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ANALGESIC SYNERGY BETWEEN PARACETAMOL AND LOW-DOSE CAFFEINE IN THE HOT-PLATE TEST

FERGUS ROWNTREE[1]*, MATHILDE ROUSSELOT[2], STEFANO CAVALIERI[3]

Affiliation [1]Department of Pharmacology, Exmoor Institute of Pharmaceutical Sciences, Taunton, United Kingdom
[2]Department of Neuropharmacology, Jura Centre for Medicinal Chemistry, Lons-le-Saunier, France
[3]Department of Neuropharmacology, Valtellina Centre for Pharmacology, Sondrio, Italy

*Corresponding author: Fergus Rowntree, f.rowntree@eips.ac.uk
ORCID iD: Fergus Rowntree 0000-0001-1117-7133; Mathilde Rousselot 0000-0001-1557-4283; Stefano Cavalieri 0000-0001-2390-1686

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Abstract

This short review examines what the published literature says about combining paracetamol with low-dose caffeine in the hot-plate test of thermal nociception, and it reports no new data. It describes the behavioural test and its main sources of variability, summarises current ideas about how each drug produces antinociception, and sets out the methods used to decide whether a combination is synergistic rather than merely additive. Rodent and human studies broadly agree that caffeine enhances the effect of paracetamol, although the size of the gain and its mechanism remain contested. Few hot-plate studies apply formal isobolographic analysis or report their methods in enough detail, which limits firm conclusions about true synergy.

1. INTRODUCTION

Paracetamol (acetaminophen) is among the most widely used analgesics in the world, and it is rarely taken alone for long. Fixed-dose products that pair it with caffeine have been sold for decades, mostly for headache and minor musculoskeletal pain [1, 2]. The pharmaceutical logic is simple. If a small amount of a second compound lets a patient get the same relief from less paracetamol, or better relief from the same dose, the combination has earned its place on the shelf.

The background to the combination is largely clinical. Paracetamol has a good tolerability record at recommended doses, but it is not especially powerful for moderate pain, and the margin between a therapeutic and a hepatotoxic dose is narrower than many users assume [3]. Anything that improves the relief

obtained from a given dose has obvious appeal, since it may reduce the temptation to take more. Caffeine fits that role because it is cheap, familiar and pharmacologically active at doses people already consume daily [4, 5]. The question for the laboratory is whether the gain seen in patients can be reproduced, measured and explained in a controlled animal test.

Whether that logic holds up is a pharmacological question that animal tests are often asked to answer first. The hot-plate test, in which a rodent is placed on a heated surface and the latency to a protective response is timed, has been used since the 1940s to look at centrally mediated antinociception [6, 7]. It is cheap, quick and easy to repeat. It is also easy to misuse. This review asks what the published record actually shows about paracetamol plus low-dose caffeine in this test, how synergy should be judged, and where the evidence is thin [8, 9]. No experiments were carried out for this article, and the discussion rests entirely on work reported by others.

Terminology is another source of confusion. Papers use synergy, potentiation, enhancement and additivity as if they were interchangeable, though they mean different things. Potentiation, for instance, is sometimes used for a drug that has no effect of its own but increases the effect of another, which is a plausible description of caffeine in some settings [10, 11].

This article is organised around that question. It begins with the hot-plate test itself, then turns to what is known about how the two drugs work, how interaction is defined in quantitative terms, what has been reported, and how future studies might be designed. A summary table collects representative published findings. Throughout, the aim is to be clear about which statements rest on direct evidence and which are inferred from related models, because the two are easy to blur in the literature on analgesic adjuvants [10, 12].

Historical context helps explain why this pairing persists. Caffeine entered analgesic mixtures in the early twentieth century, long before receptor pharmacology could say anything about how it might work, and the practice was sustained largely by clinical impression [1, 4]. Only later did controlled trials and animal experiments begin to test the idea, and the two kinds of evidence have developed rather separately. Bringing them together, and asking what the laboratory test can legitimately contribute, is the practical purpose of the discussion that follows [5, 10].

The word low-dose matters. Caffeine at high doses stimulates locomotion and can alter behaviour on a hot surface for reasons unrelated to pain, which would muddy any interpretation. Most of the clinical literature on caffeine as an analgesic adjuvant concerns single doses of roughly the amount found in a cup of coffee or two, and the preclinical studies that matter most are those that stay well below the dose at which obvious stimulation appears [10, 13]. Because many users also take caffeine in drinks across the day, the total exposure in practice may be several times the amount in a tablet, which complicates the translation of any preclinical dose [4, 14].

2. THE HOT-PLATE TEST

Woolfe and MacDonald introduced the heated-plate approach to evaluate opioid analgesics, and Eddy and Leimbach later refined it into the form that most laboratories still follow [6, 7]. The plate is held at a fixed temperature, commonly in the region of 50 to 56 °C, and the animal is confined by a clear cylinder. The endpoint is the time to a defined response, usually hind-paw licking, paw shake or a jump. A cut-off, often 30 to 60 seconds, protects the animal from tissue damage. Because the response needs supraspinal integration rather than a simple spinal reflex, the test is regarded as sensitive to centrally acting drugs [15, 16].

The choice of end-point deserves more thought than it usually gets. Paw licking is generally taken to reflect a supraspinally organised response, whereas jumping can be influenced by motor factors, anxiety and the animal's tendency to escape. Some laboratories record both, and a few use the first sign of any clear response. When a drug alters locomotor activity, as caffeine can at higher doses, jump latency may shorten or lengthen for reasons that have little to do with analgesia [15, 16]. Choosing licking as the

primary end-point, and checking spontaneous motor activity in parallel, protects against that error.

That sensitivity is uneven. Opioids perform very well. Non-steroidal anti-inflammatory drugs tend to do poorly on a normal, uninflamed paw, since there is little prostaglandin-driven sensitisation for them to reverse [15, 17]. Paracetamol sits in an awkward middle position. It has a central site of action, yet reports of its effect on hot-plate latency are mixed, with some laboratories seeing clear prolongation at moderate doses and others seeing very little. Differences in plate temperature, strain, sex and the choice of end-point probably explain a good share of the disagreement, and this inconsistency is worth keeping in mind when a combination is later said to be synergistic.

Handling and environment matter more than newcomers expect. Prior exposure to the plate produces learning, so latencies drift when the same animals are retested without an adequate interval. Stress, time of day, noise and the experimenter's sex can all shift baseline values [16, 17]. Reviews of rodent pain testing have long pointed out that behavioural end-points in this field are noisy and that apparent drug effects may reflect these background factors [15, 17]. Ethical practice adds further constraints, including humane cut-offs, limits on repeated exposure and prompt withdrawal when distress is evident [18, 19].

Positive controls are informative too. Morphine produces large, dose-dependent increases in latency and has been used for decades to confirm that a given apparatus responds as expected [6, 7]. A weak analgesic such as paracetamol will often produce changes of the order of normal day-to-day variation, so a test that cannot detect it reliably will also struggle to detect an enhancement of it.

Reporting has not always kept pace. A survey of animal research published at the time the ARRIVE guidelines first appeared found that many papers omitted basic information such as randomisation, blinding and the number of animals per group [20]. The updated guidelines place greater emphasis on these items and on the exact experimental unit [19].

For a hot-plate study of a drug combination, that means stating the plate temperature, the cut-off, the observer's blinding status and the interval between dosing and testing. Without those details a reader cannot judge whether a reported interaction is credible or a by-product of design. Pre-registration of the analysis plan, even informally within a laboratory, removes much of the flexibility that produces false positive interactions.

3. PHARMACOLOGY OF THE TWO DRUGS

The mechanism of paracetamol has been argued over for a long time. Early work focused on weak inhibition of cyclooxygenase in the brain, and a splice variant sometimes called COX-3 was proposed as the target, although its relevance in humans has been doubted [21, 22]. A more convincing line of work shows that paracetamol is deacetylated to p-aminophenol, which is then conjugated with arachidonic acid in the nervous system by fatty acid amide hydrolase to form N-arachidonoylphenolamine (AM404). That metabolite acts on transient receptor potential and cannabinoid pathways [23, 24]. Blocking cannabinoid CB_1 receptors reduces the antinociceptive effect of paracetamol in rodents, which fits this model [25].

Serotonergic descending pathways are also involved. Studies in volunteers and animals indicate that the analgesic effect of paracetamol depends in part on bulbospinal serotonin, and that blocking 5-HT_3 receptors weakens it [21, 26]. Put simply, these observations describe a drug that acts mainly in the central nervous system through several partly overlapping routes, none of which has been proved to be the single explanation. Pharmacokinetically, paracetamol is absorbed rapidly after oral dosing, undergoes extensive glucuronidation and sulfation in the liver, and has a plasma half-life of a couple of hours in healthy adults [3]. These features set the time window in which the hot-plate test should be run.

Dose-response behaviour of the two drugs is worth separating. Paracetamol typically gives a graded, rising antinociceptive effect over a moderate range in rodents, but the curve can

flatten, and very high doses add toxicity without much extra relief [3, 21]. Caffeine often shows a bell-shaped or biphasic pattern, with enhancement of analgesic action at low to moderate doses and loss of benefit, or even pronociceptive behaviour, as the dose rises [10, 12, 27]. A mixture of one drug with a flat curve and another with a biphasic one cannot be assumed to follow simple additivity, which is one more reason to test several ratios and not just one.

Caffeine is better characterised at the receptor level. At the concentrations reached after ordinary consumption, its principal action is competitive antagonism of adenosine A_1 and A_{2A} receptors [4, 27]. Adenosine has a double reputation in pain research. Activation of A_1 receptors in the spinal cord is antinociceptive, so blocking adenosine receptors might be expected to cause hyperalgesia, and sometimes it does [28]. Yet caffeine given in the right dose range frequently enhances the effect of analgesics, which has led reviewers to suggest that its adjuvant action arises from central effects on arousal, mood and attention, from actions at other sites, or from a combination of these [10, 12].

Tolerance and repeated dosing are rarely examined. Habitual caffeine intake produces adaptive changes, including up-regulation of adenosine receptors, and animals given caffeine in drinking water for days may respond differently to an acute dose than naive animals [4, 27]. Paracetamol shows little classical tolerance in experimental settings. Since most hot-plate combination experiments use a single acute administration, the findings say little about what happens in people who consume caffeine every day, who are the majority of potential users of these products [4, 13].

Safety considerations shape dose choice in both directions. Paracetamol is converted in small part by cytochrome P450 enzymes to a reactive quinone metabolite that is normally neutralised by glutathione, and overdose exhausts this protection [3]. Caffeine is itself metabolised mainly by CYP1A2, and since several isoenzymes handle both compounds, an effect of one on the clearance of the other cannot be excluded in principle [14, 29]. In practice, at the low caffeine doses under discussion, pharmacokinetic interference of this type is probably small, but it has rarely been measured in the same animals used for behavioural testing, which is a weakness of the preclinical record.

Direct antinociceptive effects of caffeine have been reported too. In mice, systemic caffeine prolonged hot-plate latency, and the effect was attributed to central cholinergic mechanisms rather than to adenosine blockade alone [30]. Reviews of the wider central actions of caffeine describe effects on dopaminergic, cholinergic and noradrenergic transmission that could plausibly influence behavioural responses to noxious heat [27, 29]. Pharmacokinetic features matter as well. Caffeine is almost completely absorbed after oral administration, with a half-life that varies widely between species and individuals, so a fixed ratio chosen in one species may not behave in the same way in another [14].

A quieter possibility is that caffeine alters the fate of paracetamol itself. If it speeds absorption, the peak plasma concentration arrives earlier and is higher for a given dose, and an apparent synergy in a time-limited behavioural test could reflect pharmacokinetics rather than a true interaction at the receptor level. Human work has reported faster absorption of paracetamol when caffeine is co-administered, and the point deserves attention in any animal study that uses a single fixed observation time [31].

4. DEFINING AND TESTING SYNERGY

Synergy is easy to claim and hard to demonstrate. Berenbaum's classic discussion made the central point that a combination can only be called synergistic relative to a stated definition of additivity [11]. Two main reference models exist. Loewe additivity, from which the isobologram derives, asks whether the doses of each drug in a combination fall below the line joining their individual equi-effective doses [32]. Bliss independence instead treats the drugs as acting through independent mechanisms and compares observed with predicted probabilities. The two can give different verdicts on the same data, so the choice must be justified before the experiment, not after it.

Tallarida's work set out a practical route for the isobolographic method in pharmacology [8, 9]. Dose-response curves are first determined for each drug alone and an equi-effective dose, usually the ED_{50}, is calculated with confidence limits. The theoretical additive dose for a chosen ratio is then computed and compared statistically with the experimentally determined dose of the mixture. A significantly lower experimental value indicates supra-additivity. This requires reasonably parallel dose-response lines, adequate numbers per dose and a defined fixed ratio, which many published combination studies lack.

Design choices shape how convincingly an interaction can be shown. A fixed-ratio design, in which the two drugs are mixed in constant proportion and serial dilutions are tested, is economical and fits the isobolographic method well [8]. Designs that vary the ratio, sometimes called ray designs, map the interaction surface more fully but need many more animals. Because hot-plate data are variable, group sizes of eight to ten per dose are often needed to detect a modest departure from additivity. Underpowered studies may report synergy by chance, and a failure to find it may reflect a lack of power rather than a lack of interaction [9, 33].

Interpretation also depends on whether the interaction is symmetrical. In some pairs each drug enhances the other. In others, one component has no effect alone, yet shifts the curve of its partner, and the isobologram then becomes awkward because there is no equi-effective dose of the inactive drug to place on its axis [8, 11]. Caffeine at low doses may well belong to this second group in the hot-plate test. Analysts then use a modified approach in which the dose-response line of the active drug is determined in the presence of a fixed dose of the adjuvant, and the shift in ED_{50} is tested directly [9].

Chou described an alternative based on the median-effect principle and a combination index, which is popular because it can be applied to a smaller set of dose levels [33]. It carries its own assumptions, and the numerical thresholds sometimes used to label synergy (an index below one) are conventions rather than statistical tests. A common weakness in the hot-plate literature is that a combination is declared synergistic simply because its effect is larger than that of either drug given alone. That finding is equally compatible with simple addition and says nothing about interaction [9, 11].

Ceiling effects need separate mention. Because the hot-plate test uses a cut-off, response curves flatten at the maximum possible latency, and the data are censored. Percent of maximum possible effect is often used to normalise results, but it transforms the scale and can hide differences when control values are high. Non-parametric or censored-data methods are more defensible in principle, although few reports use them. A sound study would also include a vehicle group and each single agent at the doses used in the mixture, and would use the same observers and apparatus for every arm [8, 9].

5. EVIDENCE FOR THE COMBINATION

Several lines of evidence suggest that caffeine amplifies paracetamol. The strongest data come from human trials rather than the hot-plate test. A pooled analysis of controlled studies of analgesic combinations found that adding caffeine in a dose of 65 mg or more increased the proportion of patients with adequate relief [1], and a later Cochrane review of caffeine as an adjuvant for acute pain concluded that the gain was real but modest, so that a small additional proportion of patients reached good relief [5]. Trials in tension-type and other headaches pointed in the same direction [2, 34].

Headache is where the combination has been tested most thoroughly. A large multicentre trial compared paracetamol, aspirin and caffeine, alone and in combination, and found that the triple combination outperformed single agents and dual combinations for episodic tension-type headache [2]. A study in tension headache had earlier found a significant benefit when caffeine was added to paracetamol-containing regimens [34]. These trials do not isolate paracetamol plus caffeine, and some products contain a third active ingredient, so they should be read as supportive rather than conclusive for this pair [1, 35].

Reviews of non-prescription analgesics help place the pair in context. They conclude that paracetamol is a useful but modest analgesic for acute pain, that combinations with caffeine or with anti-inflammatory drugs can improve outcomes, and that the choice depends on the pain type and on tolerability [36, 37]. Experimental work on the combination is therefore supporting a clinical practice that is already established, which makes careful design all the more important.

Other work supports the idea at the pharmacokinetic and benefit-risk level. Reports of faster paracetamol absorption in the presence of caffeine offer a partial explanation for quicker onset [31], while a review of benefit and risk found that adverse events with usual adjuvant doses were mostly mild, such as nervousness or sleep disturbance [13]. Reviews of oral combination analgesics have also taken a broadly favourable view of rational combinations, provided the components have different mechanisms and the ratio is justified [35]. Comparisons of paracetamol with non-steroidal drugs or with both together show that combination approaches often outperform single agents in postoperative pain [36, 37, 38].

Preclinical work is thinner. A study using the pain-induced functional impairment model in rats reported that caffeine increased the antinociceptive effect of paracetamol, with the combination showing a larger effect than predicted from either component [39]. In mice, caffeine alone shows central antinociceptive activity in thermal tests at some doses [30], and reviews of methylxanthines and pain describe a mixture of analgesic and pronociceptive findings depending on dose, route and model [10, 12]. Direct, well-controlled hot-plate studies of the paracetamol and caffeine pair, with formal interaction analysis, remain surprisingly scarce.

Safety data deserve a place beside efficacy data. At the doses used in over-the-counter products, caffeine adds little to the risk profile of paracetamol, and the main concerns are mild sleep disturbance, palpitations in sensitive individuals and, with frequent use, caffeine-withdrawal headache [13]. Overviews of non-prescription analgesics rank paracetamol-based products alongside other options and note the value of knowing the number of patients who must be treated for one to benefit [37]. Animal work cannot address most of these questions, yet it can at least show whether a low caffeine dose produces enough benefit to justify exposing people to it.

The findings are collected in table 1 and grouped by type of evidence in fig. 1. What stands out is the imbalance. Method papers and mechanistic reviews are plentiful, clinical evidence is reasonably strong, and animal studies that test the specific combination in the hot-plate test with proper interaction analysis are few. That gap matters because decisions about ratios and dose levels in fixed-dose products have mostly relied on clinical convenience rather than on systematic preclinical dose-finding [35].

Translating between species is the weakest link. Rodent caffeine doses that give plasma concentrations comparable with a cup of coffee in people are not the same as simple weight-scaled equivalents, because rodents metabolise the drug much faster [14, 40]. A dose of caffeine that is low by human standards can therefore be inactive in a mouse, while a dose that looks high in milligrams per kilogram may reproduce only ordinary human exposure. Authors who ignore this point can overstate or understate the relevance of their findings, and it is a recurring source of disagreement between laboratories [13, 40].

6. DESIGN AND REPORTING ISSUES

Dose selection is the first practical problem. Doses are usually chosen in milligrams per kilogram, and translating them between species by body surface area rather than body weight avoids large errors when relating animal doses to human ones [40]. For a combination, the ratio should be fixed in advance, and for caffeine it should be low enough that locomotor stimulation is not confounding the end-point. Where a fixed human ratio is the target, that ratio should be tested first and others added afterwards, so that the experiment speaks to the product people actually take [35].

Controls complete the design. A vehicle group is essential, a positive control such as a standard opioid confirms that the apparatus is working on the day, and single-agent groups at the doses present in the mixture allow an additivity calculation [6, 7, 8]. Solubility matters in practice. Paracetamol is only moderately soluble in water at room temperature, and suspensions can give variable absorption, so preparation methods need to be reported. Route of administration also changes the interaction: oral dosing exposes both drugs to first-pass metabolism, while intraperitoneal injection delivers them rapidly and reduces that variability [3, 14].

Table 1: Selected published findings relevant to paracetamol and caffeine in thermal nociception

Topic	Reported finding or feature	Ref.
Hot-plate origin opioid screening	Heated surface used to time protective responses to evaluate centrally acting analgesics.	[6, 7]
Model comparison nociception tests	Thermal tests favour central drugs; no single assay represents clinical pain.	[15, 16, 17]
Reporting standards animal research	Randomisation, blinding and group size often missing from older reports.	[19, 20]
Paracetamol metabolite pathway	Conversion to AM404 in the nervous system engages TRP and cannabinoid targets.	[23, 24, 25]
Paracetamol descending pathways	Effect partly depends on bulbospinal serotonin; COX-3 relevance questioned.	[21, 22, 26]
Caffeine receptor action	Adenosine A_1 and A_{2A} antagonism at usual concentrations; cholinergic effects in mice.	[4, 27, 30]
Caffeine adjuvant role	Adjuvant action debated; central arousal and other sites proposed alongside adenosine blockade.	[10, 12, 28]
Rat functional impairment model	Caffeine increased the effect of paracetamol beyond that of either drug alone.	[39]
Isobolographic analysis	Compares experimental and theoretical additive doses with confidence limits.	[8, 9]
Pooled clinical trials	Caffeine added to analgesics gave a modest gain in the proportion with adequate relief.	[1, 5]

Timing needs equal care. Paracetamol and caffeine are absorbed quickly, but peak effects may not coincide, and a single observation time can favour one drug [3, 14]. Repeated testing across a time course gives more information, though it risks learning effects on the plate. A balanced approach uses separate groups for each time point or allows long intervals, with the cut-off kept short enough to avoid tissue injury [15, 18]. Whichever route is chosen, it should be stated clearly, and the observer should not know which treatment each animal received.

Statistical analysis is the last link in the chain. Latency data are often skewed and bounded by the cut-off, so means and standard errors may mislead. Dose-response lines should be fitted with methods suited to quantal or censored data, and the confidence limits of the ED_{50} values are the quantities that feed an isobolographic test [8, 9]. Multiple comparisons across doses and time points also need correction. Authors who run many tests and report only those that reach significance create an impression of synergy that independent groups cannot reproduce [19, 20].

The test itself has well-known limits as a model of clinical pain. Acute thermal pain in a healthy animal differs greatly from the persistent, often inflammatory or neuropathic pain for which paracetamol and caffeine are used, and most reviewers recommend a battery of tests instead of reliance on a single assay [15, 17]. The formalin test, with its two phases, allows distinction between acute nociception and inflammatory pain, and it has often been used alongside thermal tests for exactly this reason [41]. A combination that appears synergistic in only one assay should be treated as a lead, not a result.

Sex and strain deserve explicit attention. Many older studies used only male animals, and sex differences in pain sensitivity, in caffeine metabolism and in analgesic

response are well recognised [16, 17]. Outbred and inbred strains differ in baseline latency and in responsiveness to adenosine-related drugs, so conclusions from one population may not carry over. Sample-size justification, allocation concealment and the pre-specification of the primary end-point are expected under current reporting standards, and they are particularly important for interaction studies, where small differences are being interpreted [19, 20].

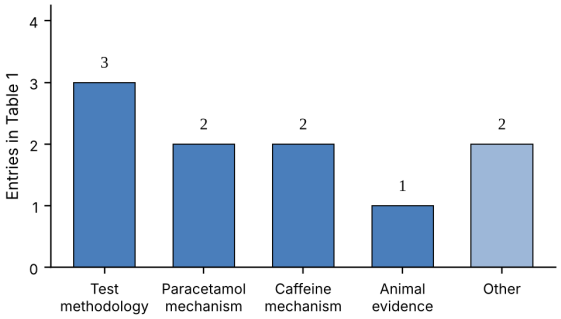


Fig. 1: Number of entries in table 1 by category of evidence

7. LIMITATIONS AND FUTURE DIRECTIONS

This review has several limits of its own. It is a narrative synthesis, not a systematic search, and so it may under-represent older or regional reports. Because the literature specific to the paracetamol and low-dose caffeine pair in the hot-plate test is small, many statements here rest on neighbouring evidence from other models, human trials or mechanistic work. Such extrapolation is reasonable as a starting point, but it should not be mistaken for direct proof, and readers should treat the conclusions about mechanism with particular caution [10, 12, 28].

There is also a conceptual limit. A positive interaction in a thermal test tells us little about whether the combination would help in chronic or inflammatory pain, where adenosine signalling is altered and where the contribution of peripheral mechanisms is greater [12, 28]. Human experimental pain models, including heat and pressure stimuli in volunteers, may offer a bridge between animal results and clinical trials, but they too have been used for this pair only sparingly [26, 31]. Closing that gap would give the field a more coherent evidence chain.

Finally, publication practice may distort the record. Studies in which a combination does nothing special are less likely to be written up, and an enthusiastic literature on adjuvants can result. Registration of animal protocols, publication of negative findings and sharing of raw latency data would all help readers judge how strong the evidence really is [19, 20]. Such steps cost little and would strengthen any later quantitative synthesis of the preclinical work on analgesic combinations [17, 35].

The most useful next step would be a transparent set of preclinical experiments done by independent groups. These would include full dose-response curves for each drug, a pre-specified fixed ratio, an isobolographic or equivalent analysis with confidence limits, measurement of plasma concentrations so that pharmacokinetic and pharmacodynamic effects can be separated, and complementary assays beyond thermal latency [8, 9, 33]. Adenosine-receptor antagonists with known selectivity and cannabinoid or serotonergic blockers could help to clarify the pathway involved [23, 24, 25, 26]. Adherence to reporting standards would make such studies far easier to compare and combine [19, 20].

8. CONCLUSION

The published evidence supports the idea that low-dose caffeine can strengthen the effect of paracetamol, and the clinical findings are the most consistent part of that picture. In the hot-plate test specifically, the case is far less secure. The test is sensitive to central actions and is practical for screening, but it responds unevenly to paracetamol, depends heavily on design details, and has seldom been used with formal interaction analysis for this pair [5, 6, 15, 39].

For researchers, the message is practical. Fix the ratio in advance, test each drug alone at the doses in the mixture, apply an accepted definition of additivity, describe the apparatus and blinding fully, and consider pharmacokinetics before concluding that two drugs interact at the receptor [9, 11, 19, 31]. Until such studies accumulate, claims of analgesic synergy between paracetamol and caffeine in this assay are better described as

promising than proven.

9. ABBREVIATIONS

AM404-N-arachidonoylphenolamine, ARRIVE-Animal Research: Reporting of In Vivo Experiments, CB_1-Cannabinoid receptor type 1, COX-Cyclooxygenase, ED_{50}-Dose producing 50% of the maximal effect, $5\text{-}HT_3$-5-Hydroxytryptamine receptor type 3, TRP-Transient receptor potential

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11. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

12. CONFLICT OF INTERESTS

Declared none

13. REFERENCES

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