M. Bite¹**¹Department of Pharmacology,²Department of Physiology, Dr. Ulhas Patil Medical College and Hospital, Jalgaon, Maharashtra, India**Received:** 19 March 2019**Accepted:** 23 April 2019***Correspondence to:**

Dr. Suyog S. Chopade

Email:

dr.suyogchopade@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.**ABSTRACT****Background:** This is an interventional study, undertaken in the experimental animal models for the evaluation of the antinociceptive potential of Physostigmine and its combination with Morphine at their sub-analgesic doses. The objective of the study was to evaluate the antinociceptive potential of Physostigmine alone and in combination with morphine.**Methods:** Antinociceptive effect of Physostigmine in three graded doses (50, 100 and 200 µg/kg) and combination of Physostigmine at low dose (50 µg/kg) with sub-analgesic dose of Morphine (0.1 mg/kg) and Morphine in analgesic dose (1 mg/kg) was evaluated by using Hot Water Bath method in albino rats.**Results:** Comparison of maximal possible effect in percentage (MPE in %) between groups at 90 minutes in control, Morphine, Physostigmine in 50, 100, 200 µg/kg doses and combination group respectively, demonstrated significant difference ($p < 0.001$) when compared by one way ANOVA test. There was no much increase in maximal possible effect in the tail withdrawal latency in Physostigmine 50 µg/kg (SC) treatment at 90 min (5.50 ± 0.88) in comparison to control (NS) treatment group. Combination treatment of low doses of both Physostigmine 50 µg/kg + Morphine 0.1 mg/kg increased in maximal possible effect the tail withdrawal latency 90 min (53.87 ± 1.38) in-comparison to control (NS) treatment group (6.17 ± 0.92).**Conclusions:** Physostigmine is more potent antinociceptive than Morphine and Physostigmine potentiated the antinociceptive activity of low dose of standard drug Morphine.**Keywords:** Antinociceptive, Morphine, Pain, Physostigmine, Tail withdrawal**INTRODUCTION**

Nociception is the mechanism by which noxious peripheral stimuli are transmitted to the central nervous system to elicit a mechanical response.¹ As long as humans have experienced pain, they have given explanations for its existence and sought soothing agents to dull or cease the painful sensation. Archaeologists have uncovered clay tablets dating back as far as 5,000 BC which reference the cultivation and use of the opium poppy to bring joy and

cease pain. A horde of opioid compounds which produce analgesia have been synthesized so far but none have been proven to be clinically superior to morphine in relieving pain. But morphine also has many side effects like physical dependence, tolerance, euphoria, sedation, respiratory depression, GI disturbance, constipation, bronchoconstriction etc. at analgesic doses. Similar to exogenous opiates we have endogenous opioid system which modulates the transmission of afferent impulses presynaptically at the level of the first-order neuron in the

dorsal horn of spinal cord. About dozen of such endogenous opiates like substances have now been found, but all are breakdown products of 3 large protein molecules - proopiomelanocortin, proenkephalin, prodynorphin. Opioids also exert a direct inhibitory effect on the postsynaptic membrane potential. In addition to the opioid descending inhibitory pathway, a monoamine pathway also originates from locations in the periaqueductal grey and reticular formation. Electrical stimulation of these pathways and intracerebral interjections of α 2-adrenergic agonists can inhibit spinal nociceptive reflexes.²

Another group of analgesics like Aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), and specific cyclooxygenase-2 (COX-2) inhibitors exert an analgesic effect by inhibiting prostaglandin synthesis and reducing prostaglandin E1-mediated and prostaglandin E2-mediated sensitization of peripheral nociceptors. They also have side effects like gastric ulceration, nephrotoxicity, hepatotoxicity etc. Hence there is always a need of development of new analgesics with less adverse effect.

Acetylcholine (ACh) is a major excitatory neurotransmitter in the nervous system of vertebrates and invertebrates.³ Central cholinergic neurons detected by choline acetyl transferase immunoreactivity are concentrated in the mediobasal forebrain, brainstem, cerebral cortex and hippocampus. Brain cholinergic system through muscarinic receptors may be involved in modulation of pain.⁴ The first observation of analgesic properties of a cholinergic drug was made by Flodmark and Wramner in 1945.⁵ It is now known that pharmacologic substances acting at the central M-cholinergic receptors can induce antinociception. Acetylcholine increased nociceptive thresholds in mice in thermal and chemical nociceptive models after intracerebroventricular (ICV) administration.⁶ This effect was abolished by systemic administration of atropine. Systemic administration of physostigmine and ICV administration of neostigmine potentiated the effect of acetylcholine.⁷ Carbachol was antinociceptive in rats and rabbits after ICV administration and in rats and cats after intracerebral (IC) microinjection.⁸⁻¹¹

Antinociceptive activity also was demonstrated for anticholinesterase agents. Systemically administered physostigmine was antinociceptive in most studies. Alessandro Bartolini showed in his study that Pretreatment with U-73122 (0.6-5 μ g per mouse i.c.v.) and anti-PLC β 1 (2-3 nmol per mouse i.c.v.) antagonizes physostigmine (0.1 mg/kg s.c.)- and oxotremorine (60 μ g per mouse i.c.v.)-induced antinociception in the mouse hot-plate test.¹² Morphine, oxotremorine and physostigmine showed antinociceptive activity in mice using the hot plate reaction time test.¹³ Antinociceptive activity of morphine and clonidine is enhanced by concurrent administration of physostigmine peripherally and intracerebroventricular injection tested by tail immersion test.¹⁴ Anticholinesterase galantamine and physostigmine

showed antinociceptive activity by hot plate method and acetic acid writhing test.¹⁵ Physostigmine significantly reduces the anaesthesia induced by ketamine tested by measured as the time from the loss to the recovery of the righting reflex but do not alter the analgesia induced by ketamine tested by tail immersion method.¹⁶ Morphine elevated the levels of ACh in the cerebellum and striatum, cold water swimming in the cerebellum, striatum and cortex, and physostigmine in the striatum and hippocampus.¹⁷ Pert showed that physostigmine was antinociceptive in primates in the electroshock test.¹⁸ These studies raised at least two questions. First, is the antinociceptive effect or part of it a result of impaired motor activity? Second, is the observed effect mediated by the action of experimental substances on the spinal cord alone? In a series of experiments performed, intra-arterial (IA) injection of bradykinin was employed as a means of nociceptive stimulation.¹⁹⁻²¹ Flexor nociceptive reflex and multispike activity, recorded in the anterolateral quadrant of the spinal cord, were measured as the behavioral and electrophysiologic correlates of nociception. Because both behavioral and electrophysiologic methods demonstrated the same degree of antinociception being produced by cholinergic substances, it is reasonable to assume that inhibition of the flexor nociceptive reflex did not result from impaired motor activity. Rather, it is a consequence of the inhibition of neuronal transmission in the spinal afferent pathways. Several more recent studies have confirmed the previous findings regarding the role of muscarinic but not nicotine receptors in spinal antinociception.^{22,23} It seems reasonable to suggest that if enhancement of central cholinergic transmission results in an antinociceptive effect, then M1 cholinomimetics and M2 cholinergic blockers must be able to induce antinociception, because M1 receptors are postsynaptic and M2 receptors cause presynaptic inhibition of acetylcholine release.

This study was taken in account to make a combination of different group of analgesic which can produce an effective level of analgesia without the side effect. Here we have tried the combination of sub-analgesic dose of morphine and physostigmine to produce the effective level of analgesia.

METHODS

Wistar albino rats of either sex weighing 150 to 200 gms were selected by the process of randomization. Wistar albino rats were divided into seven groups, each group containing six rats. Instruments required were Hot Water Bath with thermostat control. Drug Physostigmine was procured from Sigma Aldrich pharmaceuticals India and Morphine sulphate from Troika Pharmaceuticals. Study was performed in the Department of Pharmacology, KIMS, Narketpally, Andhra Pradesh, India. Source of animals was Central animal house, KIMS, Narketpally which were procured from National Institute of Nutrition (NIN), Hyderabad, India.

Design of the experiment was laboratory based randomized control trial (RCT) with prior permission of Institutional Animal Ethics Committee (IEAC) from October 2010 - September 2012. In statistical analysis, one way ANOVA was applied to maximal possible effect (MEP) in percentage at 90 min by using software SPSS v19. It was used for calculation for statistical significance in between groups. p value <0.05 was considered as statistically significant.

Tail immersion test by hot water-bath²⁴

Wistar albino rats of either sex, which showed reaction time of less than 6 sec were used in this experiment. Rats

were weighed and divided into 7 groups containing 6 animals in each group (Table 1). The tail withdrawal latency was measured at basal level i.e. at 0 minute, i.e. immediately after giving the drug and then successively at 15 min, 30 min, 60 min, 90 min of duration after drug administration. Tail withdrawal latency is the time duration from immersing the tail in hot water bath, which is maintained at 55±0.5°C temperature by using thermostat control, till the withdrawal of the tail from hot water bath. Normal saline treatment used as control. The antinociceptive activity was considered as positive when reaction time is more than 6 sec and within 15 sec. Cut-off time was taken as 15 sec in order to prevent the damage to the rat tail.

Table 1: Different group of rats with respective drugs administered in specific dose and route of administration.

Group No.	Groups (N = 6)	Drug	Dose and route of administration
1	Control	Normal saline	0.5 ml/rat i.p
2	Sub analgesic dose of standard	Morphine	0.1 mg/kg i.p
3	Analgesic dose standard	Morphine	1 mg/kg i.p
4	Test drug	Physostigmine	50 µg/kg s.c
5	Test drug	Physostigmine	100 µg/kg s.c
6	Test drug	Physostigmine	200 µg/kg s.c
7	Combination with test drug	Physostigmine + morphine sub analgesic dose	50 µg/kg s.c + 0.1 mg/kg i.p

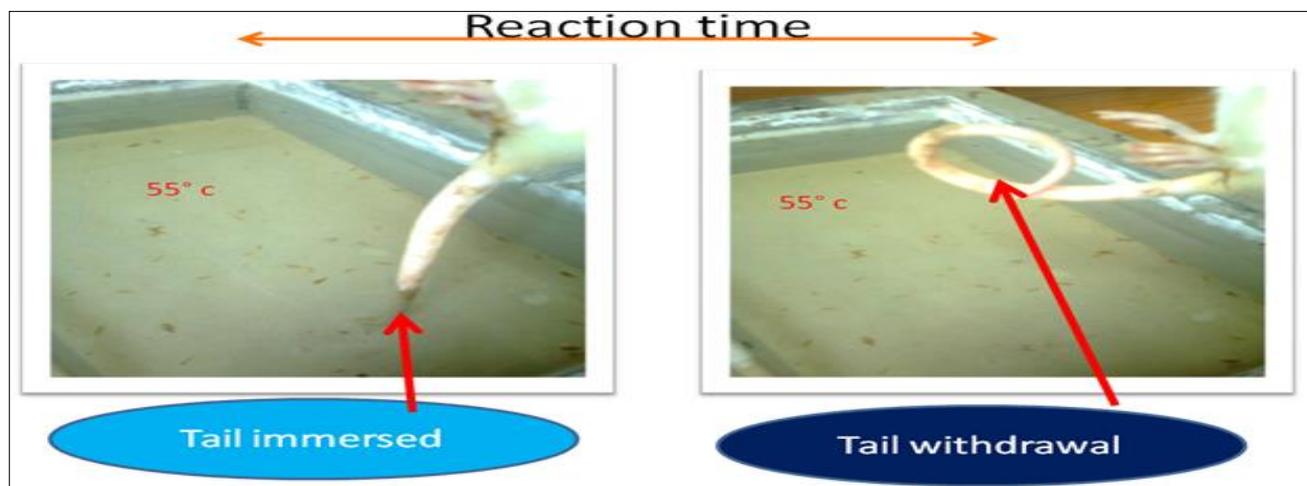


Figure 1: Tail immersion test showing reaction of rat in the form of tail withdrawal from hot water bath.

Table 2: Comparison of tail withdrawal latency (sec) of Physostigmine group with different groups (Mean±SE).

	0 min	15 min	30 min	60 min	90min
Control (NS)	2.75±0.17	3.08±0.15	3.25±0.11	3.17±0.11	3.58±0.15
Morphine (0.1 mg/kg)	3.42±0.15	3.67±0.25	4.17±0.11	4.17±0.21	4.08±0.15
Morphine (1 mg/kg)	3.08±0.08	7.67±0.17	10.67±0.33	14.00±0.37	14.67±0.21
Physostigmine (50µg/kg)	2.83±0.11	3.08±0.15	3.08±0.15	3.00±0.13	3.50±0.18
Physostigmine (100µg/kg)	2.75±0.11	3.50±0.13	5.33±0.17	6.67±0.11	8.92±0.24
Physostigmine (200 µg/kg)	3.00±0.13	5.33±0.42	7.83±0.49	10.42±0.42	14.25±0.11
Physostigmine 50µg/kg + Morphine 0.1mg/kg	3.08±0.08	3.92±0.24	4.92±0.27	7.25±0.28	9.50±0.18

RESULTS

Table 3: Comparison of Mean±SE and SD of maximal possible effect in % of tail withdrawal latency of different groups.

Group no.	Groups	Mean±SE	Std. deviation
1	Normal Saline (Control) (NS)	6.17±0.92	2.25
2	Morphine 0.1 mg/kg (MOR 0.1)	5.74±0.88	2.15
3	Morphine 1mg/kg (MOR 1)	97.22±1.76	4.30
4	Physostigmine 50 µg/kg (PHYSO 50)	5.50±0.88	2.19
5	Physostigmine 100 µg/kg (PHYSO 100)	50.39±1.61	3.95
6	Physostigmine 200 µg/kg (PHYSO 200)	93.78±0.89	2.19
7	Physostigmine 50 µg/kg+Morphine 0.1 mg/kg (PHYSO 50 + MOR 0.1)	53.87±1.38	3.38

Tail flick latency in seconds of normal saline as control group in 6 rats at 0 min, 15 min, 30 min, 60 min and 90 minutes showed no significant difference when their mean is calculated (Table 2). Physostigmine 200 µg/kg (s.c) produces increase in tail withdrawal latency (sec) at 30

min, 60 min, and 90 min in comparison to Normal Saline (control) 0.5 ml i.p.

Morphine 1 mg/kg i.p. produces increase in tail withdrawal latency (sec) at 15 min, 30 min, 60 min, and 90 min in comparison to Normal Saline (control) 0.5 ml i.p.

Physostigmine 100 µg/kg (s.c) produces increase in tail withdrawal latency (sec) at 30 min, 60 min and 90 min in comparison to Normal Saline (control) 0.5 ml i.p.

Table 3 shows MPE of increased tail withdrawal latency in % is increased in Morphine 1 mg/kg, Physostigmine 100 µg/kg, Physostigmine 200 µg/kg and combination group Physostigmine 50 µg/kg + Morphine 0.1 mg/kg in comparison to control group. Further comparison showed increase in MPE in % of tail withdrawal latency of combination group of physostigmine 50 µg/kg + Morphine 0.1 mg/kg in comparison to Physostigmine 50 µg/kg alone and Morphine 0.1 mg/kg alone.

Physostigmine 50 µg/kg + Morphine 0.1 mg/kg produce increase in tail withdrawal latency (sec) at 60 min, 90 min in comparison to Normal Saline (control) 0.5 ml (i.p), Physostigmine 50 µg/kg alone and Morphine 0.1 mg/kg alone.

From the observed data the maximum possible effect in percentage of increased tail flick latency at 90 min is calculated which is shown in Table 3. Formula of Maximum Possible Effect (MPE) in percentage = (post drug latency - pre drug latency/ cut-off time - pre drug latency) x 100.

Table 4: Intergroup comparison of MPE in % of Physostigmine group of Tail withdrawal latency by One Way ANOVA test.

ANOVA for physostigmine tail immersion test					
	Sum of squares	df	Mean square	F	P value (Sig.)
Between groups	58935.785	6.000	9822.631	1060.873	0.0001***
Within groups	324.065	35.000	9.259		
Total	59259.851	41.000			

*** p<0.005 indicating highly significant difference

Table 4 shows that p value is less than 0.005 i.e. 0.0001 which is highly significant. This indicates that there is highly significant difference among the comparison groups.

DISCUSSION

In the present study, three graded doses of Physostigmine (50 µg/kg, 100 µg/kg, 200 µg/kg) (s.c) and combination of Physostigmine (50 µg/kg) (s.c) + sub-analgesic dose of Morphine (0.1 mg/kg) (i.p) was compared with standard drug Morphine analgesic dose (1 mg/kg) (i.p) and control

group Normal Saline (NS) (0.5ml) (i.p). Tail withdrawal latency (sec) was recorded at 0 min, 15 min, 30 min, 60 min and 90 min after drug administration. Subcutaneous (s.c) administration of Physostigmine increased the tail withdrawal latency period (sec) (Mean±SE) in the doses of 100 µg/kg and 200 µg/kg at 60 min (6.67±0.11, 10.42±0.42 respectively) and 90 min (8.92±0.24, 14.25±0.11 respectively) interval in-comparison to control (NS) treatment group (3.17±0.11, 3.58±0.15 respectively), indicating Physostigmine produces antinociceptive effect in tail immersion test by hot water bath.

Intraperitoneal (i.p) administration of Morphine in the antinociceptive dose of 1 mg/kg produced increase in the tail withdrawal latency (sec) at 15, 30, 60, 90 min (7.67 ± 0.17 , 10.67 ± 0.33 , 14.00 ± 0.37 , 14.67 ± 0.21 respectively) in comparison to control (NS) treatment group (3.08 ± 0.15 , 3.25 ± 0.11 , 3.17 ± 0.11 , 3.58 ± 0.15 respectively).

Combination treatment of low doses of both Physostigmine 50 $\mu\text{g}/\text{kg}$ + Morphine 0.1 mg/kg increased the tail withdrawal latency at 60 and 90 min (7.25 ± 0.28 , 9.50 ± 0.18 respectively) in-comparison to control (NS) treatment group (3.17 ± 0.11 , 3.58 ± 0.15 respectively) or Physostigmine 50 $\mu\text{g}/\text{kg}$ (3.00 ± 0.13 , 3.50 ± 0.18 respectively) alone or Morphine 0.1 mg/kg (3.17 ± 0.11 , 3.58 ± 0.15 respectively) alone.

Maximal possible effect (MPE) in tail withdrawal latency in percentage (%) at 90 min was calculated in Physostigmine 100 $\mu\text{g}/\text{kg}$, Physostigmine 200 $\mu\text{g}/\text{kg}$, Morphine 1 mg/kg and combination treatment of Physostigmine 50 $\mu\text{g}/\text{kg}$ + Morphine 0.1 mg/kg (50.39 ± 1.61 , 93.78 ± 0.89 , 97.22 ± 1.76 , 53.87 ± 1.38 respectively) which is more and statistically significant in comparison to control group (6.17 ± 0.92). These results suggest that Physostigmine 100 $\mu\text{g}/\text{kg}$, Physostigmine 200 $\mu\text{g}/\text{kg}$, Morphine 1 mg/kg and combination treatment of Physostigmine 50 $\mu\text{g}/\text{kg}$ + Morphine 0.1 mg/kg can produce significant antinociceptive effect in the tail immersion test model in albino rats.

Further intergroup comparison of MPE (%) showed that Physostigmine 200 $\mu\text{g}/\text{kg}$ (93.78 ± 0.89) is comparable with Morphine 1 mg/kg (97.22 ± 1.76) indicating that Physostigmine is more potent than Morphine. MPE (%) of combination group Physostigmine 50 $\mu\text{g}/\text{kg}$ + Morphine 0.1 mg/kg (53.87 ± 1.38) is significantly more than Physostigmine 50 $\mu\text{g}/\text{kg}$ (5.50 ± 0.88) alone or Morphine 0.1 mg/kg (5.74 ± 0.88) alone indicating Physostigmine can potentiate antinociceptive effect of Morphine.

The results of the present study indicated that cholinergic drugs can produce antinociceptive effect in the tail flick test. Nemirovsky et al, Gillberg et al, Gordh et al, Yaksh et al, also reported antinociceptive effect of cholinomimetics and anticholinesterases in the experimental animal models.^{20-22,25-29}

The results of the present study indicated Physostigmine can potentiate the antinociceptive effect of low dose of Morphine in Tail Immersion Test models. Peterson J et al, Beilin B et al, also reported enhancement of analgesic effect of Morphine by Physostigmine in post operative patients.^{30,31}

CONCLUSION

Present study suggests that there is involvement of cholinergic system in antinociceptive action which is evaluated by administration of Physostigmine in Tail

Immersion Test in Albino Rats. Physostigmine is more potent antinociceptive than Morphine. Physostigmine potentiated the antinociceptive activity of low dose of standard drug Morphine. Further studies are required to evaluate the analgesic effect of combination treatment of cholinergic drugs with Morphine in human beings.

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Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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