Central dopaminergic system plays a role in the analgesic action of paracetamol: Preclinical evidence

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Abstract:

Objective: Even after 100 years of discovery, the exact mechanisms for the analgesic action of paracetamol are under scanner. It was recently proposed that paracetamol may act through different mechanisms, especially altering the serotoninergic system. The main objective of this preclinical study was to verify the role of drugs modulating dopaminergic system (l-dopa, bromocriptine, olanzapine) on the analgesic effect of paracetamol.

Materials and Methods: Thirty adult male albino mice were divided into five groups: distilled water (0.5 ml/25 g), paracetamol (200 mg/kg), levodopa (10 mg/kg) + paracetamol, bromocriptine (5 mg/kg) + paracetamol (200 mg/kg), and olanzapine (2 mg/kg) + paracetamol (200 mg/kg). All drugs were administered orally for 14 days. Eddy’s hot plate and tail immersion tests were used to determine analgesic activity. Tests were conducted 1 h after the drug administration on the 14th day. After that, animals were sacrificed and brains were dissected out, to measure the levels of dopamine. Statistical comparisons among the groups were performed by one-way analysis of variance followed by Tukey-Kramer test.

Results: Coadministration of l-dopa and bromocriptine with paracetamol increased the antinociceptive activity of paracetamol significantly, whereas coadministration of olanzapine with paracetamol decreased the analgesic activity of paracetamol in the Eddy’s hot plate and tail immersion tests considerably. There was a significant increase (P < 0.001) in the levels of dopamine in the brains of mice, which received levodopa, bromocriptine, and paracetamol. However, it was opposite in the brains of animals which received olanzapine.

Conclusion: The results suggest that analgesic action of paracetamol is influenced by dopaminergic system.

Key words: Analgesic, dopamine, mice, paracetamol

Pain is an unpleasant, but an alarming signal for an underlying problem associated with potential tissue damage. Both central and peripheral factors are implicated in the pain mechanisms. Discovery of analgesics, such as narcotics and nonnarcotics, which interact with the transmitters and modulators of the pain system have enabled us to make a huge leap in the field of pain management.[1,2]

Nonnarcotic (nonopioid) analgesics are the commonly used agents, both clinically and over the counter for mitigating pain. Unlike the potent narcotic analgesics, these nonopioid analgesics also have anti-inflammatory and antipyretic properties. Inhibition of prostaglandin synthesis by blocking cyclooxygenase (COX) enzyme is responsible for both the beneficial and undesirable effects of this class of analgesic drugs. They include both nonselective and selective COX-2 inhibitors and paracetamol.[3]

Paracetamol (acetaminophen), a commonly used analgesic and antipyretic, was discovered 100 years ago. Its discovery by Harmon Northrop Morse in 1878, through the chemical reaction of P-nitrophenol with tin in glacial acetic acid, is one of the most important discoveries in field of medicine. However, due to a misinterpretation of its side effect profile, paracetamol was not used for therapeutic purposes for many years. The scenario changed in the early 1950s. Now, its use is considered as the first step in the management of pain in many clinical conditions.[4-10]

Even after 100 years of discovery, it is an irony that the exact mechanism of action of paracetamol is not fully understood. Like any other nonopioid, it was thought that paracetamol primarily acts by inhibiting COX enzyme, especially in the brain. However, the absence of anti-inflammatory action made the researchers to think about its mechanism of action from

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a different perspective. Over the past two decades, there is enough evidence to say that this potent analgesic, antipyretic drug acts through different mechanisms such as effects on monoaminergic, cholinergic, opioid, nitric oxide (NO), and cannabinoi d pathways.\[3,5,6\]

Among the monoaminergic pathway, effect on serotonergic pathway is studied in detail. Many studies have shown that paracetamol increases the level of serotonin in the brain, which in turn add to its pharmacological actions.\[15\] However, the effect of paracetamol on the other monoaminergic pathways is not yet known.

In view of its increasing clinical significance, further research works are warranted to study its effect on other mediators involved in pain pathways.\[9\] With this in mind, the current research was undertaken to evaluate the analgesic role of paracetamol through dopaminergic system using rodent models of pain - Eddy’s hot plate test and tail immersion test.

Testing for analgesic activity of an agent using rodent models raises ethical, philosophical, and technical issues. Main hurdle is pain cannot be observed directly in animals but can only be measured by watching their reaction to a nociceptive stimulus. The animal models normally used for screening analgesic activity are pain state models based on the use of thermal stimuli, mechanical stimuli, electrical stimuli, and chemical stimuli. The neuronal base of these models is poorly understood. However, they are helpful in guessing the pain-relieving activity of a particular chemical. Eddy’s hot plate test and tail immersion test are commonly used rodent models for screening analgesic activity of an agent. These two animal models using thermal stimuli are considered to involve a supraspinally organized response. The involvement of prostaglandins as a mediator of pain is negligible in these models.\[6,11,12\] Hence, we chose these models to assess analgesic mechanism of paracetamol through dopaminergic system.

Using the above two rodent models, which are specific for centrally acting drugs, we conducted this study to investigate and compare the analgesic potential of paracetamol when used alone or in combination with drugs acting on dopaminergic system in mice.

### Materials and Methods

The Institutional Animal Ethical Committee clearance (YU-IAEC 2/9.6.2016) was obtained before the initiation of the research work.

#### Animals

Four-month-old healthy Swiss albino male mice with an average weight of 25 g were selected for the study. They were kept under standard housing conditions in the animal house (Reg No: 347/CPCSEA). The mice were divided into five groups of six animals each as follows:

- **Group I:** Distilled water (0.5 ml/25 g)
- **Group II:** Paracetamol (200 mg/kg)\[8\]
- **Group III:** Levodopa (10 mg/kg)+paracetamol (200 mg/kg)
- **Group IV:** Bromocriptine (5 mg/kg)+paracetamol (200 mg/kg)
- **Group V:** Olanzapine (2 mg/kg)+paracetamol (200 mg/kg)

All drugs were administered orally for 14 days. The drugs levodopa, bromocriptine, and olanzapine were selected because they are known to have modulatory role on dopaminergic system. First two are dopamine facilitator/agonist; the third one is a blocker of dopamine with low potency. On 14th day, after 1 h of drug administration of test compounds, the animals were taken for the following tests for screening their central analgesic role.

#### Eddy’s Hot Plate Test

The mouse was placed on the Eddy’s hot plate, with a temperature of 55°C. Time taken by the mouse to lick its paw or to jump was noted as reaction time. The cut off time was kept as 15 s to prevent injury to the paw.\[12\]

#### Tail Immersion Test

The mouse was held by the left hand gently and the tip of its tail (the last 1.5 cm, which was marked earlier) was dipped in hot water bath of temperature 48°C ± 0.5°C. The time taken for tail curling or flicking was recorded.\[12\]

#### Estimation of Dopamine in Mouse Brain

On 14th day, after the pharmacological experiments, animals were euthanized and brain dopamine levels were estimated using the method described by Bhat \textit{et al}.\[10\] The method is described briefly.

Dissected brain was weighed and homogenized in HCl–butanol for about 1 min (in 1:10 ratio). The homogenized sample was then centrifuged for 10 min at 3000 rpm. 1 ml aliquot of supernatant phase was removed and then added to centrifuge tube containing 2.5 ml hexane and 0.3 ml of 0.1 M HCl. The aqueous phase was then used for dopamine estimation. All steps were carried out at 0°C. To the 0.2 ml of aqueous phase, 0.05 ml of 0.4 M HCl, and 0.1 ml of sodium acetate buffer (pH 6.9) were added, followed by 0.1 ml iodine solution (0.1 M iodine in ethanol) for oxidation. This reaction was stopped after 2 min by adding 0.1 ml sodium sulfite solution. After 1.5 min, 0.1 ml 10 M acetic acid is added. The solution was then heated to 100°C for 6 min. When the sample again reached room temperature, excitation and emission spectra were read from the spectrophotometer at 330 to 375 nm. Tissue blanks for dopamine were prepared by adding the reagents of the oxidation step in reversed order (sodium sulfite before iodine).

#### Calculation

The neurotransmitter level is calculated using the following formula.

\[
X_{dopamine} = \frac{(\text{Sample O.D} - \text{Blank O.D}) - (\text{Standard O.D} - \text{Blank O.D}) \times \text{Concentration of Standard (500 μg/ml)}}{	ext{μmoles/g tissue}}
\]

Where, O.D is optical density. This gave the amount of dopamine present in 1 ml of the sample.

The final reading of neurotransmitter level is expressed as μmoles/g tissue.

#### Data Analysis

Results are depicted as mean ± standard deviation. One-way analysis of variance was carried out and the statistical
comparisons among the groups were performed with Tukey-Kramer test with the help of InStat–GraphPad software (Graph Pad software Inc., CA, USA). P < 0.05 was considered statistically significant.

**Results**

**Analgesic Activity of Paracetamol Was Increased in Both Thermal Stimuli-Evoked Pain State Models When Combined with Drugs Which Increase Dopaminergic Activity in Brain**

The Eddy’s hot plate test [Table 1] showed that the withdrawal time of the paw was significantly prolonged (P < 0.001) in the paracetamol, paracetamol + levodopa, and paracetamol + bromocriptine groups (Groups II–IV) on comparing with normal group (Group I).

When comparing the analgesic activity among the Groups II, III, and IV, it was seen that analgesic activity was more in paracetamol + levodopa (Group III), followed by paracetamol + bromocriptine (Group IV) than paracetamol alone (Group II) treated animals. Similar results were seen in tail immersion test, where latency of curling or flicking of the tail in contact with the hot water was significantly prolonged (P < 0.001) in Groups II, III, and IV [Table 2].

**Analgesic Activity of Paracetamol Was Decreased in Both Thermal Stimuli-Evoked Pain State Models When Combined with Drug Which Decreases Dopaminergic Activity in Brain**

The Eddy’s hot plate test [Table 1] showed that the withdrawal time of the paw was significantly decreased (P < 0.05) in the paracetamol + olanzapine group (Group V) on comparing with normal group (Group I). Similar results were seen in tail immersion test, where latency of curling or flicking of the tail in contact with the hot water was significantly decreased (P < 0.05) in Group V on comparing with normal group [Table 2].

**Dopamine Levels were Increased in the Brains of Mice, Which Received Paracetamol and Drugs Which Increase Dopaminergic Activity in Brain**

The levels of dopamine was significantly increased in the brains of animals which received paracetamol (P < 0.05), paracetamol + levodopa (P < 0.001), and paracetamol + bromocriptine groups (P < 0.001) (Groups II–IV) on comparing with normal group (Group I). When dopamine levels among the Groups II, III, and IV were compared, it was seen that dopamine levels were more in paracetamol + levodopa (Group III), followed by paracetamol + bromocriptine (Group IV) than paracetamol alone (Group II) treated animals [Table 3].

**Dopamine Levels were Decreased in the Brains of Mice, When Paracetamol was Combined with Drug Which Decreases Dopaminergic Activity in Brain**

The levels of dopamine were significantly decreased (P < 0.001) in the brains of animals which received paracetamol + olanzapine (Group V), on comparing with normal group (Group I) [Table 3].

**Discussion**

Paracetamol is a useful and effective analgesic in a wide range of clinical conditions. It is one of the commonly used drugs worldwide. One of the important highlighting features of this drug is its minimal toxic profile. Because of its efficacy and high therapeutic index, it is used in almost all age groups.\(^1,2,6\)

It is an astonishing fact, even after several years of its discovery and clinical utility, the exact mechanism responsible for the therapeutic beneficial of paracetamol is not completely known. Due to several research works carried out to reveal its unknown mechanisms of action, several new mechanisms have come to the limelight apart from its conventional prostaglandin

![Table 1: Effect of drugs on paw withdrawal time of mouse in eddy’s hot plate](image1)

| Group          | Paw withdrawal time (s) |
|----------------|-------------------------|
| Group I: Normal | 3.1±0.89                |
| Group II: PCT  | 7.83±1.32\(^6\)         |
| Group III: PCT+LDOPA | 10.5±1.04\(^6,7\) |
| Group IV: PCT+BRO | 9.84±0.75\(^6\)       |
| Group V: PCT+OLA | 1±0.63\(^6\)           |

\(n=6\); Results are expressed as mean±SD. One-way ANOVA followed by Tukey-Kramer multiple comparisons test. *P<0.001; considered highly significant on comparing Group II, III, IV with Group I, P<0.008; considered significant on comparing Group V with Group I, P<0.001; considered highly significant on comparing Group III with Group II, P<0.008; considered significant on comparing Group IV with Group II, *P<0.001; considered highly significant on comparing Group II with Group V, \(P<0.03\); considered not significant on comparing Group III with Group IV. PCT=Paracetamol, LDOPA=Levodopa, BRO=Bromocriptine, OLA=Olanzapine, SD=Standard deviation, ANOVA=Analysis of variance

![Table 2: Effect of drugs on tail curl/flick time of mouse in tail immersion test](image2)

| Group          | Tail curl/flick (s)    |
|----------------|------------------------|
| Group I: Normal | 2.16±0.75              |
| Group II: PCT  | 6.14±0.67\(^6\)        |
| Group III: PCT+LDOPA | 7.84±1.16\(^6,7\) |
| Group IV: PCT+BRO | 7.67±0.51\(^6\)       |
| Group V: PCT+OLA | 0.66±0.50\(^6\)        |

\(n=6\); Results are expressed as mean±SD. One-way ANOVA followed by Tukey-Kramer multiple comparisons test. *P<0.001; considered highly significant on comparing Group II, III, IV with Group I, P<0.0022; considered significant on comparing Group V with Group I, P<0.0111; considered significant on comparing Group III with Group II, P<0.0012; considered significant on comparing Group IV with Group II, *P<0.001; considered highly significant on comparing Group II with Group V, \(P<0.74\); considered not significant on comparing Group III with Group IV. PCT=Paracetamol, LDOPA=Levodopa, BRO=Bromocriptine, OLA=Olanzapine, SD=Standard deviation, ANOVA=Analysis of variance

![Table 3: Effect of drugs on mouse brain dopamine levels](image3)

| Group          | Dopamine levels (µmoles/g tissue) |
|----------------|-----------------------------------|
| Group I: Normal | 77.57±3.95                        |
| Group II: PCT  | 82.08±1.72\(^6\)                  |
| Group III: PCT+LDOPA | 159.03±2.23\(^6,8\) |
| Group IV: PCT+BRO | 124.51±1.93\(^6,8\)  |
| Group V: PCT+OLA | 47.08±2.32\(^6\)                  |

\(n=6\); Results are expressed as mean±SD. One-way ANOVA followed by Tukey-Kramer multiple comparisons test. *P<0.0282; considered significant on comparing Group II with Group I, P<0.001; considered highly significant on comparing Group III, IV with Group I, P<0.001; considered highly significant on comparing Group III, IV with Group II, P<0.001; considered highly significant on comparing Group III with Group II, P<0.001; considered highly significant on comparing Group III with Group IV. PCT=Paracetamol, LDOPA=Levodopa, BRO=Bromocriptine, OLA=Olanzapine, SD=Standard deviation, ANOVA=Analysis of variance, ANOVA=Analysis of variance
Apart from its effect on serotonergic system, cholinergic, opioid, NO, and cannabinoid pathways,[3,5,6] this preclinical study is the first of its kind, where a possible role of dopaminergic system involved in the analgesic action of paracetamol is investigated.

From our results, it is clear that modulation of dopaminergic system is also involved in the analgesic action of paracetamol. As seen in the Eddy’s hot plate test and tail immersion test, analgesic activity of paracetamol was increased when combined with drugs which facilitate or increase the dopaminergic activity in the brain. On the other hand, the antinociceptive activity of this unconventional nonopioid was diminished when combined with olanzapine, a dopamine blocker in the central nervous system. Biochemical results also add new dimensions into the above findings. Analgesic activity was more in paracetamol + levodopa group, in which the dopamine levels were higher than the other groups. Whereas analgesic activity was diminished in paracetamol + olanzapine group, in which the brain dopamine levels were lower than the other groups. It was surprising that the animals which received only paracetamol also have an elevated dopamine levels in their brains. This clearly shows that analgesic activity of paracetamol involves a modulation of dopaminergic system.

The elevation of brain dopamine levels by paracetamol can be answered by the following two mechanisms.

Once administered into the body, it is converted to an intermediary p-aminophenol in the liver. This intermediary undergoes conjugation in the brain with arachidonic acid in the presence of fatty acid amide hydroxylase to release N-arachidonoyl phenolamine (AM404), an active metabolite. This compound inhibits the reuptake of anandamide, an endocannabinoid.[3,5] Anandamide in turn can increase the levels of endogenous dopamine by regulating dopamine transporter function.[10]

Another mechanism by which paracetamol can increase dopamine levels is by inhibiting the inducible nitric oxide synthase expression, which in turn decrease the synthesis of a pronociceptive, (NO).[16] NO has also an effect on dopaminergic neurons. This regulatory molecule which has an important role in homeostasis process can induce oxidative stress in cerebral neurons if produced in excess leading to neurotoxicity. Studies have shown that it has role in the pathogenesis of Parkinsonism, a neurodegenerative disease associated with the damage of dopaminergic neurons.[17‑20] Apart from its direct effect on dopaminergic neurons, NO also can activate endocannabinoid transporter leading to a decrease in the level of anandamide, which in turn adds up to the decrease in brain dopamine levels.[3,16]

Our results are further underlined by the previous facts that analgesic action of paracetamol at submaximal doses was potentiated when combined with selective and nonselective NOS inhibitors. It was also seen that analgesic activity of paracetamol was diminished when combined with endocannabinoid antagonists.[3]

One of the common factors where endocannabinoid and NO system converge is their role in dopaminergic system.

Indeed, latest findings have proven that dopamine at spinal and supraspinal levels has a role in analgesia. Numerous clinical data indicate that dopamine diminution by 6-OHDA injection into the brain results in hyperalgesic responses, whereas the injection of the dopamine reuptake inhibitor GBR12935 into the brain increased pain thresholds. In another study, apomorphine, a mixed dopamine agonist has shown antinociceptive effects in rodents. This action was blocked by sulpiride, a central-acting D2-receptor antagonist. It was also reported that intrathecal administration of dopamine has shown antinociceptive effects in rat tail flick test.[1,21]

From the above facts and findings, it is summarized that paracetamol acting through endocannabinoid and NO system increases the levels of dopamine, which in turn add up to the analgesic action of this commonly used nonopioid [Figure 1].[3,5]

Conclusion
The results suggest that analgesic action of paracetamol is influenced by dopaminergic system. Further studies are ongoing to unearth the exact mechanism by which this commonly used analgesic increases dopamine levels in the brain.

Simultaneously, further research works are also required to see whether paracetamol has any role in Parkinsonism or other neurodegenerative diseases by increasing the levels of dopamine through modulating endocannabinoid and NO pathways.

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Conflicts of Interest
There are no conflicts of interest.

References
1. Bhat SS, Hegde KS, Chandrashekhar S, Rao SN, Manikoth S. Preclinical screening of *Phyllanthus amarus* ethanolic extract for its analgesic and antimicrobial activity. Pharmacognosy Res 2014;7:378-84.
2. Sundeep Hegde K, Sham Bhat S, Chandrashekar S, Manikoth S. Pre-clinical screening of indigenous medicinal plant *Phyllanthus amarus* for its analgesic activity. Int J Appl Biol Pharm 2014;5:215-8.
3. Hamza M, Dionne RA. Mechanisms of non-opioid analgesics beyond cyclooxygenase enzyme inhibition. Curr Mol Pharmacol 2009;2:1-14.
4. Ghaffar UB, Tadvi NA. Paracetamol toxicity; a review. J Contemp Med A Dent 2014;2:12-5.
5. Sharma CV, Mehta V. Paracetamol: Mechanisms and updates. Continuing Education in Anaesthesia, Critical Care and Pain 2014;14:153-58.
6. Karandikar YS, Belsare P, Panditrao A. Effect of drugs modulating serotonergic system on the analgesic action of paracetamol in mice. Indian J Pharmaco 2016;48:281-5.
7. Machado GC, Maher CG, Ferreira PH, Pinheiro MB, Lin CW, Day RO, *et al.* Efficacy and safety of paracetamol for spinal pain and osteoarthritis: Systematic review and meta-analysis of randomised placebo controlled trials. BMJ 2015;350:h1225.
8. Chiam E, Weinberg L, Bellomo R. Paracetamol: A review with specific focus on the haemodynamic effects of intravenous administration. Heart Lung Vessel 2015;7:121-32.
9. Borges RS, Barros TG, Pereira GA, Batista J Jr., Beleza Filho RF, Veiga AA, *et al.* A structure and antioxidant activity study of paracetamol and salicylic acid. Pharmacol Pharm 2014;5:1185-91.
10. Asha PK, Shasthri V. Review on oxidation of paracetamol. Int J Pharm 2016;7:1-5.
11. Milind P, Monu Y. Laboratory models for screening analgesics. IRJP 2014;4:15-9.
12. Al-Ali AM, Jawad AM. The effect of chlorpromazine, diclofenac and their combination on four models of induced pain in mice. J Bahrain Med Soc 2009;21:240-5.
13. Anandpara R, Sojitra B, Nakhya V, Ganatra T. Evaluation and comparison of anti-Parkinson activity of methanolic extract of *Phaseolus vulgaris* with L-dopa. IJPR 2015;5:103-8.
14. Onaolapo OJ, Onaolapo AY. Subchronic oral bromocriptine methanesulfonate enhances open field novelty-induced behavior and spatial memory in male Swiss Albino Mice. Neurosci J 2013;2013:948241.
15. Jayaraj M, Rao SN, Shyamjith M. Proconvulsant effect of Olanzapine, an atypical antipsychotic on maximal electroshock induced seizures in Wistar albino rats. Int J Appl Biol Pharm 2015;6:162-4.
16. Oz M, Jaligam V, Galadari S, Petroianu G, Shuba YM, Shippenberg TS. The endogenous cannabinoid, anandamide, inhibits dopamine transporter function by a receptor-independent mechanism. J Neurochem 2010;112:1454-64.
17. Mahindrakar YS, Thorat PA, Iyer CM. Oxidant and antioxidant status in Parkinson’s disease. Indian Med Gaz 2014;195-202.
18. Tieu K, Ischiropoulos H, Przedborski S. Nitric oxide and reactive oxygen species in Parkinson’s disease. IUBMB Life 2003;55:329-35.
19. Hunot S, Hirsch EC. Neuroinflammatory processes in Parkinson’s disease. Ann Neurol 2003;53 Suppl 3:S49-58.
20. Lorenc-Koci E, Czarnecka A. Role of nitric oxide in the regulation of motor function. An overview of behavioral, biochemical and histological studies in animal models. Pharmacol Rep 2013;65:1043-55.
21. Hache G, Coudore F, Gardier AM, Guiard BP. Monoaminergic antidepressants in the relief of pain: Potential therapeutic utility of triple reuptake inhibitors. Pharmaceuticals 2011;4:285-342.