

Advanced in vitro Research on Modulation of Tumor Necrosis Factor Alpha (TNF- α) by Piperine and Pirfenidone on Naloxone Precipitated Opioid Withdrawal Syndrome

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Abstract: *The study evaluates the modulation of TNF-alpha (α) by piperine and pirfenidone on opioid withdrawal syndrome in mice. Withdrawal syndrome also called a discontinuation syndrome occurs on discontinuation or reducing certain medications. The sedative withdrawal disorder arises after administration of heroin, morphine, and followed by several days. In human, the signs and symptoms of withdrawal symptoms are stomach cramps, rhinorrhea, perspiring, raised pulse, and expanded circulatory strain, touchiness, dysphoria, hyperalgesia, and a sleeping disorder. In this study, we used two models: precipitated and spontaneous withdrawal. Naloxone was administered to precipitate morphine withdrawal symptoms in the precipitated model whereas in case of spontaneous model, morphine was terminated on 6th day after the completion of 5 days morphine administration. Naloxone is the most well-known μ opioid receptor antagonist which is used to precipitate withdrawal symptoms. In the precipitated model, various opioid withdrawal syndromes have been observed, the best response was observed in synergistic effect of both the drugs i.e. pirfenidone and piperine treated group. The effect of each drug at a low dose was noted to be significantly lower than that of the drug at high dose. The combination of pirfenidone and piperine showed the most promising results. The study suggests that the use of both drugs may offer a synergistic effect in managing opioid withdrawal symptoms.*

Keywords: Opioid Withdrawal, Piperine, Pirfenidone, TNF-alpha, Naloxone, Precipitated Withdrawal

1. Introduction

Opioids drugs are used in the treatment of pain or used as analgesics. But, their clinical utilization could be inadequate, secondary to undesirable and unfavorable effects such as toleration, helplessness, reward, and alteration in behavior. Opioid compulsion is long-term utilization, degenerated disease. Left untreated, high morbidity and mortality rates are seen (Cruts *et al.*, 2008; Clausen *et al.*, 2009). Psychosocially assisted pharmacological treatment of opiate dependence is used to reduce illicit opiate abuse, reduce the harms related to opiate abuse and improve quality of life (Amato *et al.*, 2008; WHO, 2009). Withdrawal syndrome, also called a discontinuation syndrome is a set of symptoms occurring on discontinuation or dosage reduction of some types of medications. The opiate withdrawal syndrome emerges after repeated administration of heroin, morphine, and lasts for hours to a few days, depending upon the specific drug and the duration and dose of prior administration. In humans, the signs and symptoms of withdrawal include stomach cramps, diarrhea, rhinorrhea, sweating, elevated heart rate and increased blood pressure, irritability, dysphoria, hyperalgesia, and insomnia (Morgan and Christie 2011).

Opiate drugs exert their effects by binding to three opioid receptor types (μ , γ , and κ) and mimicking the actions of endogenous opioid peptides, the endorphins, endomorphins, enkephalins, and dynorphins. The μ opioid receptor (MOR)

subtype is critical for the rewarding effects of heroin and morphine. The most prominent neuroadaptive changes during morphine induced dependence include desensitization of MORs and up regulation of the cAMP pathway. The mitogen activated protein kinase (MAPK) pathway as well as Ca²⁺ signaling are also affected during morphine dependence. The primary consequence of morphine withdrawal is „super activation" of adenylyl cyclase (AC) and a subsequent overproduction of its downstream signaling molecule, cAMP. Other cAMP actions during withdrawal include PKA-mediated enhanced GABAergic synaptic transmission in areas such as periaqueductal grey (PAG), ventral tegmental area (VTA), nucleus accumbens (NAcc.) and dorsal raphe (Williams *et al.*, 2001; Bailey and Connor 2005). Among the brain regions implicated in opiate dependence and withdrawal, the periaqueductal gray area (PAG) appears to be critical in regulating the complex signs and symptoms of opioid withdrawal. Numerous neurochemical mechanisms in the PAG have been identified that may contribute to the opioid withdrawal syndrome (Handong Ouyang *et al.*, 2012). Other receptors like glutamate, muscarinic, nicotinic and toll like receptors are also involved in morphine withdrawal syndrome.

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2. Materials and Methods

2.1 Materials

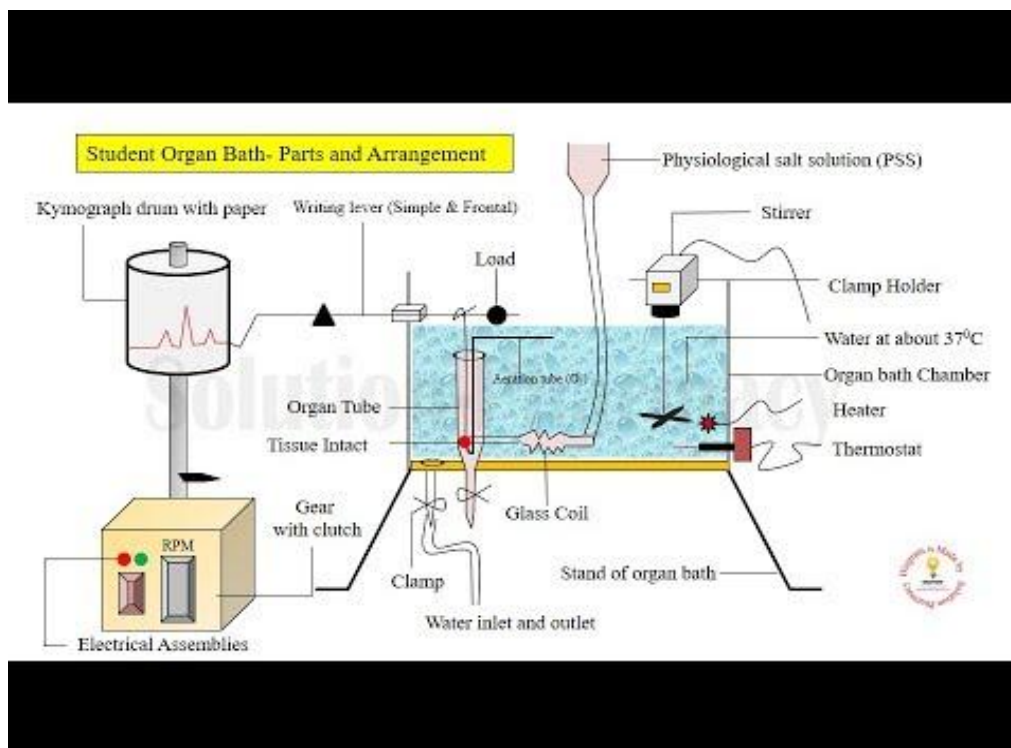
Drugs and chemicals which were used for the present research paper were morphine sulfate from (Jackson Laboratories Ltd. Amritsar, Punjab, India), Naloxone (Jackson Laboratories Ltd. Amritsar, Punjab, India), Pirfenidone (Sigma-Aldrich, St. Louis, Missouri, United States), Piperine (Sigma-Aldrich, St. Louis, Missouri, United States), Memantine (Sigma-Aldrich, St. Louis, Missouri, United States), Krebs solution ($\text{NaCl} 118$, $\text{KCl} 4.75$, K_2HPO_4 1.2, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, NaHCO_3 25 and glucose 11.1mM) at 37°C , aerated with 95% O_2 and 5% CO_2

2.2 Equipments

Student Organ Bath is an apparatus widely used in pharmacology laboratory and various educational institutes, research and development sector. It is not single apparatus but it is a combination of many small units which include are – bath chamber, organ tube, glass coil, PSS reservoir, heater, thermostat, stirrer or mixer, oxygen tube, aerator, lever, load, Sherrington rotating drum (Kymograph Drum) and Kymograph paper.

Working: Firstly isolate the organ bath to perform the experimental study. Once organ bath is isolated it should be transferred into physiological salt solution (PSS). Here we used the Krebs's solution. The following standard steps have to be followed:

Clean the organ bath assembly. Attaching or arranging the all hooks, clamp and water pipes. Fixing the lever and stick the kymograph paper on rotating drum. Kymograph paper should be either smoked or to use color ink at the end of lever, Fill the water into 2/3 portion of organ bath or up to suitable length depending upon the unit of organ bath. Switch on the mains and heater. Wash the organ tube with PSS once. Fill the PSS into organ bath and hold it. Start providing oxygen by aerator machine. Attach the aeration tube into the organ bath. Now tie the tissue of rat ileum Tie the other end of thread into writing lever, Attach the lever with rotating drum and wait resting or constant line appears., Inject the drug into the organ bath and carefully examine the changes in muscle strength by observing kymograph paper. Release the clamp of organ bath to let the PSS flow outside the organ tube. For every new dose of drug, we have to change the PSS. Also ensure the temperature that should not increase than optimum temperature.



2.3 Animals

Wistar rat of either sex ranging in the weight of 200-300 g was brought from NIPER, S. A. S. Nagar, Punjab a CPCSEA registered breeding facility. The food and water were stored in suitable containers in adequate quantity and more water during transit. It was kept in the central animal house facility at Lovely Professional University Phagwara, Punjab registered with CPCSEA with registration number 954/PO/Re/S/06/CPCSEA. Each animal was given a 12 hour

dark and 12 hour light cycle and was kept at circling humidity and temperature conditions.

2.4. In vitro rat ileum study

Adult rats weighing 250 ± 200 g, fasted for 24 h, were sacrificed by a high intraperitoneal dose of thiopental sodium followed by carotid bleeding. A small 2–3 cm section of the ileum was isolated from the intestine. The rat ileum preparation was prepared by tying a loop on one end of the tissue, and another thread was tied on a diagonally

opposite aspect in order to ensure the complete opening up of the lumen of the tissue while the same was mounted in the tissue bath. The tissue was then suspended in an organ bath containing 20 ml of Krebs solution (NaCl 118, KCl 4.75, K₂HPO₄ 1.2, CaCl₂ 2.6, MgSO₄ 1.2, NaHCO₃ 25 and glucose 11.1 mM) at 37°C, aerated with 95% O₂ and 5% CO₂. A resting tension of 1 g was applied to the tissue. The tissue was allowed to equilibrate for 40–60 min, and the response to acetylcholine (ACh) was determined for three times (10–6 M) so that withdrawal response could be expressed as percentage of a particular mean ACh response. Morphine (10 M) was added to the bath, and the tissue was exposed to the opioid agonist for a period of 4 min. Naloxone (10–5–5 M) was then added in the bath to elicit a strong opioid withdrawal contracture in the rat ileum. After washout, another ACh response was obtained (to verify whether the ileum responsiveness was modified after withdrawal contracture). After 10 min of resting period, test drug/vehicle (varying concentration as per the protocol) was added in the bath along with the 4 - min exposure of the ileum to the opiate [morphine (10 M)]. Naloxone (10–5 M) was then added to elicit a response. Following washout, ACh response was repeated to affirm the functional ability of the tissue. Moreover, in order to avoid the development of tolerance to repeated morphine exposure, each preparation was exposed only to three challenges with morphine and naloxone. Naloxone per se did not produce any effect on naive preparations or those washed after morphine contact.

Naloxone challenge immediately followed a brief period of 4-min morphine exposure, elicited a strong contracture in the rat ileum preparation in terms of the tension ratio results. Administration of piperidine significantly and dose dependently attenuated this naloxone - induced withdrawal response in morphine withdrawn rat ileum preparation as assessed in terms of tension ratio in morphine/naloxone group, when compared to that of control group. Further, the administration of piperidine significantly and dose dependently attenuated naloxone-induced withdrawal response in morphine withdrawn rat ileum. Memantine which was taken as standard drug also attenuated the morphine withdrawal syndrome but not as good as achieved in piperidine and piperidine. The effect of each drug at a low dose was noted to be significantly lower than that of the drug at high dose. But the best result was attained by the combination of both the drugs i.e. piperidine + piperidine.

3. Result

Action of piperidine and piperidine on naloxone precipitated opioid withdrawal on contracture of rat ileum.

Table 1: In vitro Effect of piperidine and piperidine and its combination

Group no.	Group name	In vitro Mean ± SD
1.	Control	78.33± 9.33
2.	Memantine	60.67±6.86 ^{SS}
3.	Piperidine low dose	56.83±9.91 ^{SSS}
4.	Piperidine high dose	45.33±6.19 ^{SSS, ^^}
5.	Piperidine low dose	51.33±5.35 ^{SSS}
6.	Piperidine high dose	36.67±4.89 ^{SSS, ^^}
7.	Piperidine + Piperidine low dose	19.17±5.85 ^{SSS, ^^}

^{SS}p<0.01, ^{SSS}p<0.001 represent significant difference in group 1 vs group 2, 3, 4, 5, 6, 7; ^{^^}p<0.01, ^{^^^}p<0.001 represent significant difference in division 2 vs division 3, 4, 5, 6, 7 one-way analysis of variance succeed by Tukey's various prominent examination.

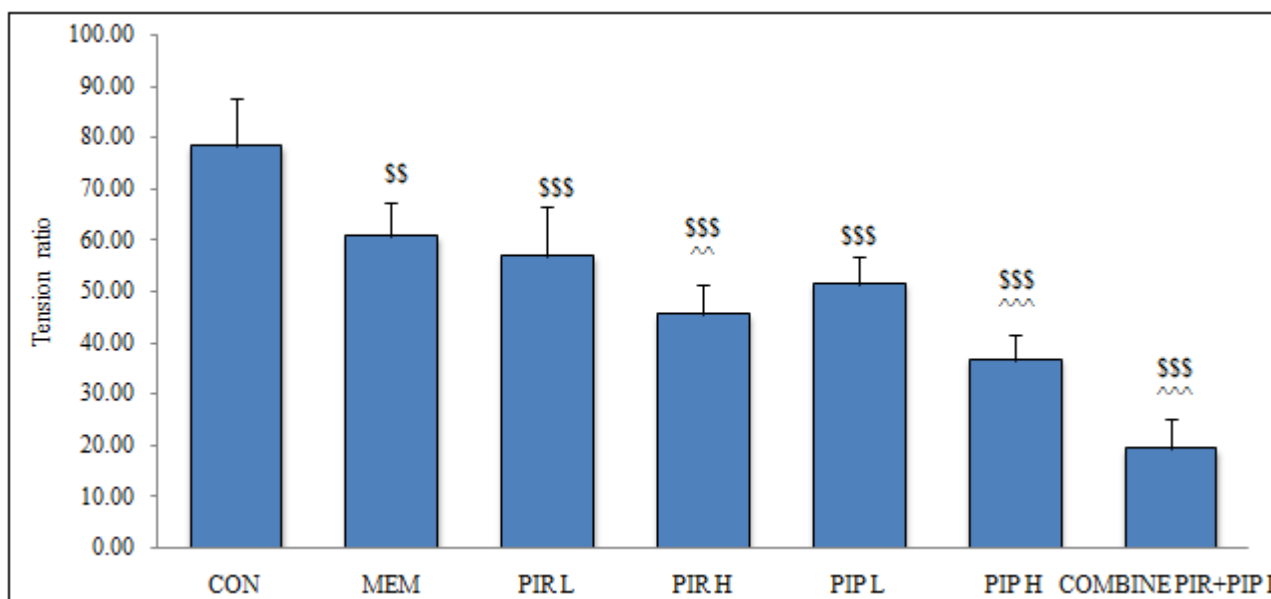


Figure 1: In vitro Effect of piperidine and piperidine and its combination

Data represented as mean ± SD. CON: Control; PIR: Piperidine; PIP: Piperidine; MOR: Morphine; NAL: Naloxone; MEM: Memantine; L: low dose; H: high dose. ^{SS}p<0.01, ^{SSS}p<0.001 represent significant difference in group 1 vs group 2, 3, 4, 5, 6, 7; ^{^^}p<0.01, ^{^^^}p<0.001

represent significant difference in division 2 vs group 3, 4, 5, 6, 7 one-way analysis of variance come succeed by Tukey's comparison test.

4. Discussion

In the present investigation, administration of piperine and pifrenidone, anti inflammatory drugs during the morphine treatment protocol dose dependently attenuated the precipitation of naloxone-precipitated opioid withdrawal syndrome in vitro in morphine withdrawn rat ileum. Various receptors and mechanisms are involved in the pathophysiology of opioid withdrawal. Opiate drugs exert their effects by binding to three opioid receptor types (μ , γ , and κ) and. The μ opioid receptor (MOR) subtype is critical for the rewarding effects of heroin and morphine. The primary consequence of morphine withdrawal is super activation of adenylyl cyclase (AC) and a subsequent overproduction of its downstream signaling molecule, cAMP. Other cAMP actions during withdrawal include PKA-mediated enhanced GABAergic synaptic transmission in brain area such as periaqueductal grey (PAG). (Williams *et al.*, 2001; Bailey and Connor 2005). The brain regions contributing to the physical signs of opiate withdrawal in all the stages include periaqueductal gray (PAG) area, the locus coeruleus (LC), amygdala, ventral tegmental area (VTA), nucleus accumbens (NAcc) (McPhie and Barr, 2009). Among these brain regions, the peri aqueductal gray area (PAG) appears to be critical in regulating the complex signs and symptoms of opioid withdrawal (Handong Ouyang *et al.*, 2012).

5. Conclusion

The study observed a promising synergistic effect of piperine and pifrenidone on the modulation of TNF-alpha in opioid withdrawal syndrome. This finding suggests a potential therapeutic strategy for managing withdrawal symptoms effectively. Further research is warranted to explore the underlying mechanisms and to optimize the dosages of these drugs for better clinical outcomes.

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