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Original Research Article

Effect of Paracetamol on the Activity of Drugs Modulating the Serotonergic System

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ABSTRACT

Introduction: Paracetamol (PCM) is one of the most commonly used over-the-counter drugs. Its mechanism of action is postulated to involve the serotonergic system. If that be the case, it could have interactions with other drugs that act through this system. The aim of this study was to explore the possible effects of Paracetamol on the anti-anxiety effects of Buspirone and anti-depressant effects of Fluoxetine, which both act through the serotonergic system.
Methodology: Study was conducted using animal models of anxiety and depression. Elevated Plus Maze anxiety model was used, in which adult rats were assigned to five groups: Control, Diazepam, Buspirone, Buspirone+PCM, Paracetamol. Similarly in Forced Swim depression model, rats were divided into four groups: Control, Fluoxetine, Fluoxetine+PCM, Paracetamol. Statistical analysis was done using ANOVA followed by Tukey's test.
Results: It was seen that co-administration of Paracetamol with Buspirone decreased the percentage preference for open arm compared to Buspirone alone thereby increasing anxiety ($p<0.001$). In Forced swim model, Paracetamol with Fluoxetine decreased the immobility of animals which indicates enhanced anti-depressant activity of Fluoxetine ($p<0.001$).
Discussion: Paracetamol may have increased Serotonin turnover which stimulated the 5-HT_{1A} autoreceptors which antagonizes anti-anxiety activity of Buspirone. Similarly, increase in the extracellular Serotonin concentrations in the vicinity of the cell body and the dendrites of Serotonin neurons due to Paracetamol may have enhanced action of Fluoxetine.
Conclusion: The result of this experiment suggests that Paracetamol interferes with the activity of drugs modulating the Serotonergic System.

Key words: Buspirone, Fluoxetine, Rodents, Elevated plus Maze, Forced Swim Test.

INTRODUCTION

Paracetamol (PCM), a non-steroidal anti-inflammatory agent, is one of the most commonly used over the counter drugs. It is put to use as an anti-pyretic, analgesic, even anti-inflammatory, without even fully understanding its underlying mechanism of action. [1] Many hypotheses have been put forth to explain the central as well as peripheral mechanisms of Paracetamol - inhibition of Cyclo-Oxygenase enzyme, inhibition of Prostaglandin H2 synthase, activity via Cannabinoid receptor, Nitric oxide synthase inhibition, effects on the endogenous opioid system, etc. [2] But, no hypothesis has been explored as extensively as the mechanism of Paracetamol on Serotonergic system, which suggests that it may have a modulatory effect on the serotonergic system in the brain and spinal

cord. [2] This implication becomes most relevant clinically when other drugs that act via the serotonergic pathway may be used in conjunction with Paracetamol.

Serotonin is a versatile neurotransmitter which is involved in a myriad of functions in the body - learning and memory, mood regulation, pain processing and modulation, cardiovascular functioning, gastrointestinal motility and many others. [3] This diversity of actions of this one neurotransmitter is reflected in the diversity of classes of drugs which modulate it - for example, antidepressants like Selective Serotonin Reuptake Inhibitors (SSRIs) like Fluoxetine, Citalopram; anti-emetics like Serotonin ($5\text{-}HT_3$) antagonists like Granisetron, Ondansetron; anti-anxiety drugs like Buspirone ($5HT_{1A}$ partial agonist), Sumatriptan; anti-migraine drugs like Ergotamine, Methysergide; appetite suppressants like Chlorphentermine; to name a few.

Accordingly, the side effects of such drugs, or their combinations thereof, could be a matter of concern in the clinical scenario. SSRIs alone can cause osteoporosis in the elderly, sleep disturbances, suicidal thoughts and the fatal Serotonin syndrome. [4] If these drugs are combined with other agents that potentiate Serotonin synthesis or secretion, the adverse effects could be multiplied. On the other hand, if they are combined with agents that inhibit the serotonergic pathway in any way, the therapeutic benefits may be hampered.

If Paracetamol, in fact, does have modulatory activity on the serotonergic system, it may be prudent to deduce the possible interactions it may have, with other drugs that act via the serotonergic system. The results could be translated to benefit patients and doctors in the clinical setting.

Also, being an over-the-counter and easily available drug, Paracetamol may be consumed, unknowingly, by the patients who are also on treatment with other serotonergic drugs and may face undesirable effects of the subsequent drug interactions. If the interactions between these drugs are known and established, the patients can be educated beforehand and the adverse effects can be prevented.

With this in mind, the current study was undertaken. The aim was to study the effect of Paracetamol on the activities on two drugs - Fluoxetine and Buspirone - in animal models of depression and anxiety respectively.

MATERIALS AND METHODS

Study was initiated after Institutional Animal Ethics Committee approval. (BVDUMC/1797/2014-15)

Animals: 30 adult Wistar rats weighing 250 - 300 g of either sex were used. Housing was done in standard cages (3 animals per cage) with food (standard chow) and water *ad libitum*, maintaining a 12-hr light-dark cycle. Animal coding was done according to standard protocol and animals were randomly allocated to different experimental groups. All tests were performed between 09:00 a.m. - 04:00 p.m. to minimize the confounding effects of circadian rhythms.

Drugs used for experiments: Drugs used for the study were -

Paracetamol - 200 mg/kg orally
Buspirone - 10 mg/kg orally
Fluoxetine - 10 mg/kg orally
Diazepam - 1 mg/kg orally

Animals were pretreated with Fluoxetine for 7days before day of the experiment. On the day of the experiment, Buspirone or Diazepam or Fluoxetine were administered 30 mins. Prior to Paracetamol depending on the group. 60mins. after administration of Paracetamol, animals were subjected to the experiments.

Procedures:

Elevated Plus Maze: [5]

30 adult albino mice were randomly allocated into 5 groups of 6 animals each:

Group 1: Control (No treatment)
Group 2: Treatment with Diazepam
Group 3: Treatment with Buspirone
Group 4: Treatment with Buspirone + Paracetamol
Group 5: Treatment with Paracetamol

The drugs were administered as described above to the respective groups. The plus-maze consists of four arms with 50 × 10 × 40 cm dimensions out of which two arms are open and two are closed. Both open arms face each other and are perpendicular to the closed arms, which also face each other. For closed arms, all walls are closed with an open roof, whereas open arms have only the base without any walls. The maze is elevated to a height of 50 cm. The rat was placed in the centre of the maze, facing the open arm opposite to the experimenter.

The procedure was conducted in a dark room with a 15W bulb over the central area as the source of illumination. The observations were made from an adjacent room. An entry was recorded when all four limbs of the animal entered the arm. The apparatus was wiped with a cloth and then cleaned with ethanol soaked cotton after each animal.

Observations - During the 5 min test period the total number of entries and the time spent in the open and closed arms were recorded. Percentage time spent in open arm was calculated using the formula:

$$\% \text{ time spent} = \frac{\text{Time spent in open arm}}{\text{Total time spent}} \times 100$$

Modified Forced Swim Test: [5-7]

20 adult albino mice were randomly allocated into 4 groups of 6 animals each:

Group 1: Control (No treatment)

Group 2: Treatment with Fluoxetine

Group 3: Treatment with Fluoxetine + Paracetamol

Group 4: Treatment with Paracetamol

For the study, modified Forced Swim Test using Water wheel was used as described by Nomura et al. (1982), [5,6] as a modification to the original test described by Porsolt et al. (1977). [5,7] The water wheel apparatus consists of a transparent rotating Plexiglass wheel (30cm in height, 15 cm in diameter) with ribs submerged in a clear Plexiglass tank of water (column of water = 15 cm). The axis of the wheel has a digital counter to record the number of rotations completed by the wheel. The water is kept lukewarm at 25° C.

One day prior to the test, a pretest session was conducted. The rats were placed individually in the water filled apparatus and allowed to swim for 15 mins. Animals placed in the apparatus for the first time were initially highly active, trying to climb the ribs of the wheel in an attempt to find a route of escape and resulting in more number of rotations of the wheel. After 2-3 min activity began to subside and was interspersed with phases of immobility or floating of increasing length. After 5-6 mins, immobility reached a plateau where the animals remain immobile for approximately 80% of the time, where the wheel hardly moved. An animal was considered to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose just above the surface. After 15 mins in the water the animals were removed and dried off before being returned to their home cages.

On the day of the test, the drugs were administered as described above. The animals were forced to individually swim in the apparatus for a period of 5 minutes each time and the number of rotations was recorded.

Statistical analysis

All values in the study were expressed as Mean ± Standard Error of Mean (M ± SEM). One way Analysis of Variance (ANOVA) followed by post hoc Tukey's test was used for statistical analysis using Graph Pad Prism version 5. Differences were considered significant when p value was <0.05.

RESULTS

I] Elevated Plus Maze:

Table I shows the time spent (in seconds) in the open and closed arms out of a total time of 5 minutes (300 sec). All results displayed are compared to the results from the Control group and Busp+PCM group. Diazepam showed the most

significant activity as compared to Control ($p<0.001$), followed by Buspirone ($p<0.001$). The combination of Buspirone and Paracetamol spent slightly more time in open arm as compared to the Control, however this difference was not significant ($p=0.1012$). Paracetamol alone group showed results lower than Control, though there was no significant difference ($p=0.412$). The combination of Buspirone and Paracetamol showed significantly less activity as compared to Diazepam and Buspirone alone ($p<0.001$), but significantly more activity than Paracetamol alone ($p<0.05$).

Table I: Time spent (in sec.) in Open and Closed arms of Elevated Plus Maze by rats:

Sr. no.	Groups	Open Arm	Closed Arm
1	Control	71 ± 9.7	229 ± 9.7
2	Diazepam	138.3 ± 8.7* §	161.7 ± 8.7* §
3	Buspirone	125.4 ± 2.6* §	174.6 ± 2.6* §
4	Busp+PCM	90.5 ± 4.6	209.5 ± 4.6
5	Paracetamol	62.2 ± 3.3 §	237.8 ± 3.3 §

Data represented as Mean ± Standard Error of Mean (SEM). One way ANOVA followed by post hoc Tukey's test was done to compare differences between Control, Standard and test groups. Total time = 300 seconds
p value <0.05 considered statistically significant
*: comparison with Control was significant ($p<0.05$)
§: comparison with Buspirone+PCM was significant ($p<0.05$)

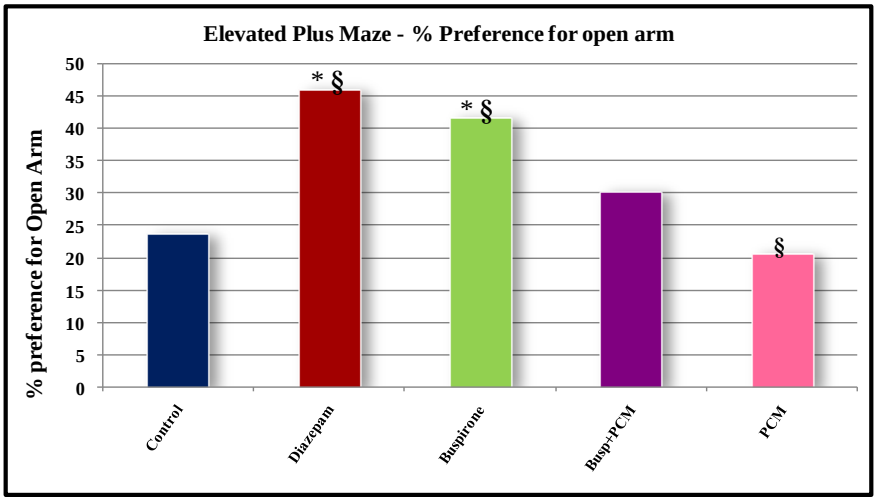

Fig. 1: Percentage Preference for Open Arm of Elevated Plus Maze by rats:

Data represented as Mean ± Standard Error of Mean (SEM). One way ANOVA followed by post hoc Tukey's test was done to compare differences between Control, Standard and test groups. p value <0.05 considered statistically significant, *: comparison with Control was significant ($p<0.05$), §: comparison with Buspirone+PCM was significant ($p<0.05$)

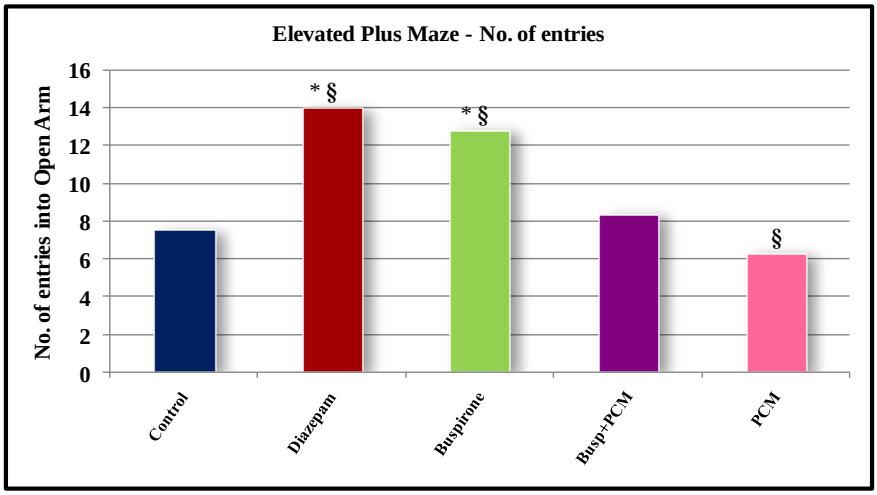

Fig. 2: Number of entries into the open arm:

Data represented as Mean ± Standard Error of Mean (SEM). One way ANOVA followed by post hoc Tukey's test was done to compare differences between Control, Standard and test groups. p value <0.05 considered statistically significant, *: comparison with Control was significant ($p<0.05$), §: comparison with Buspirone+PCM was significant ($p<0.05$)

The percentage preference for open arm basically gives a measure of time spent by the animal in the open arm. All results were compared to the results from the Control group. As is evident from Fig. 1, Diazepam showed the most significant activity as compared to Control ($p<0.001$), followed by Buspirone ($p<0.001$). The combination of Buspirone and Paracetamol showed slightly higher preference as compared to the Control, however this difference was not significant ($p=0.1012$). Paracetamol alone group showed least preference for the open arm, and its results were lower than Control, though there was no significant difference ($p=0.412$). The combination Buspirone+PCM showed significantly less activity as compared to Diazepam ($p<0.0001$) and Buspirone ($p<0.001$), whereas it showed significantly more activity as compared to Paracetamol ($p<0.05$).

Number of entries into the arms gives an assessment of the exploratory behavior of the animals. Fig. 2 illustrates the results of the number of entries of the animals into the open arm of the EPM. All results were compared to the results from the Control group. Diazepam showed the most significant activity as compared to Control ($p<0.05$), followed by Buspirone ($p<0.05$). The combination of Buspirone and Paracetamol showed slightly more number of entries as compared to the Control, however this difference was not significant ($p=0.6459$). Paracetamol alone group showed least number of entries into the open arm, and its results were lower than Control, though there was no significant difference ($p=0.459$). The combination Buspirone+PCM showed significantly less number of entries as compared to Diazepam and Buspirone ($p<0.05$ for both), but significantly more entries as compared to Paracetamol ($p<0.05$).

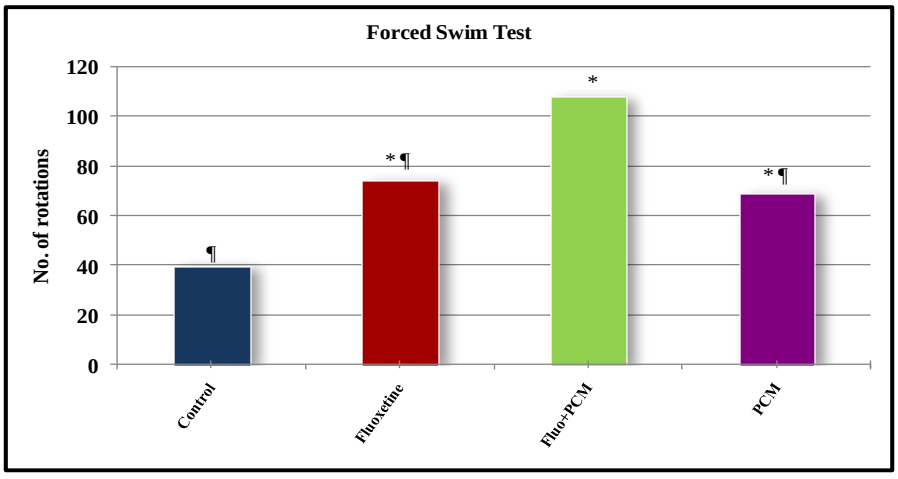

Fig. 3: Number of rotations of water wheel in Forced Swim Test:

Data represented as Mean ± Standard Error of Mean (SEM). One way ANOVA followed by post hoc Tukey's test was done to compare differences between Control, Standard and test groups. p value <0.05 considered statistically significant, *: comparison with Control was significant ($p<0.05$), ¶: comparison with Fluoxetine+PCM was significant ($p<0.05$)

The parameter assessed in this modification of the Forced Swim test was number of rotations of the water wheel. More the number of rotations, more the antidepressant activity. All results were compared to the results from the Control group. As is seen in Fig. 3, both Fluoxetine and Paracetamol showed significant activity as compared to Control ($p<0.001$ for both). However, the most significant activity was seen with the combination of Fluoxetine and Paracetamol ($p<0.001$). In fact, the combination showed significantly more activity than Fluoxetine or Paracetamol alone ($p<0.001$ for both).

DISCUSSION

Paracetamol or acetaminophen (N-acetyl-p-aminophenol), was synthesized in 1877 at Johns Hopkins University by Harmon Northrop Morse and was introduced into clinical practice as an analgesic as early as 1893. [8] It has enjoyed the repute of being one of the safest and most commonly used analgesics, antipyretics and even anti-inflammatory agents available over-the-counter since then. [1]

However, the mechanisms underlying these actions are still largely a mystery. Paracetamol, classified as a Non-Steroidal Anti-Inflammatory agent, for all practical purposes, is a weak inhibitor of the COX enzyme, its effect very closely affected by presence of peroxide in the surrounding tissue. [2,9] This peroxide-dependent COX inhibition justifies better activity of Paracetamol in the brain, where peroxide concentrations are low, as opposed to peripheral sites of inflammation with high peroxide levels. [9] This observation led to the postulation of a central mechanism of action of Paracetamol. [2]

Among the numerous theories put forth regarding the central actions of Paracetamol, the most popular theory is the modulation of serotonergic system, especially the Descending Serotonergic Pathway. [2] This pathway, which originates from the Nucleus Raphe Magnus (NRM) passing through the Peri-Aqueductal Grey area (PAG), is an inhibitory pain pathway and Serotonin is its main neurotransmitter. [2,8] Studies by Sandrini et al (2003) and Tiippana et al (2013) have shown that the analgesic activity of Paracetamol is significantly reduced when lesions are produced in the Serotonergic pathway or by inhibiting synthesis of Serotonin in animal models. [11-13] Conversely, Dogrul et al (2012) showed that Paracetamol treatment increased the central levels of Serotonin and reduced the density of cortical Serotonin receptors. [10]

Studies by Pe´lissier et al. (1996) and Raffa and Codd (1996) have shown that Paracetamol may not have affinity for 5-HT receptors or neuronal 5-HT reuptake sites, but there is evidence to suggest an indirect mechanism. [13,14] Chen and Bazan (2003) demonstrated an inhibition of hippocampal synaptic plasticity via a 5-HT2 receptor by Paracetamol, suggesting the involvement of a release of endogenous 5-HT in this effect. [15] A similar effect was seen in studies by Tjolsen et al. (1991), [16] Pini et al. (1996) [17] and Courade et al. (2001) [18] who proposed a mobilization of endogenous Serotonin by Paracetamol and showed that 5-HT receptor antagonists similarly influence the antinociceptive effect of 5-HT and that of Paracetamol (Courade et al., 2001). [18]

Dogrul et al. (2012) demonstrated that Paracetamol administration (100- 400 mg⁄ kg p.o. and 200-400 mg⁄ kg i.p.) increased 5-HT levels in various regions of (rat) brain (cortical, pontine, hypothalamus, striatum, hippocampus, brain stem) and caused subsequent downregulation of 5-HT_{2A} receptors. [10] When the spinal 5-HT pathway was lesioned in rats, using 5, 6-Dihydroxytryptamine (5, 6- DHT) injected intrathecally, Paracetamol induced antinociception in the formalin test was reduced, whereas lesioning the noradrenergic pathway, using 6-Hydroxydopamine (6-OHDA) had no effect on Paracetamol antinociception. Likewise, depletion of 5-HT in cortical and pontine regions (12% and 19% of baseline) using p-Chlorophenylalanine significantly decreased Paracetamol induced antinociception in rats. [10]

Libert et al demonstrated negative effect of intrathecal Tropisetron on analgesic activity of Paracetamol. They concluded that this result should be taken into consideration, especially when administering 5-HT_3 antagonists as antiemetics together with Paracetamol for analgesia in the post-operative period. [19] Pickering et al conducted a similar study in human volunteers with similar results. [20] In another study, Pickering et al. deduced that

paracetamol reinforces descending inhibitory pain pathways. [21]

As mentioned above, Serotonin plays an integral role in the central processing of pain. In addition to this, it is important in learning and memory, mood regulation, cardiovascular functioning, gastrointestinal motility, bone metabolism and many others. [3] Drugs acting via modulation of Serotonin, while exerting their intended effects, may also cause many side effects due to the widespread area of action of Serotonin. Furthermore, if a combination of such drugs is used, it may lead to some undesirable drug interactions. For example, Mir et al. (2012), found that co-administration of SSRIs and 5-HT_3 antagonists led to increased emesis as a results of inhibition of the 5-HT_3 antagonists by SSRIs. [22] On the other hand, a combination of SSRIs like Fluoxetine or Sertraline with an agent like Buspirone can lead to development of the fatal Serotonin Syndrome. [23]

It is because of phenomena like these, that one needs to be aware of the possible drug interactions that may result from a combination of two drugs that act via the serotonergic system. This was the basis for undertaking the current study. In doing so, we strived to uncover any possibility of drug interactions between Paracetamol and two drugs acting via the serotonergic system using animal models of depression and anxiety. The drugs selected were the SSRI Fluoxetine and the 5-HT_{1A} partial agonist Buspirone. Fluoxetine is one of the most commonly used drugs in the first line treatment of Major Depressive Disorder, whereas Buspirone is used as an anti-anxiety drug. [24,25] The animal models used were the Elevated Plus Maze method for anxiety and the modified Forced Swim Test for depression.

The Elevated Plus Maze (EPM) is the most commonly employed animal model of anxiety. The apparatus is raised above floor level and is composed of two closed arms perpendicular to two open arms. [5] The test is based on the conflict between the natural tendency of rodents to explore novel environments and their innate avoidance of unprotected, bright and elevated places (represented by the open arms). When an animal is placed on the maze, initially it explores the open arm, but eventually goes to the closed arm. [26] The two parameters assessed are the number of entries in the arms, which analyses the exploratory behavior of the animal, and the percentage preference to the open arms, which tests the anxiety of animal. Administration of classical anti-anxiety drugs, such as benzodiazepines, increases exploration of the arms as well as the time spent in the open arms.

Buspirone is a 5-HT_{1A} receptor partial agonist used as an anti-anxiety drug. It acts on both pre- and post-synaptic receptors in various areas of the brain. The anti-anxiety action is attributed to the post-synaptic receptor whereas the presynaptic receptor is an auto receptor and by stimulating these, there is a decrease in the secretion of Serotonin. [24,27] Hence, when it is co-administered with Paracetamol, which exerts its effects via serotonergic system, probably by enhancing release of Serotonin, this effect of Buspirone may result in decreased total secretion of Serotonin. This may, in turn, also attenuate the anti-anxiety activity of Buspirone itself. This is reflected in our study.

As is evident in Fig. 1 and 2, Buspirone alone exerts significant anti-anxiety activity as compared to the Control ($p<0.001$), which is comparable to the activity of the standard drug, Diazepam ($p>0.05$). Paracetamol alone had significantly less activity as compared to Diazepam and Buspirone alone ($p<0.001$), and was less than Control though not significantly ($p=0.4$). When the combination Busp+PCM was given, the anti-anxiety activity was significantly decreased as compared to Diazepam and Buspirone alone ($p<0.001$), and was comparable to Control ($p=0.1$). However, the combination Busp+PCM still had significantly more activity than PCM alone ($p<0.05$),

suggesting that Buspirone may be acting on some post-synaptic receptors to exert its effect.

To the best of our knowledge and after an elaborate literature search, we found that there are no studies that have results similar to the current study on effect of Paracetamol on anti-anxiety activity of Buspirone. Anderson et al. (1996) found that chronic treatment with SSRI Fluvoxamine attenuated response to Buspirone in normal male volunteers. [28] Many studies in humans and animals, however, show that Buspirone attenuates activities of drugs like SSRIs, Opioids and NSAIDs when given in combination. [29] Roca-Vinardell et al (2003), showed that subcutaneous administration Buspirone antagonized the analgesic effect of Paracetamol and conversely antinociceptive effect of Paracetamol is increased both by the selective blockade of 5-HT_{1A} receptors with WAY 100635 and by the selective blockade of 5-HT_{1B} receptors with SB 216641, both administered systemically. [30]

A study done by Kiev et al (1989) where 150 patients on Buspirone were given NSAIDs showed that the combination proved to be synergistic. [31] This may be because the authors used NSAIDs other than Paracetamol, which may be acting via different mechanisms altogether. Camborde et al. (2003) showed additive analgesic effects of the combination of Buspirone and Paracetamol. [30] Giordano et al. (1992) and Alhaider et al. (1993) have shown that Buspirone itself has good analgesic activity. [33,34] However, the studies have implicated the analgesic activity of Buspirone to have additional mechanisms other than on 5-HT receptors. [33-35] These studies did not consider the anti-anxiety activity of Buspirone.

For evaluation of effect of Paracetamol on the antidepressant activity of Fluoxetine, modified Forced Swim Test using Water wheel was done. The Forced Swim Test and all its modifications are the most commonly used tests for evaluating the antidepressant potential of drugs. The method employed in the current study is a modification of the original Porsolt test [5,7] described by Nomura et al. (1982). [5,6] The principle of the Forced Swim Test is to assess response to an acute inescapable stressor, provoking despair-based behavior or a stress coping behavior in the form of immobility in the animal, which is most commonly a rodent. When the animal is placed in a column of water from which there is no escape, it will initially try to escape, but will eventually give up and remain immobile, only floating enough to keep its snout above water in an attempt to conserve energy. With repeated exposures, the animal will give up faster and will show classical signs of helplessness. [5,36] Antidepressants prevent this development of helplessness even with a single dose and the animal keeps trying to escape. In the Water wheel modification, the number of rotations gives a measure of the antidepressant activity. More the number of rotations on the wheel, more is the antidepressant activity.

Fluoxetine is an SSRI which decreases the reuptake of Serotonin from the synaptic junction by the presynaptic receptors, allowing more Serotonin to be available for use. A combination of Paracetamol (which increases the secretion of Serotonin) and Fluoxetine (which decreases reuptake of Serotonin) should be at least additive, if not synergistic. The results in this study support this hypothesis.

As is seen in Fig. 3, Fluoxetine and Paracetamol showed significant antidepressant as compared to Control group ($p<0.001$), however the difference between the two was not significant ($p>0.05$). The combination Fluo+PCM showed statistically more significant activity as compared to Fluoxetine alone and Paracetamol alone.

Similar results were seen in the study by Manna et al. (2015) which showed that Paracetamol potentiates the antidepressant and anticonvulsant effects of Fluoxetine in mice. [37] In a study by Mackay et al. (1999), it was seen that in

patients of suspected Serotonin syndrome due to SSRI use, Paracetamol was a commonly used concomitant drug. [38] It would be interesting to assess the antidepressant activity of Paracetamol alone in models of acute and chronic depression.

Warner-Schmidt et al. (2011) studied the effects of concomitant use of NSAIDs Aspirin and Ibuprofen and SSRIs Citalopram and Fluoxetine in healthy human volunteers. They found that NSAIDs significantly decreased the effects of the antidepressants. [39] However, it is uncertain, whether these results can be extrapolated to our study. Aspirin and Ibuprofen, which are potent inhibitors of COX enzyme, may have different central mechanisms as compared to Paracetamol.

While it may seem to be a beneficial combination and may reduce the requirement of dosage of both drugs, the combination of Fluoxetine with Paracetamol may have adverse effects of its own, which need to be elicited and established. This may be a matter of concern for the clinician, in patients who are already on antidepressant treatment and consume Paracetamol. Also, such patients would have to be educated about the inadvertent use of these drugs in combination, as Paracetamol is an over-the-counter drug and easily available to the masses. Theoretically, similar synergism should exist between Paracetamol and other SSRIs, however, it is difficult to comment on the same without adequate evidence.

Another implication of these findings can be that, with increased age or liver disease, the metabolism of Paracetamol gets decreased, which may lead to increased serum levels of the drug. In such cases, concomitant administration of drugs like SSRIs could be dangerous. Numerous studies have shown that Serotonin syndrome and other side effects of SSRIs are mainly seen in the elderly. [23] Therefore, caution must be exercised while using these agents in such cases.

Results from this study and evidence collected from previous studies confirm that Paracetamol modulates the serotonergic system. This effect may be direct or indirect, the exact mechanism and sites of action are yet to be conclusively elucidated. Also, this effect may just be one of the mechanisms by which this drug exerts its actions. However, the fact remains that Paracetamol can interfere with the actions of other drugs which act via the Serotonergic system. The interactions may be antagonistic or additive or even synergistic. According to the results in this study, increase in the extracellular Serotonin concentrations in the vicinity of the cell body and the dendrites of Serotonin neurons due to Paracetamol may have potentiated the antidepressant activity of Fluoxetine. This may be a matter of concern with respect to development of side effects, especially the fatal Serotonin syndrome. Therefore, when the two are used together, it may be prudent to temper the doses of one or both of the drugs. Conversely, it may increase Serotonin turnover which stimulates the $5\text{-}HT_{1A}$ autoreceptors which antagonizes anti-anxiety activity of Buspirone. Therefore, when used with drugs like Buspirone, clinicians may have to tailor the dosage of Buspirone or may consider an alternative agent altogether.

Limitations

The use of Buspirone may be debated. Although used in many parts of the world, Buspirone is not commonly used in the treatment of anxiety in India. Also, it is not the first line treatment for Generalized Anxiety. For this purpose, we included the standard drug Diazepam for comparison in our study.

Another limitation of the current study was not using other SSRIs for comparison along with Fluoxetine. The inclusion would have given a clearer picture of the drug interactions that may result from the combination of Paracetamol with SSRIs. The adverse effects of the combination of Paracetamol and Fluoxetine in chronic doses were not assessed. Although, there was no mortality during the study, it may have been educational to see what adverse

effects may result from a continued use of the drugs.

Further studies have been planned to study the effects of Paracetamol on chronic Fluoxetine dosage in longer antidepressant models like the Unpredictable Chronic Mild Stress model. Also, it would have been prudent to actually measure the levels of Serotonin in the CNS and at the spinal level after the administration of Paracetamol with and without the other drugs. This, too, has been proposed as a further study.

CONCLUSION

Paracetamol may have a multifaceted mechanism of action, however, it certainly acts through the Serotonergic system, most probably by enhancing release of Serotonin, directly or indirectly. As a result, it may stimulate the 5-HT_{1A} autoreceptors. Given in conjunction with the SSRI Fluoxetine, it potentiates the SSRI, but with Buspirone, it attenuates the anti-anxiety action of Buspirone. Paracetamol interacts with drugs acting via the serotonergic system, and should be used with caution in patients who may already be on medication with such drugs.

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Conflict of interest: The authors declare no conflict of interest.

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