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Novel Synergistic Antinociceptive Mechanisms and Anxiolytic Effects of Orphenadrine and Diclofenac Combination in Mice: *In silico*, Molecular and Histological Insights.

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ABSTRACT

Background: Orphenadrine is a centrally acting muscle relaxant, while diclofenac is a non-steroidal anti-inflammatory drug. This study evaluated the putative synergistic antinociceptive and anxiolytic effects of their combination and explored underlying mechanisms.

***Methods*:** Mice were allotted into four groups (n=6): control (distilled water, 10 mL/kg, po), diclofenac (50 mg/kg ip), orphenadrine (20 mg/kg, po), and the combination. Acetic acid-induced writhing and hole-board assays were conducted. Brain tissues were isolated and analyzed using biochemical, histological, *in silico* and semi-quantitative RT-PCR assays.

***Results*:** Orphenadrine and diclofenac reduced writhing, while the combination exerted a ~90 inhibition ($p<0.01$). The combination significantly increased head-dipping behavior ($p<0.05$). Tumour necrosis factor (TNF)-α expression was reduced compared to control ($p<0.05$) but higher than individual treatments. Cyclooxygenase (COX) expression was significantly reduced. Histology revealed renal and hepatic distortions. *In Silico* studies showed moderate binding of orphenadrine to $5HT_2$, and COX.

***Conclusions*:** The combination exerts synergistic antinociceptive and anti-anxiety effects, possibly via serotoninergic activation and COX inhibition, but may induce tissue toxicity.

***Keywords**:* Orphenadrine, diclofenac, synergistic, antinociceptive, anti-anxiety, tumour necrosis factor.

1. INTRODUCTION

Orphenadrine is a muscle relaxant with parasympatholytic, antihistamine, and antinociceptive activities [1]. It is usually used either alone or in combination with acetaminophen for the management of pain. The antinociceptive actions of orphenadrine have been reported to largely involve supraspinal mechanisms [2]. One of the reported mechanisms is through an interaction with serotonergic transmission. It has been documented that orphenadrine increases the concentration of serotonin in the rat brain; however, the exact interactions involved are yet to be fully elucidated [3]. Orphenadrine has been shown to exert neuroprotective effects and is used as an adjunct therapy in Parkinson's disease. Its anticholinergic and antagonistic actions at N-methyl-D-aspartate (NMDA) glutamate receptors are reported to underline the neuroprotective effects [4]. Although orphenadrine exerts an antinociceptive effect as a single agent, its synergistic action when combined with acetaminophen has been exploited therapeutically [5]. The cyclooxygenase (COX)-3 inhibiting action of acetaminophen, together with the supraspinal actions of orphenadrine, resulted in enhanced antinociceptive effect. Non-steroidal anti-inflammatory

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(NSAIDS) drugs are among the most prescribed and potent antinociceptive agents [6]. While NSAIDS are very effective, their inhibitory effect on prostaglandin synthesis is associated with deleterious adverse effects that could be devastating and has limited their clinical usefulness. Some of the deleterious effects include renal injury and gastrointestinal ulceration [7, 8]. This study evaluated the potential synergistic effects of a combination of NSAIDS and orphenadrine. We suggest that this combination could provide improved analgesia while reducing the potential deleterious effects associated with NSAID therapy.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological materials

Albino Swiss mice (18-23 g)

2.1.2 Chemicals and reagents

Orphenadrine (Norflex® tablets), acetic acid (BDH, England), Diclofenac injection, Trizol solution, formosaline, antibodies of cyclooxygenase (COX) and tumour necrosis factor (TNF)-α.

2.2.3. Equipment and other materials

Hole board apparatus, Image J software, primers for TNF and COX, spectrophotomer, cDNA synthesis kit 127 (ProtoScript II®, New England BioLabs), and glidev7.5 programme of Maestro-v12.5 (for docking studies)

2.2 Methods

2.2.1 Animal handling

Albino Swiss mice (18-23 g) were inbred and obtained from the animal facility of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, under controlled conditions [9]. The mice were fed standard pelletized feed and had free access to clean drinking water. The animals were handled in accordance with the procedures approved by the Faculty of Pharmacy Ethics committee (EC/FP/025/015).

2.2.2. Antinociceptive study

2.2.2.1 Acetic acid-induced mouse writhing assay.

Mice were randomly allotted into four groups, each containing six animals, as follows:

I. Control (distilled water; 10 ml/kg. po)
II. Diclofenac (50 mg/kg, ip)
III. Orphenadrine (20 mg/kg, po)
IV. Orphenadrine (20 mg/kg, po) + Diclofenac (50 mg/kg, ip)

The mice were treated and after 1 hour (30 mins for diclofenac), they were injected with 0.6 % acetic acid (10 mL/kg, ip). The number of abdominal constrictions (writhes) were recorded for thirty (30) minutes in a quiet chamber [10]. Twenty-four hours after the acetic acid-induced mouse writhing assay, the mice were subjected to the hole board assay.

2.2.3. Anti-anxiety study

2.2.3.1. Hole board assay

The hole board apparatus consisted of a wooden board (40 × 40 × 25 cm) with 16 holes (each 3.5 cm in diameter), and elevated to a height of 25 cm. The groups were as previously described in the writhing assay. Each treated mouse was placed on the board and the number of head dips (ears passing below the surface of the board) during a 5-minute observation period in a quiet room was recorded. The board was wiped with 70% alcohol in between observations to remove olfactory cues [11]. Immediately after the hole board assay, mice were euthanized using ketamine (100 mg/kg. ip) anesthesia followed by cervical dislocation. The head was carefully severed, and a pair of sterile scissors was used open the cranium to excise the brain. The brain was immediately transferred into Eppendorf tubes containing Trizol® for molecular analyses. The kidneys and livers were also excised and kept in 10% formosaline for histological screening.

2.2.4 Protein concentration assay

The brain was homogenized, and the protein content was determined using the previously described method [12].

2.2.5 In Silico Study

2.2.5.1 Ligand Preparation

The structure of orphenadrine and diclofenac was obtained from Pub Chem (https://pubchem.ncbi.nlm.nih.gov/) and modified for the docking study. The Ligprep® panel was used to prepare each compound using an OPLS3 force field (to minimize structures, generate putative states at pH 7.0 ± 2.0 with Epik®. Other modifications including desalting (remove molecules with large number of atoms), isomeric changes (generating tautomers), and bond order changes [13].

2.2.5.2. Preparation of Protein Targets

The protein crystal tructures of target proteins, 5-HT_2 receptor, and COX-2 with respective PDB ID: 6BQX and 6COX, were obtained from the protein databank (http://www.rcsb.org/pdb) and populated on the Schrodinger suite. The proteins were modified with the preparation wizard of Schrodinger [14].

2.2.5.3. Molecular Docking

The "receptor grid generation" option in the glidev7.5 programme of Maestro-v12.5. was applied and used to create a "receptor site" on the structure of respective protein target (5HT_2 and COX). The modified ligand (orphenadrine or diclofenac) was docked into the receptor site using the extra precision (XP) workflow module of the Schrödinger suite®. XP docking analyses the ligand-site integration to measure level of "fit". The results of the XP docking were quantified using the docking scores tools such as Glide emodels ® [15].

2.2.5.4. Docking Pose Analysis

Docking pose was assessed with the pose-viewing mode of the Maestrov11.5®. The H-bond and Van der Waals receptor interactions were visualized.

2.2.6 Semi-Quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) assay

2.2.6.1 Isolation and purification of RNA

Ribonucleic acid (RNA) was isolated from the brain and the removal of contaminant (DNA) was as previously described. The isolated and purified RNA was quantified at 260 nm using an A&E Spectrophotometer (A&E Lab. UK).

2.2.6.2 RT conversion of RNA

One (1 µg) RNA was converted to cDNA by reverse transcriptase reaction with the aid of cDNA synthesis kit (ProtoScript II®, New England BioLabs) in a 3-step reaction involving varying temperatures (65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min) [16].

2.2.6.3 PCR, amplification, and gel electrophoresis

The previously described method was modified and adopted to amplify the target gene. Briefly, a 25 µl of the reaction mixture containing cDNA, primer of TNF or COX (forward and reverse) and PCR master mix® were subjected to an initial denaturation at 95 ◦C for 5 min. This was repeated in cycles (30x) of amplification (denaturation at 95 ◦C for 30 s, hardening for 30 s and extension at 72 ◦C for 60 s) and a final extension at 72 ◦C for 10 min [9].

The primers were purchased from Inqaba Biotec, Hatfield, South Africa.

TNF-α; Forward primer: ACCACGCTCTTCTGTCTACTG

Reverse primer: CTTGGTGGTTTGCTACGAC,

COX2; Forward primer GATTGACAGCCCACCAACTT

Reverse primer CGGGATGAACTCTCTCCTCA.

2.2.6.4 Agarose Gel electrophoresis

The amplified genes were subjected to 1.0% agarose gel electrophoresis. The house keeping gene (GAPDH) was used to normalize the relative expression of each gene, and the band intensity were quantified using the "image J" software [17].

2.3 Statistical analysis

Data are presented as mean±SEM. One way analysis of variance was used to determine the differences between groups, followed by Tukey's post hoc analysis which was used to determine the differences among groups mean. $p<0.05$ was set as the level of significance.

3. RESULTS

3.1 *Orphenadrine and diclofenac combination acted synergistically to mitigate the peripherally mediated nociceptive stimulus in the mouse writhing assay*

Fig. 1 shows that the intraperitoneal administration of 0.6 % acetic acid in the control group increased the number of writhes from 9.0 ± 0.5 at 5 minutes to 18.6±2.4 at 30 minutes. There was a significant reduction in the number of writhes (5.0±2.1) in animals treated with only orphenadrine, compared to control (18.6±2.4, $p<0.001$), at 30 minutes. Similarly, treatment with only diclofenac reduced the number of writhes at all times, exerting peak effect at 30 minutes (2.3±0.9). The combination of orphenadrine + diclofenac significantly suppressed writhes at all times with peak reduction at 30 minutes vs control (18.6±2.4 vs 0.75±0.48). Cumulatively the combination evoked a significant peak reduction in the number of writhes compared to control (1.3±0.6. vs 71.7±13.1, $p<0.001$), orphenadrine (1.3±0.6 vs 13.3±3.3, $p<0.05$) and diclofenac (1.3±0.6 vs 12.8±2.3, $p<0.05$) groups.

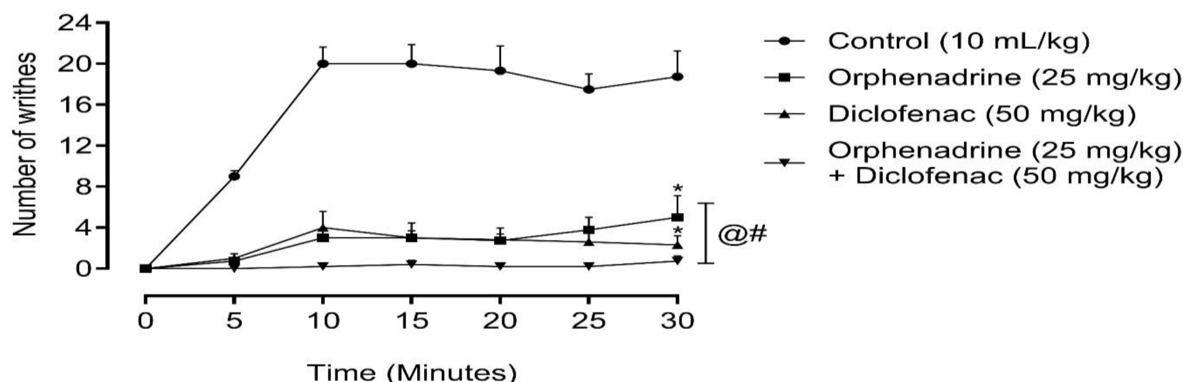

Fig 1: Acetic acid-induced mouse writhing assay: Effect of a diclofenac, orphenadrine, and orphenadrine + diclofenac on the number of writhes in the mouse writhing assay. *p<0.01 vs control, @p<0.01 vs diclofenac group, #p<0.05 vs orphenadrine group.

3.2 *The combination of orphenadrine and diclofenac increased the number of head dips in mice treated with 0.6% acetic acid in the hole board assay.*

Fig. 2 illustrates that the mice treated with orphenadrine tended to reduce the number of head dips from 22.5±1.1 in the control group to 18.1±0.8, $p>0.05$. Similarly, mice treated with diclofenac showed a significant reduction in head dips, compared to the control (22.5±1.1 vs 15.7±1.6, $p<0.001$). There was no significant difference in the number of head dips between the control and the combination (22.5±1.1 vs 24.3±1.6, $p>0.05$). However, the combination increased the number of head dips compared to diclofenac (24.3±1.6 vs 15.7±1.6, $p<0.01$) or orphenadrine (24.3±1.1 vs 18.1±0.8, $p<0.05$) group.

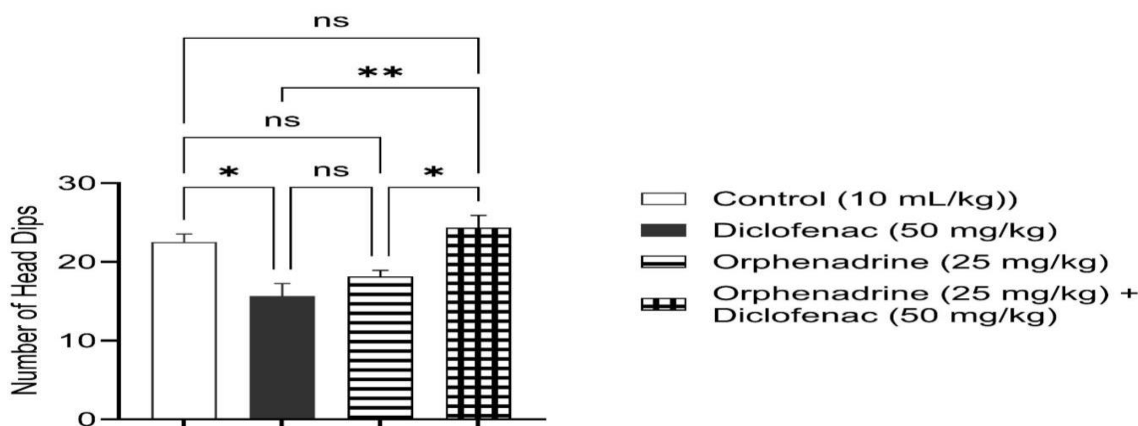

Fig 2: The hole board assay: Effect of a diclofenac, orphenadrine, and orphenadrine + diclofenac on the number of head dips in the hole board assay. *p<0.05 vs control. *p<0.05 vs orphenadrine group, **p<0.01 vs diclofenac group. ns=not significant.

3.3 *Tumour necrosis factor expression in mice*

Fig. 3 illustrates that TNF-α expression was significantly elevated in the brain of mice that received only acetic acid. The administration of diclofenac, orphenadrine and the combination significantly reduced TNF-α expression. Mice that received only orphenadrine has the lowest levels of TNF-α. TNF-α expression in mice that received the combination was highest compared to diclofenac and orphenadrine.

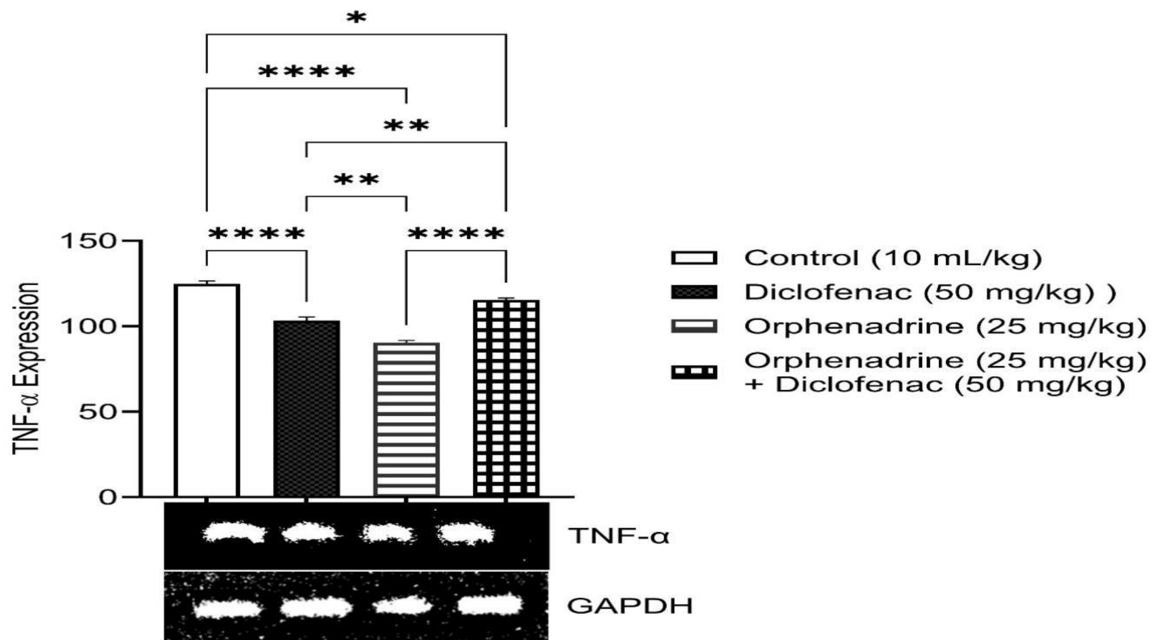

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Fig 3: Tumour necrosis factor (TNF)-α expression: Effect of diclofenac, orphenadrine or its combination on TNF-α expression in the brain of mice subjected to the mouse writhing assay. *p<0.05, ***p<0.001 vs control. **p<0.01 vs diclofenac group. ****p<0.001 vs orphenadrine group.

3.4 *Cyclooxygenase expression in mice*

As shown in Fig. 4, treatment with diclofenac, orphenadrine or the combination significantly reduced COX expression in the control group. The peak significant reduction was observed in the orphenadrine group compared to control, diclofenac and the combination groups.

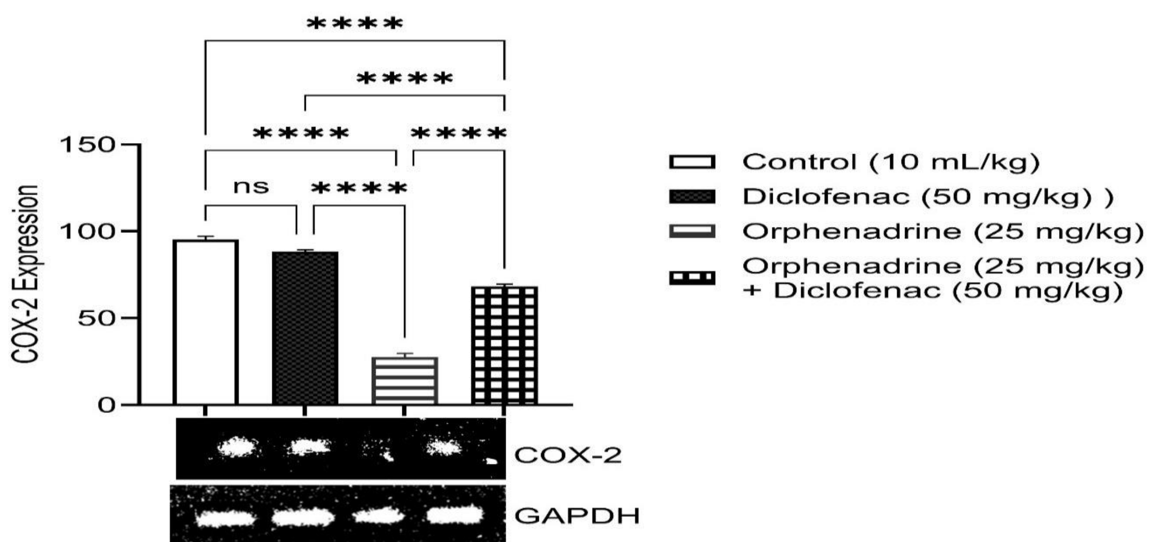

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Fig 4: Cyclooxygenase (COX)-2 expression in the brain of mice subjected to the mice writhing assay and treated with orphenadrine, diclofenac or the combination. ****p<0.001 vs control, ****p<0.001 vs diclofenac group, ****p<0.001 vs orphenadrine group. ns=not significant.

3.5 *Orphenadrine showed moderate affinity for serotonin receptor, and cyclooxygenase.*
Table 1 shows that orphenadrine and diclofenac had binding energies of -5.92 and -5.27 kcal/mol for serotonin ($5HT_2$) receptors, respectively. The binding energy for COX-2 was -4.76 kcal/mol for orphenadrine and -9.50 kcal/mol for diclofenac.

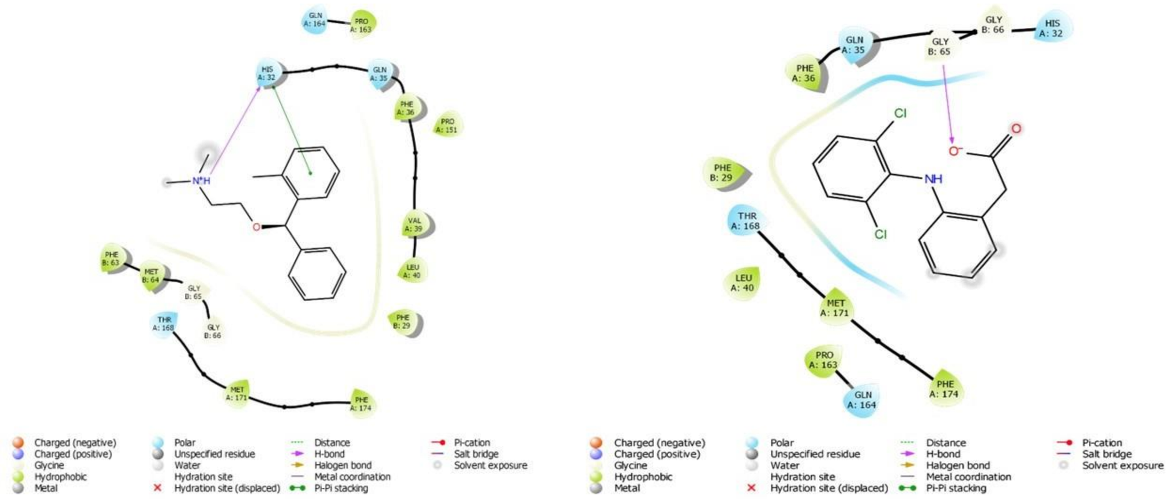

Fig 5: Ligand-receptor interactions, binding affinities and residues interactions between orphenadrine or diclofenac and serotonin (5HT2) and cyclooxygenase (COX2).

3.6 Histological assay
Liver sections from the control mice showed normal tissue architecture, with well-defined hepatocytes, sinusoids, portal vein and bile duct. There was evidence of the presence of the local immune system of the liver (periportal mobilization of lymphocytes). Mouse that received diclofenac, orphenadrine, or the combination of diclofenac and orphenadrine all showed severe hepatocyte haemorrhagic necrosis. This may probably result in substantial loss of liver tissue. There was additional activation of the local immune system (Kupffer cell activation) in the group that received the combination (Fig. 5).

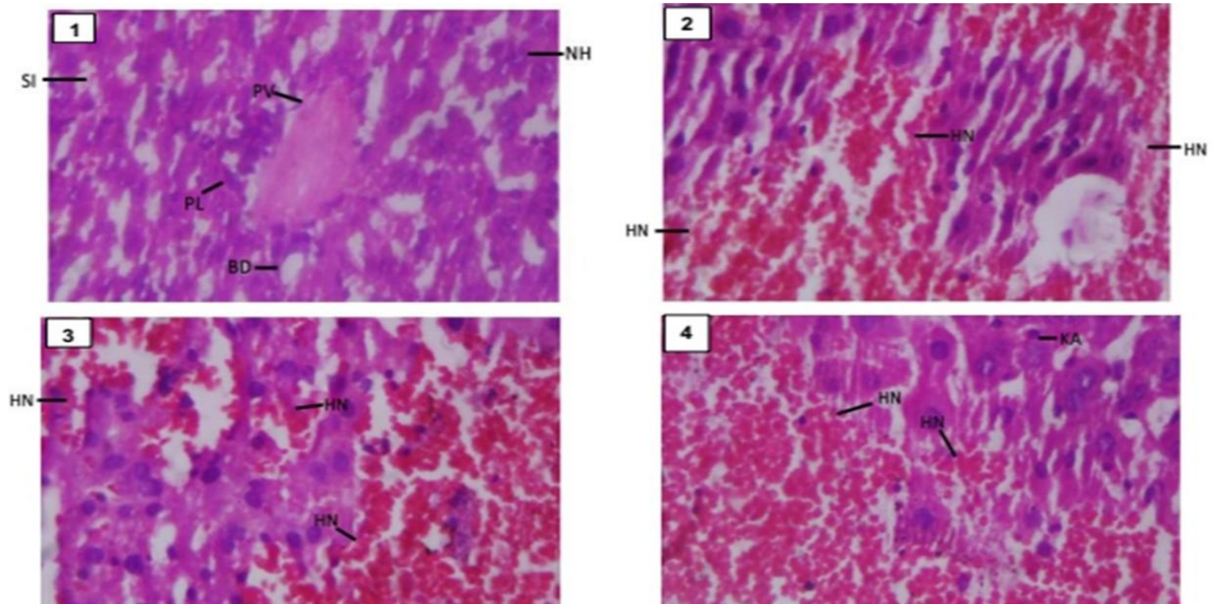

Fig 5: Mouse liver, 1: control shows normal hepatocytes (NH), sinusoids (SI), portal vein (PV), bile duct (BD) and mild periportal mobilization of lymphocytes. 2: Mouse liver given orphenadrine only shows severe hepatocyte

haemorrhagic necrosis. 3: Mouse liver given diclofenac only shows severe hepatocyte haemorrhagic necrosis (HN): 4: Mouse liver given diclofenac (AA) + orphenadrine shows severe hepatocyte haemorrhagic necrosis (HN) and Kupffer cell activation (KA): H&E 400 X.

Histological sectioning of the kidneys from the control mouse shows normal architecture, with well-defined tubules, glomeruli and interstitial space. The kidneys of mice that received diclofenac showed severe interstitial haemorrhage and inflammation (pyelonephritis). There were similar observations in rats treated with orphenadrine, but to a lower degree. However, in mouse treated with a combination of diclofenac and orphenadrine, the degree of interstitial haemorrhage and tubular necrosis was higher (Fig 6).

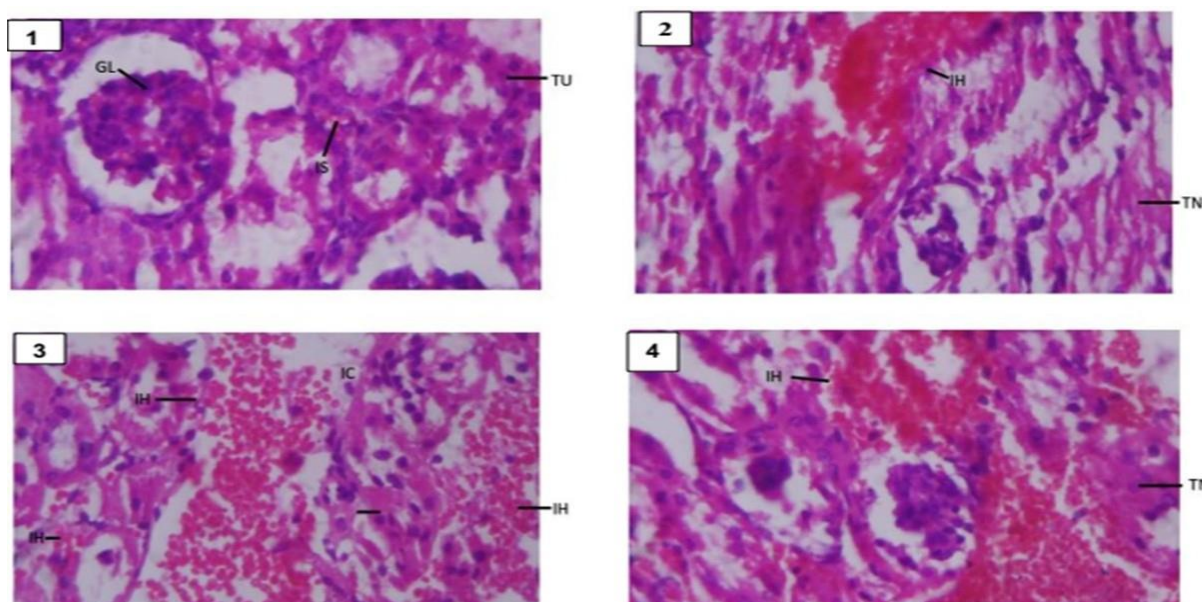

Fig 6: Mouse kidney: control shows normal architecture: glomerulus (GL), tubules (TU) and interstitial space (IS): 2: Mouse kidney given orphenadrine (AA) only shows focal interstitial haemorrhage (IH) and tubular necrosis (TN): 3: Mouse kidneys, given diclofenac (AA) only shows severe interstitial haemorrhage (IH) and infiltrates of inflammatory cells (IC). 4: Mouse kidneys given diclofenac + orphenadrine shows severe interstitial haemorrhage (IH) and tubular necrosis.

4. DISCUSSION

Hunskaar et al reported that orphenadrine possesses central antinociceptive effects that may involve activation of the serotonergic pathway [1, 5]. In their study, they posited that orphenadrine lacks peripheral antinociceptive activity. However, due to its complex interactions with nociceptive pathways, it is possible that orphenadrine may exert peripheral effects. Hence, our current study employed a peripheral model of nociception to assess any peripherally mediated antinociceptive effect and the possible mechanistic interactions involved. It is tenable that due to its centrally mediated antinociceptive effect, orphenadrine may possess superior peripheral actions involving novel pathways. Data from this study affirmed that orphenadrine exhibits peripherally mediated antinociceptive effect, as evidenced by its activity in the acetic acid-induced mouse writhing assay. The acetic acid-induced mouse writhing assay is commonly used to assess compounds for peripherally mediated antinociceptive effect [18]. In this model, injection of acetic acid activates nociceptive reflexes via the release of local mediators such as prostaglandin and substance P, which stimulate local nociceptors and produce characteristic abdominal “stretching” or “writhing”. The number of “writhe(s)” is directly proportional to the pain intensity. Recent evidence suggests that central mechanisms may also contribute to responses observed in the acetic acid writhing assay [19]. Results from the acetic acid assay indicate that orphenadrine may interact with local nociceptive mediators such as prostaglandins or substance P. Nociception is a complex response with a behavioural component [20, 21]. As a result, the nociceptive stimuli may trigger an “anxiety-like state”. Such anxiety could be unsettling and may lead to psychological deficits [20]. Consequently, agents with antinociceptive properties that also exert anxiolytic effects could provide improved therapeutic outcomes. Orphenadrine has been reported to interact with several neural pathways and mediators that regulate behaviour [4, 22, 23], necessitating an evaluation of its putative anxiolytic effect using the hole board model. The hole board assay measures exploratory behaviour by recording the number of “head dips”. These “head dips” into circular holes on the board

is an exploratory behaviour which is reduced in an "anxious state" [11]. Hence, the more head dips, the less anxiety. Orphenadrine did not produce any significant effect on the number of head dips. Synergism is a pharmacological phenomenon often exploited in therapy to improve therapeutic outcomes [24]. Compounds exhibiting synergistic actions may achieve potentiated effects at reduced doses. The present study evaluated the potential synergistic interaction between the combination of orphenadrine and diclofenac, a non-steroidal anti-inflammatory drug (NSAID), used to manage pain. The findings revealed that the combination of orphenadrine and diclofenac acted synergistically. For instance, the combination reduced the number of writhes by approximately 90 % in the acetic acid-induced assay, producing a potentiated antinociceptive effect than either agent administered alone. Consistently the insignificant anti-anxiety effect observed with either diclofenac or orphenadrine alone was significant when both agents were combined. Thus, the combination exerted synergistic antinociceptive and anxiolytic effects. These actions of the combination were somewhat consistent at the molecular level. Tumour necrosis factor (TNF) is a proinflammatory cytokine involved in both peripheral and central sensitization of nociceptors [25]. TNF aggravates the nociceptive response and serves as a link between the immune system and nociception. The observed increase in TNF expression by the combination was contrary to its antinociceptive effects. However, reports indicate that, in addition to its pronociceptive actions, TNF is a vital mediator of tissue repair due to its pro-angiogenic and fibrotic properties [26]. It is therefore possible that the increased expression in TNF may have contributed to tissue repair. In *silico* docking studies are computer-aided approaches used to predict binding affinities of ligands to target proteins, with more negative docking scores indicating a stronger binding affinity [15]. In this study, orphenadrine and diclofenac were docked with cyclooxygenase (COX), a principal regulator of the arachidonic pathway involved in nociception. COX catalyses the formation of prostaglandins, a diverse set of inflammatory mediators. Diclofenac exerts its antinociceptive effect by inhibiting COX activity. Accordingly, diclofenac demonstrated strong binding affinity for COX in the docking analysis. Interestingly, there was a different finding at the molecular level. Molecular analysis showed that COX expression was highest in the diclofenac-treated group compared to orphenadrine or combination groups. The inhibitory action of diclofenac may have induced a compensatory upregulation of COX expression at the molecular level to counteract the deficiency at the biochemical level. Conversely, the distinct reduction in COX expression in the orphenadrine group was novel and unexpected. It opens a new mechanistic possibility that the antinociceptive action of orphenadrine may involve a direct suppression of COX. The observations from the docking study supports this hypothesis as orphenadrine exhibited direct interaction with COX. Furthermore, the binding affinity of the combination appeared to be additive, suggesting that the combination may exhibit stronger COX inhibition *in vivo* biological systems. The serotonergic system is involved in the descending control of nociception, and experimental evidence supports an interaction between orphenadrine and the serotonergic system [8, 27]. Our current study provides novel *in silico* evidence supporting this interaction between orphenadrine and serotonin as orphenadrine demonstrated moderate binding affinity with serotonin ($5HT_2$) receptors. One of the major adverse effects associated with diclofenac therapy is nephrotoxicity, particularly on prolonged use [8]. Hunskaar et al also reported that orphenadrine exhibits toxic effect only at doses higher than 25 mg/kg [5]. However, the observations in this study were at variance to these reports. The histoarchitecture of the kidney of mice in the diclofenac group showed distortions with nephritis, an effect that was worsened in the orphenadrine-treated and combination groups. These observations were extended to the hepatic system. These findings indicate that although the combination was effective, it also exacerbated the adverse outcomes associated with acute, single use of both agents. These toxic effects may be dose-dependent, highlighting the need to conduct comparative dose-response studies to ascertain safe doses. Furthermore, it is also necessary to evaluate the actions of the combination in chronic antinociceptive and toxicity models. Finally, the docking studies are largely predictive and the findings require further validation in an *in vivo* system.

5. CONCLUSION

The findings demonstrate that orphenadrine exhibits peripherally mediated antinociceptive and anxiolytic activity that is significantly enhanced when combined with diclofenac. The effects may involve serotonergic pathways, and COX modulation. However, the observed renal and hepatic toxicity raises safety concerns, necessitating further dose optimization and chronic toxicity studies.

DECLARATIONS

Acknowledgment.
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Conflict of interest

The Authors declare no conflict of interest

Authors contribution

Conceptualization, OE; methodology, OE, KGN, BAO, IIM, AJE, II; validation, VE, OE, AJE. and II.; investigation, OE, KGN, BAO, IIM, NOO, AFO, OTE, AW, and AJE.; resources, OE and AJE.; data curation, OE, VE and AJE.; writing—original draft preparation, OE, VE, AJE and II.; writing—review and editing, OE, VE and AJE.; visualization, OE and AJE.; supervision, OE, AJE, VE and II.; project administration, OE and AJE.; funding acquisition, OE, KGN, BAO, IIM, NOO, AFO, OTE, AW, VE and AJE. All authors have read and agreed to the published version of the manuscript.

6. REFERENCES

[1] Hunskaar S, Berge O-G, Hole K. Antinociceptive effects of orphenadrine citrate in mice. *Eur J Pharmacol* 1985; 111: 221–226.

[2] Chen YW, Tzeng JI, Chen YC, et al. Intrathecal orphenadrine elicits spinal block in the rat. *Eur J Pharmacol* 2014; 742: 125–130.

[3] Hunskaar S, Rosland JH, Hole K. Mechanisms of orhenadrine-induced antinociception in mice: a role for serotonergic pathways. *Eur J Pharmacol* 1989; 160: 83–91.

[4] Sureda FX, Gabriel C, Pallàs M, et al. In vitro and in vivo protective effect of orphenadrine on glutamate neurotoxicity. *Neuropharmacology* 1999; 38: 671–677.

[5] Hunskaar S, Berge O -G, Hole K. Orphenadrine Citrate Increases and Prolongs the Antinociceptive Effects of Paracetamol in Mice. *Acta Pharmacol Toxicol (Copenh)* 1986; 59: 53–59.

[6] Shirakawa K, Takeno M, Kuma H, et al. Comparative Evaluation of Cyclooxygenase Inhibition Profiles Across Various NSAIDS Forms and Doses: Implications for Efficacy and Adverse Effects. *Pain and Therapy 2024 14:1* 2024; 14: 329–338.

[7] Rainsford KD, Bjarnason I. NSAIDs: take with food or after fasting? *Journal of Pharmacy and Pharmacology* 2012; 64: 465–469.

[8] Mishra D, Ghosh G, Sudhir Kumar P, et al. An experimental study of analgesic activity of selective COX-2 inhibitor with conventional NSAIDS. *Asian Journal of Pharmaceutical and Clinical Research* 2011; 4: 78–81.

[9] Edosuyi O, Osagiede EB, Eguasa N, et al. Fumarate attenuated doxorubicin-induced cardiac injury through modulation of endothelial nitric oxide synthase expression and transforming growth factor-β1 signalling. *Prospects in Pharmaceutical Sciences*. 2025; 11: 1-8.

[10] Edosuyi O, Igbe I, Iniaghe LO. Antinociceptive and antioxidant activities of *Hunteria umbellata* stem bark: Possible role of the serotonergic, opioidergic and dopaminergic pathways. *J Complement Integr Med* 2018; 15: 1–15.

[11] Ozolua RI, Ajibade MA, Uwaya DO, et al. A Report on the Antidepressant-like Activity of *Paullinia pinnata* Methanol Leaf Extract in Mice and Possible Involvement of Monoaminergic Mechanisms. *Targets* 2025; 3: 22.

[12] Edosuyi O, Choi M, Igbe I, et al. Fumarate exerted an antihypertensive effect and reduced kidney injury molecule (KIM)-1 expression in deoxycorticosterone acetate-salt hypertension. *Clin Exp Hypertens* 2021; 43: 555–564.

[13] Elekofehinti OO, Iwaloye O, Molehin OR, et al. Identification of lead compounds from large natural product library targeting 3C-like protease of SARS-CoV-2 using E-pharmacophore modelling, QSAR and molecular dynamics simulation. *In Silico Pharmacol* 2021; 9: 49.

[14] Olawale F, Iwaloye O, Elekofehinti OO, et al. A Multi-target Approach for the Discovery of Anti Breast Cancer Agents from Plants Secondary Metabolites. *Lett Drug Des Discov* 2021; 18: 1009–1023.

[15] Meng X-Y, Zhang H-X, Mezei M, et al. Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery. *Current Computer Aided-Drug Design* 2011; 7: 146–157.

Vol.15 (1): 8-17; March 2026 ISSN: 2971-737X (Print); ISSN: 2971-7388.
Available at www.nijophasr.net https://doi.org/10.60787/nijophasr-v15-i1-641

[16] Olumegbon LT, Lawal AO, Oluyede DM, et al. Hesperetin protects against diesel exhaust particles-induced cardiovascular oxidative stress and inflammation in Wistar rats. *Environmental Science and Pollution Research* 2022; 29: 52574–52589.

[17] Elekofehinti OO, Lawal AO, Ejelonu OC, et al. Involvement of fat mass and obesity gene (FTO) in the anti-obesity action of Annona muricata Annonaceae: in silico and in vivo studies. *J Diabetes Metab Disord* 2020; 19: 197–204.

[18] Naghizadeh B, Mansouri MT, Ghorbanzadeh B. Ellagic acid enhances the antinociceptive action of carbamazepine in the acetic acid writhing test with mice. *Pharm Biol* 2016; 54: 157–161.

[19] Edosuyi O, Ekanem S. The Antinociceptive Property of Brachystegia eurycoma (Fabaceae) Partly Involves Stimulation of Opioid Pathways and Mitigation of the Action of Reactive Radicals. *Journal of Science and Practice of Pharmacy* 2022; 9: 481–488.

[20] Gameiro GH, Gameiro PH, da Silva Andrade A, et al. Nociception- and anxiety-like behavior in rats submitted to different periods of restraint stress. *Physiol Behav* 2006; 87: 643–649.

[21] Woo AK. Depression and Anxiety in Pain. *Rev Pain* 2010; 4: 8–12.

[22] Balhara P, Ahmed S, Vasudeva N, et al. Neuroprotective potential of orphenadrine in focal cerebral ischemia: A neurobehavioral and mechanistic study. *Toxicol Appl Pharmacol* 2026; 510: 117757.

[23] Van Der Zee P, Hespe W. Influence of orphenadrine HCl and its N-demethylated derivatives on the in vitro uptake of noradrenaline and 5-hydroxytryptamine by rat brain slices. *Neuropharmacology* 1973; 12: 843–851.

[24] Pan Y, Ren H, Lan L, et al. *Life*. Review of Predicting Synergistic Drug Combinations. 2023; 13: 1878.

[25] García-Domínguez M. The Role of TNF-α in Neuropathic Pain: An Immunotherapeutic Perspective. 2025; 15: 785.

[26] Ashcroft GS, Jeong MJ, Ashworth JJ, et al. Tumour necrosis factor-alpha (TNF-α) is a therapeutic target for impaired cutaneous wound healing. *Wound Repair Regen* 2012; 20: 38–49.

[27] Millan MJ. Descending control of pain. *Prog Neurobiol* 2002; 66: 355–474.