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Research Article

Study of anti-nociceptive potential of physostigmine and its combination with morphine in albino rats

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ABSTRACT

Background: The cholinergic drugs are having antinociceptive potential but are under investigation because of their serious side effects. It is difficult to accept them as an analgesic. This study is 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 effect of Physostigmine and its combination with subanalgesic dose of morphine and comparing their effect with analgesic dose of Morphine.
Methods: Antinociceptive effect of Physostigmine in three graded doses (50, 100 & 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 tail flick 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 the tail flick latency in Physostigmine 50 µg/kg (SC) treatment at 90 min (3.08±0.15) in comparison to control (NS) treatment group. Combination treatment of low doses of both Physostigmine 50 µg/kg + Morphine 0.1 mg/kg increased the tail flick latency 90 min (7.08±0.15) in-comparison to control (NS) treatment group (3.33±0.11).
Conclusions: Physostigmine is more potent antinociceptive than Morphine and Physostigmine potentiated the antinociceptive activity of low dose of standard drug Morphine.

Keywords: Pain, Antinociceptive, Physostigmine, Morphine, Tail flick

INTRODUCTION

Pain is an ill-defined, unpleasant sensation usually evoked by an external or internal noxious stimulus experienced by human beings. The taxonomy committee of International Association for the study of pain defines pain as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage".[1] 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. The analgesics are of two types a) opioid and b) non-opioid.

Opioids are the most potent and commonly used group of analgesic drugs mostly used for visceral pain, e.g. Morphine and Pethidine. But their analgesic action is associated with a greater degree of dose dependent adverse drug reactions including drug dependence.[2]

Non-steroidal anti-inflammatory drugs (NSAIDS) are mainly used for treatment of integumental pain and act mainly by peripheral mechanism of action by inhibiting prostaglandin synthesis. These drugs are more effective against pain associated with inflammation and adverse effects like CNS depression and dependence are less when compared to opioids. However these drugs are known to produce gastric irritation including peptic ulceration. Hence there is always a need of development of new analgesics with less adverse effect.

Several areas distributed throughout the neural axis exert a top-down modulation of pain sensation according to the nature of the painful stimulus and the behavioural state of the individual, both in normal and pathologic conditions. This modulation is largely mediated by descending monoaminergic pathways that either inhibit or facilitate transmission of nociceptive information at the level of the dorsal horn.[3-8] Monoamines including serotonin, norepinephrine and dopamine act via different receptor subtypes to exert a complex modulation of neurotransmitter release from nociceptive afferents and excitability of dorsal horn neurons. These monoaminergic systems have an important role in mechanisms of inflammatory and neuropathic pain and are a target for pharmacologic management of this conditions.[5]

Recently it has been noticed that Acetylcholine (ACh) is a major excitatory neurotransmitter in the nervous system of vertebrates and invertebrates.[9] 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.[10] Muscarinic receptor agonists has been demonstrated to be potent and efficacious analgesics in mice. This action of ACh is mediated by the muscarinic ACh receptors (mAChRs).[11] The cholinesterase inhibitor Physostigmine increased the pain threshold in man.[12] Muscarinic ACh receptors have also been shown as potential mediators of pain-related neuroplasticity, especially within the spinal cord. Intrathecal administration of cholinergic muscarinic agonists or acetyl cholinesterase inhibitors produces the analgesia in both animals and humans.[13]

The hippocampus is an important part of the mammalian brain, and it is involved in the regulation of many functions, such as memory, learning, avoidance and pain response. Electrophysiological, pharmacological, behavioural and clinical data indicate that the hippocampal formation is an integral component of the limbic system, and plays an important role in the affective and motivational components of pain perception.[14] The hippocampus receives cholinergic projections from the medial septal nucleus and Broca's diagonal band, which terminate in the CA1, CA3, & dentate gyrus regions.[15]

Though cholinergic drugs are having antinociceptive potential but are under investigation because of their serious side effects. It is difficult to accept them as an analgesic. This study is 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. So that if their combination will show the anti-nociceptive activity then side effects will be less and analgesia will be achieved.

The objective of the study was to evaluate antinociceptive activity of Physostigmine in graded doses and its combination with Morphine at sub analgesic doses by using Radiant heat induced pain by using analgesiometer and to compare its antinociceptive potential with standard drug Morphine.

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 analgesiometer for tail flick method. 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; AP. Source of animals was Central animal house, KIMS, Narketpally which were procured from National Institute of Nutrition (NIN), Hyderabad.

Design of the experiment was laboratory based randomized control trial (RCT) with prior permission of Institutional Animal Ethics Committee (IEAC).

Table 1: Grouping of animals and drug schedule.

Group No	Groups Each group 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

i.p = intraperitoneal, s.c = subcutaneous.

Tail flick method

The instrument used in this method was Analgesiometer. This test was performed only on those rats that had shown the reaction time less than 6 seconds. The cut off time was taken as 10 seconds to avoid the injury to the sensory nerve endings. Wistar albino rats of either sex were selected by the process of randomization and placed in separate cages. After selecting the rats the drug was administered and the reaction time was recorded at 0 min (basal level i.e. immediately after administration of drug) 15 min, 30 min, 60 min and at 90 min of administration of drug. Reaction time between 6 sec to 10 seconds was considered as antinociceptive effect.

Statistical analysis

One way ANOVA was applied only to maximal possible effect 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 is considered as statistically significant.

RESULTS

Tail flick latency in seconds of normal saline as control group in 6 rats at 0 min, 15 min, 30 min, 60 min & 90 minutes showed no significant difference when their mean is calculated. Like this reading are taken from all groups and compared. Only mean readings are considered in further for comparison and calculation (Table 1).

Table 2: Tail flick latency in seconds of normal saline (NS) 0.5 ml intraperitoneally (control group).

SL NO	Tail Flick Latency in seconds				
	0 min	15 min	30 min	60 min	90 min
1	3.00	3.00	3.00	3.50	3.50
2	2.50	2.50	3.00	3.00	3.00
3	3.00	3.00	3.50	3.00	3.50
4	3.00	3.50	3.00	3.50	3.50
5	3.00	3.00	3.50	3.00	3.00
6	3.00	3.50	3.50	3.00	3.50
Total	17.50	18.50	19.50	19.00	20.00
Mean	2.92	3.08	3.25	3.17	3.33
SD	0.20	0.38	0.27	0.26	0.26
SE	0.08	0.15	0.11	0.11	0.11

Table 3: Comparison of mean tail flick latency in seconds of physostigmine with different groups (mean ± SE).

	0 min	15 min	30 min	60 min	90 min
Control (NS)	2.92±0.08	3.08±0.15	3.25±0.11	3.17±0.11	3.33±0.11
Morphine (0.1 mg/kg)	2.83±0.11	3.08±0.15	3.25±0.11	3.17±0.11	3.33±0.11
Morphine (1 mg/kg)	3.08±0.08	5.50±0.18	7.42±0.15	9.67±0.17	9.83±0.11
Physostigmine 50 µg/kg	2.83±0.11	3.08±0.15	3.08±0.15	3.00±0.13	3.17±0.11
Physostigmine 100 µg/kg	3.17±0.11	3.17±0.11	4.08±0.27	6.25±0.28	7.50±0.18
Physostigmine 200 µg/kg	3.17±0.11	3.92±0.33	6.25±0.21	8.00±0.18	9.50±0.18
Physostigmine 50µg/kg + Morphine 0.1mg/kg	3.17±0.11	3.08±0.15	4.08±0.27	6.33±0.17	7.25±0.11

Subcutaneous (s.c) administration of Physostigmine increased the tail flick latency period (sec) (Mean ± SE) in the doses of 100 µg/kg and 200 µg/kg at 60 min (6.25±0.28, 8.00±0.18 respectively) and 90 min (7.50±0.18, 9.50±0.18 respectively) interval in-comparison to control (NS) treatment group (3.17±0.11, 3.33±0.11 respectively) indicating Physostigmine produces antinociceptive effect in tail flick test. However there is no much increase in the tail flick latency in the Physostigmine 50 µg/kg (s.c) treatment at 60 and 90 min (2.83±0.11, 3.08±0.15 respectively) in comparison to control (NS) treatment group (3.17±0.11, 3.33±0.11 respectively) (Table 2).

Table 4: Maximal possible effect (MPE) in % of increased tail flick latency of physostigmine.

SL NO	NS	MOR 0.1	MOR 1	PHYSO50	PHYSO100	PHYSO200	PHYSO50 + MOR 0.1
1	7.14	6.67	100.00	6.67	57.14	85.71	64.29
2	6.67	1.33	100.00	7.14	61.54	100.00	61.54
3	7.14	7.14	92.86	6.67	71.43	92.86	57.14
4	7.14	7.14	100.00	0.00	71.43	100.00	64.29
5	0.00	0.00	92.86	0.00	61.54	92.31	53.85
6	7.14	7.14	100.00	7.14	57.14	85.71	57.14
Total	35.23	29.42	585.17	27.62	380.22	556.59	358.25
Mean	5.87	4.90	97.53	4.60	63.37	92.77	59.71
SD	2.88	3.31	3.83	3.57	6.55	6.39	4.31
SE	1.18	1.35	1.56	1.46	2.67	2.61	1.76

NS – Normal Saline; MOR 0.1 – Morphine 0.1 mg/kg; MOR 1 – Morphine 1 mg/kg; PHYSO 50 – Physostigmine 50 µg/kg; PHYSO 100 – Physostigmine 100 µg/kg; PHYSO 200 – Physostigmine 200 µg/kg; PHYSO 1 + MOR 0.1 – Physostigmine 50 µg/kg + Morphine 0.1 mg/kg

Intraperitoneal (i.p) administration of Morphine in the antinociceptive dose of 1 mg/kg produced increase in the tail flick latency 15, 30, 60, 90 min (5.50±0.18, 7.42±0.15, 9.67±0.17, 9.83±0.11 respectively) in comparison to control (NS) treatment group (3.08±0.15, 3.25±0.11, 3.17±0.11, 3.33±0.11 respectively) (Table 2).

Table 5: Comparison of mean ± S.E. and S.D. of MPE in % of tail flick latency of Physostigmine.

Groups	Mean± SE	Std. Deviation
Normal Saline (Control) (NS)	5.87±1.18	2.88
Morphine 0.1 mg/kg (MOR 0.1)	4.90±1.35	3.31
Morphine 1 mg/kg (MOR 1)	97.53±1.56	3.83
Physostigmine 50 µg/kg (PHYSO 50)	4.60±1.46	3.57
Physostigmine 100 µg/kg (PHYSO 100)	63.37±2.67	6.55
Physostigmine 200 µg/kg (PHYSO 200)	92.77±2.61	6.39
Physostigmine 50 µg/kg + Morphine 0.1 mg/kg (PHYSO 50 + MOR 0.1)	59.71±1.76	4.31

However there is no much increase in the tail flick latency in the Morphine 0.1 mg/kg (i.p) treatment at 15, 30, 60 and 90 min (3.08±0.15, 3.25±0.11, 3.17±0.11, 3.33±0.11 respectively) in comparison to control (NS) treatment group (3.08±0.15, 3.25±0.11, 3.17±0.11, 3.33±0.11 respectively) (Table 2).

Combination treatment of low doses of both Physostigmine 50 µg/kg + Morphine 0.1 mg/kg increased the tail flick latency at 60 and 90 min (6.33±0.17, 7.08±0.15 respectively) in-comparison to control (NS) treatment group (3.17±0.11, 3.33±0.11 respectively), Physostigmine 50 µg/kg (3.00±0.13, 3.17±0.11 respectively) alone or Morphine 0.1 mg/kg (3.17±0.11, 3.33±0.11 respectively) alone (Table 2).

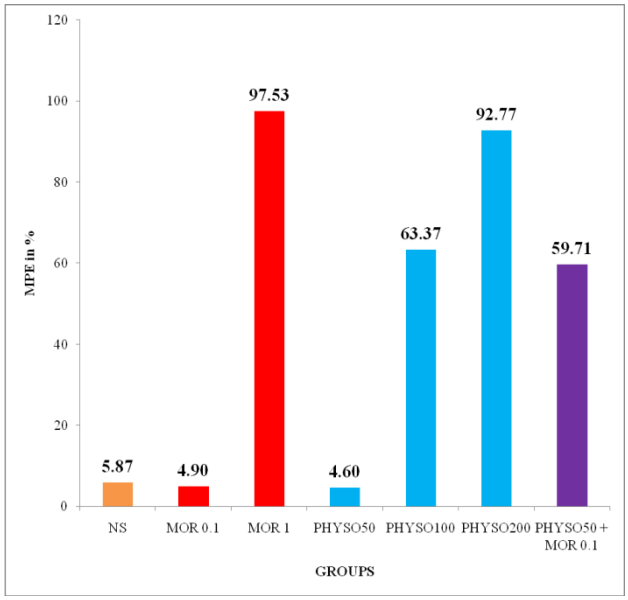

Figure 1: MPE in % of increased tail flick latency of physostigmine.

From the observed data the maximum possible effect in percentage of increased tail flick latency at 90 min is calculated.

Maximum Possible Effect (MPE) in percentage = (post drug latency – pre drug latency/ cut-off time – pre drug latency) x 100 (Table 3).

Maximal possible increase (MPE) in tail flick latency (%) at 90 min was calculated in Physostigmine 100 µg/kg, Physostigmine 200 µg/kg, Morphine 1mg/kg and combination treatment of Physostigmine 50 µg/kg + Morphine 0.1 mg/kg (63.37±2.67, 92.77±2.61, 97.53±1.56, 59.71±1.76 respectively) which is more and statistically significant in comparison to control group (5.87±1.18) (Table no.3). These results suggest that Physostigmine 100 µg/kg, Physostigmine 200 µg/kg, Morphine 1 mg/kg and combination treatment of Physostigmine 50 µg/kg + Morphine 0.1 mg/kg can produce significant antinociceptive effect in the tail flick test model in Albino Rats (Table 3).

Table 6: Intergroup comparison of MPE in % of physostigmine by one way ANOVA test.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	62028.983	6.00	10338.164	485.492	0.0001***
Within Groups	745.297	35.00	21.294		
Total	62774.281	41.00			

Further intergroup comparison of MPE (%) showed that Physostigmine 200 µg/kg (92.77±2.61) is comparable with Morphine 1 mg/kg (97.53±1.56) (Table no.3) indicating that Physostigmine 200 µg/kg is more potent than Morphine 1 mg/kg. MPE (%) of combination group Physostigmine 50 µg/kg + Morphine 0.1 mg/kg (59.71±1.76) is significantly more than Physostigmine 50 µg/kg (4.60±1.46) alone or Morphine 0.1 mg/kg (4.90±1.35) alone indicating Physostigmine can potentiate antinociceptive effect of Morphine (Table 3).

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 flick 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 flick latency period (sec) (Mean ± SE) in the doses of 100 µg/kg and 200 µg/kg at 60 min (6.25±0.28, 8.00±0.18 respectively) and 90 min (7.50±0.18, 9.50±0.18 respectively) interval in-comparison to control (NS) treatment group (3.17±0.11, 3.33±0.11 respectively), indicating Physostigmine produces antinociceptive effect in tail flick test.

Intraperitoneal (i.p) administration of Morphine in the antinociceptive dose of 1 mg/kg produced increase in the tail flick latency (sec) at 15, 30, 60, 90 min (5.50±0.18, 7.42±0.15, 9.67±0.17, 9.83±0.11 respectively) in comparison to control (NS) treatment group (3.08±0.15, 3.25±0.11, 3.17±0.11, 3.33±0.11 respectively.

Combination treatment of low doses of both Physostigmine 50 µg/kg + Morphine 0.1 mg/kg increased the tail flick latency at 60 and 90 min (6.33±0.17, 7.08±0.15 respectively) in-comparison to control (NS) treatment group (3.17±0.11, 3.33±0.11 respectively) or Physostigmine 50 µg/kg (3.00±0.13, 3.17±0.11 respectively) alone or Morphine 0.1 mg/kg (3.17±0.11, 3.33±0.11 respectively) alone.

Maximal possible effect (MPE) in tail flick latency in percentage (%) at 90 min was calculated in Physostigmine 100 µg/kg, Physostigmine 200 µg/kg, Morphine 1 mg/kg and combination treatment of Physostigmine 50 µg/kg + Morphine 0.1 mg/kg (63.37±2.67, 92.77±2.61, 97.53±1.56, 59.71±1.76 respectively) which is more and statistically significant in comparison to control group (5.87±1.18). These results suggest that Physostigmine 100 µg/kg, Physostigmine 200 µg/kg, Morphine 1 mg/kg and combination treatment of Physostigmine 50 µg/kg + Morphine 0.1 mg/kg can produce significant antinociceptive effect in the tail flick test model in albino rats.

Further intergroup comparison of MPE (%) showed that Physostigmine 200 µg/kg (92.77±2.61) is comparable with Morphine 1 mg/kg (97.53±1.56) indicating that Physostigmine is more potent than Morphine. MPE (%) of combination group Physostigmine 50 µg/kg + Morphine 0.1 mg/kg (59.71±1.76) is significantly more than Physostigmine 50 µg/kg (4.60±1.46) alone or Morphine 0.1 mg/kg (4.90±1.35) 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 (1985, 1988, 1990), Yaksh et al (1985, 1995), Gillberg et al (1986, 1989, 1990 1991), Gordh et al (1989) also reported antinociceptive effect of

cholinomimetics and anticholinesterases in the experimental animal models.[16-24]

The results of the present study indicated that Physostigmine can potentiate the antinociceptive effect of low dose of Morphine in tail flick test model. Peterson J et al, Beilin B et al also reported enhancement of analgesic effect of Morphine by Physostigmine in post-operative patients.[25,26]

CONCLUSION

Present study suggests that there is involvement of cholinergic system in antinociceptive action which is evaluated by administration of Physostigmine in tail flick test in albino rats.

Physostigmine (100 µg/kg & 200 µg/kg) (s.c) has antinociceptive action by tail flick test in albino rats.

The antinociceptive effect of Physostigmine 200 µg/kg is comparable to Morphine 1 mg /kg in Tail Flick Test model, indicating that Physostigmine is more potent than Morphine.

Combination of low doses of Physostigmine (50 µg/kg) potentiated antinociceptive effect of low dose of Morphine (0.1mg/kg) in Tail Flick Test model, indicating that cholinergic drugs like Physostigmine can be combined with Morphine for enhancement of Morphine action.

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