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The Analgesic and Anti-inflammatory Properties of Caffeine Co-administration with Nefopam in Mice

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A B S T R A C T

This study aimed to evaluate the analgesic properties of nefopam and the adjuvant effects of caffeine. It also investigated the anti-inflammatory effect of co-administering nefopam and caffeine on Prostaglandin E2 level (PGE2). The study involved 56 female albino mice (Mus musculus), divided into seven groups, eight mice per each group. Group A which served as the control group received distilled water; Group B received 30 mg/kg BW of nefopam orally; Group C received 28.5 mg/BW of nefopam and 5 mg/BW of caffeine orally; Group D received 27 mg/BW of nefopam and 10 mg/BW of caffeine orally; Group E received 25.5 mg/BW of nefopam and 15 mg/BW of caffeine orally; Group F received 24 mg/BW of nefopam and 20 mg/BW of caffeine orally; Group G received 22.5 mg/BW of nefopam and 25 mg/BW of caffeine orally. The nociceptive response was assessed using the formalin test, showing a significant (P<0.05) reduction in pain response (accumulative time of flinching and licking) was the first phase (0-5 minutes) in Group E as well as, a significant (P<0.05) decrease in nociceptive response in flinching and licking in second phase (20-60 minutes) was observed in Group D. However, the Prostaglandin level was significantly (P<0.05) lower in Group C. The study concluded that adding low dosages of caffeine to analgesics can boost pain relief in smaller quantities. Caffeine co-administration with nefopam may exert

Keywords: pain, nefopam, caffeine, mice, formalin test, PGE2

INTRODUCTION

Pain is an unpleasant sensory and affective experience associated with actual or tissue damage within the body or articulated in relation to such damage (1). Classification of pain into 4 groups, pain nociception, perception of pain, pain suffer, and behaviors of pain, to each grounded taking place anatomy, physiology, and psychology factor toward underset and dissimilar pain types (2). Inflammation stands for the reaction of the body for injury or other tissue infection (3). Different mediators are included in the inflammation, as prostaglandins (4). Injection of formalin, causing painful inflammation stimulus triggered with painful stimulation dependent on existence on prostaglandin-E2 (PGE2). Cyclooxygenase-2(COX-2) on nervous cell in charge of creating prostaglandin (PG) (5). The need with recall emergency depending on pain severity (6). Healthcare providers must possess a comprehensive understanding of pain in order to effectively conduct a thorough pain evaluation (7). Pain is considered to be a significant hurdle in various contexts (8). Analgesic, or pain reliever, is a medication that targets pain without disrupting nerve signal transmission, altering sensory perception, or affecting consciousness (9). Classification is based on their primary function, which is primarily related to nociception and pain perception. These

two aspects are closely intertwined and contributed to the overall pain experience (10). Analgesic is a category of drugs that are extensively utilized and subjected to misuse on a global scale (11), especially with experiences a high level of pain (12). Around half of the patients requiring surgical intervention were provided with analgesic medications (13). The analgesics have been awarded formal permission for usage (14). The incidence of analgesia subsequent to experimental operations is undertaken in laboratory settings (15). Non-opioid medications are generally considered safe comparing to other non-steroidal anti-inflammatory drugs (16). Nefopam, a nonopioid analgesic, works by inhibiting the reuptake of dopamine, noradrenaline, and serotonin in both the spinal and supra spinal areas (17). This medication is classified in the benzoazocine group. It is commonly used as a substitute for opioid analgesic medications in order to relieve moderate to severe pain (18). Caffeine is present in coffee, tea, and soft drinks (19). Caffeine is a stimulant that is commonly used to enhance neurological responses in both humans and animals (20).The neuroprotective properties of caffeine and its potential therapeutic applications in animal models, specifically in inhibiting the A1, A2, and A3 adenosine receptors in the CNS(21). Nefopam monotherapy in humans may cause drowsiness and sweating, and patients with renal dysfunction may experience compromised medication elimination. Proper oral dosages are crucial for effective treatment (22). Therefore, this study is interested in using a co-administration approach with caffeine by adding tiny amounts of caffeine to medications to boost their analgesic impact in order to reduce the required oral doses of nefopam, which is highly desirable for enhancing pain management.

MATERIALS AND METHODS

Ethical Approval and Animals

All procedures in this study was reviewed and approved by the local Committee on Animal Use and Care, College of Veterinary Medicine, University of Baghdad under the reference number 2289 dated in October 19, 2023. The study involved 56 female albino mice (*Mus musculus*), aged two months and weighing 25 g, housed at the University of Baghdad's College of Veterinary Medicine. The mice were housed in plastic cages measuring 20×30×50 cm^3 and given a two-week adjustment period before starting the experiment. The standard rodent diet was provided, and the environmental conditions were carefully regulated to a temperature of 20 (±5) °C. A 14/10-hour light/dark cycle was implemented to mimic natural day and night patterns. Ventilation vacuums were used for regular air replacement, and the litter was routinely replaced weekly.

Experimental Design

The animals were divided into seven groups; each group consisted of eight mice, the duration for each group was a day and the frequency of each medicine was once a day. Group A, which served as the control group, received distilled water orally. Group (B), received nefopam alone (30 mg/kg BW) orally (23), then decreasing the dose of nefopam about 5% in each group based on the original dose (23), and caffeine dose starting with 5% on the next groups based on the original dose (100 mg/kg BW) (24). Group (C), received 95% of original dose of nefopam at dose (28.5 mg/kg BW) and 5% of caffeine original dose at dose of (5 mg/kg BW) orally; Group (D), received 90% of total original dose of nefopam at dose of (27 mg/kg BW) and 10% of total original dose of caffeine at a dose of (10 mg/kg BW) orally. Group (E), received 85% of total dose of nefopam at (25.5 mg/kg BW) and 15% of total dose of caffeine at a dose of (15 mg/kg BW) orally ; Group (F), received 80% of total dose of nefopam at dose of (24 mg/kg BW) and 20% of total dose of caffeine at dose of(20mg/kg BW) orally; Group (G), received 75% of total dose of nefopam at dose of (22.5 mg/kg BW) and 25% of total dose of caffeine at a dose of (25 mg/kg BW) orally.

Preparation of Dosage Solutions and Doses

The stock solution of nefopam (REX pharma, India) was prepared at a concentration of 30 mg, diluted with 10 mL of distilled water (DW) with a final dose at 0.1 mL/10 g BW mouse (23). Similarly, the caffeine (Alpha chemika, India) stock solution had a concentration of caffeine 100 mg, diluted with 10 mL of DW with a final dose at 0.1 mL/10 g BW mouse (24). In order to prepare stock solutions with varying concentrations of nefopam, a series of dilutions were performed using the original nefopam stock solution and a caffeine stock solution. To prepare a 95% nefopam stock solution, 9.5 mL of the original nefopam stock solution and 0.5 mL of the original caffeine stock solution (5% concentration) were used. Similarly, for the preparation of a 90% nefopam stock solution, 9 ml of the original nefopam stock solution and 1 mL of the original caffeine stock solution (10% concentration) were used. The process was repeated to prepare 85% nefopam stock solution, 8.5 mL of the original nefopam stock solution and 1.5 mL of the original caffeine stock solution (15% concentration) were used, 80% nefopam stock solution, 8 mL of the original nefopam stock solution and 2 mL of the original caffeine stock solution (20% concentration) were used, and 75% nefopam stock solution, 7.5 mL of the original nefopam stock solution and 2.5 mL of the original caffeine stock solution (25% concentration) were used.

Evaluation of Analgesic Formalin Induced Acute Pain

The study involved mice given a subcutaneous injection of 10 µL of 4% formalin (Central Drug House, India) to assess acute pain, on the right side of the hind paw of mice.

The formalin model reveals the biphasic nociceptive response which means of frequency of licking of the injected paw or flinching, a pain response characterized by spontaneous, fast, transient shaking or raising of the paw. Data were recorded the average frequency of licking and flinching during the initial acute phase (phase I) within the first five minutes and the lasting phase (phase II) between 20 and 60 minutes after the injection (25).

Assessment of Serum Prostaglandin E2 (PGE2) Level After Induced Pain

Upon the end of the experiment, five mice from each group were euthanized using chloroform, and blood samples were collected from the heart using a sterile 1 mL syringe that was fitted with a 25-gauge needle. The blood samples were collected in gel tube for serum isolation. The serum was then pipetted and transferred to sterile centrifuge tubes for ELIZA analysis. The serum levels of prostaglandin E2 (PGE2) were assessed using a mouse ELISA kit catalog number: MBS729260 (MyBioSource, USA). The kit contains a polyclonal anti-PGE2 antibody and a PGE2-HRP conjugate. The PGE2-HRP conjugate was incubated with the assay sample and buffer for one hour. After decantation and washing, the wells were used for the incubation of the horseradish peroxidase (HRP) enzyme substrate. A final solution was introduced, resulting in a noticeable yellow coloration. The intensity of the color was measured at 450 nm using a spectrophotometer (HUMA READER HS, 65205 Wiesbaden, Germany), in a microplate reader. The intensity of the color is inversely related to the concentration of PGE2, as there is competition between PGE2 in the samples and the PGE2 HRP conjugate for binding to the antiPGE2 antibody site.

Statistical Analysis

Program SAS2018 applied for detecting influence with numerous aspects for parameters of the study. This study applied (LSD) Least Significant Difference $P \leq 0.05$, horizontally in addition vertically in which means usually with Analysis of Variance two-way ANOVA, for effective comparison way also significance statistical (26).

Results And Discussion

Formalin Test

Formalin test persuaded pain consumes 2 phase's initial phase as well as, late phase. The blockade of serotonergic or adrenergic receptor showed the analgesic effects of nefopam in the first phase of the formalin test while, the late phase is mainly related to the suppression of prostaglandin production, which is involved in pain regulation (27). The study assessed the inflammatory pain response by measuring PGE2 levels. The initial phase of this study involved comparing all treated groups to determine which group exhibited the lowest nociceptive response, measured by the average frequency of licking and flinching. In the second phase, a comparison was again made between all treated groups to identify which group displayed the lowest nociceptive response during the inflammatory phase. The investigation revealed the nociception response (average of frequency of licking and flinching) in phase 1 (0-5 min) was significantly ($P<0.05$) higher in group A, the control group than other groups. The lowest value was in Group E, Group D and Group F, showed a significant ($P<0.05$) decrease in average of frequency of licking and flinching when compared to group C and Group B and Group A the control group ,however, Group C and Group B, showed a significant ($P<0.05$) decrease in average frequency of licking and flinching when compared with Group A the control group, lastly, Group G was insignificant difference when compared to Groups C, D, E, and F and significant decrease when compared to Group B and Group A the control group. In phase 2 (20-60 min), the nociception response (average frequency of licking and flinching) was significantly ($P<0.05$) higher in the control group (Group A),than other treated groups ,while in Group D and Group E showed a significant ($P<0.05$) decrease in average frequency of licking and flinching when compared to Group B and Group A the control group, however, Group C ,Group G and Group F was insignificant different when compared with Group D, Group E and Group B. On the other hand, all treatment groups showed a significant decrease in average frequency of licking and flinching when compared to Group A the control group as depicted in Figure 1.

The findings of phase 1 (0-5 min) are consistent with the prior study by (27), which found that nefopam, derived from diphenhydramine, inhibits histamine H1 receptor and monoamine reuptake, causing elevated levels of noradrenaline, dopamine, and serotonin. Also found that spinal noradrenaline depletion decreases nefopam's analgesic effect during phase 1 but not phase 2 of the formalin tests, providing analgesic relief for pain which explain significant decrease in accumulation time of licking and flinching in all groups treated with nefopam alone or in combination. The results of phase 2 (20-60 min) the investigated result showed that caffeine used as an adjuvant to enhance the effectiveness of pain relief showed antinociceptive response in second phase, with doses ranging which aligns with the findings of previous study conducted by (28), who explain a significant ($P<0.05$) decrease shown in Group D and Group E when compared to groups B and the control group. However, the results showed high doses of caffeine have antinociceptive effects in the formalin test and produce a comparable analgesic outcome for pain which agreed with prior study (29), which explain insignificant different in groups with high dose of caffeine. According to a previous study (30), caffeine administration at therapeutic doses is unlikely to have any noticeable effects or modifications. Furthermore, notable variances may be attributed to the dispensation of elevated quantities within the recommended therapeutic range of

caffeine. This may explain the negligible variances observed in certain cohorts administered distinct co-administrations of nefopam and caffeine in both phases.

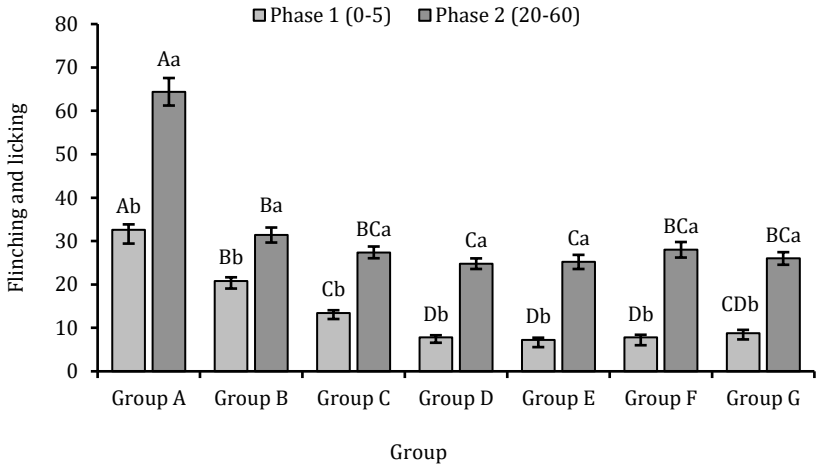

Figure 1. Biphasic nociceptive response (Average frequency of licking and flinching) comparison between different groups co-administered with nefopam and caffeine, as determined by the formalin test. The error bars represent the standard error of the mean (SEM). Mean ± SEM with statistical significance denoted as follows: different uppercase letters indicate significant differences between groups within the same phase, and different lowercase letters indicate significant differences across different phases for the same group ($P \leq 0.05$). n=8. LSD value=5.483

Serum PGE2

The study found a significant ($P<0.05$) increase in PGE2 levels in Group B. In contrast, Group C, had a statistically significant decrease in PGE2 levels when compared with other groups and an insignificant difference between other groups and the control group as demonstrated in the Figure 2.

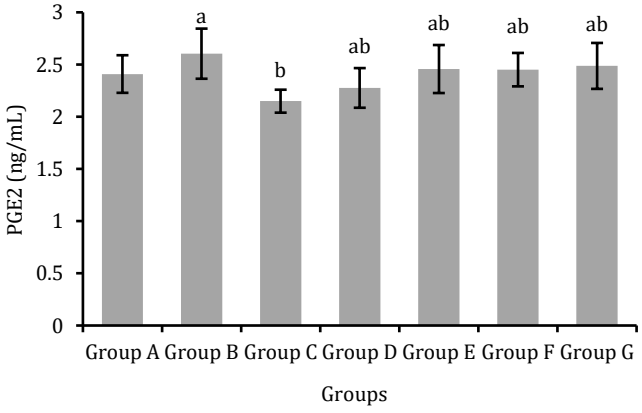

Figure 2. Comparison between different groups Co-administrated with nefopam and caffeine are determined by PGE2 level. Mean±SEM with different letters in are significantly different. * $P \leq 0.05$. n=8, LSD=0.407

A previous investigation (31), which confirmed the findings, showed that the inhibition of glutamate release may be responsible for Nefopam's analgesic effects, at least in part. Injecting formalin into the hind paw leads to a notable increase in the levels of glutamate outside the cells in the first phase of the experiment. This release of glutamate is likely responsible for the initial effects and contributes to the rise of pain-promoting chemicals, such as prostaglandin E2. Such chemicals are expected to intensify the processing of pain signals in the second phase of the formalin test. This might explain the significant rise in PGE2 levels seen in Group B, which only received nefopam. Nefopam is often used as an alternative to opioid analgesics for the treatment of moderate-to-severe pain. This medicine is classified as a member of the benzoxazine chemical family and exerts its analgesic effects by acting on the central nervous system. This drug is not classified as an opioid and does not bind to opioid receptors or have anti-inflammatory effects. However, it does exhibit an improved analgesic response compared to other groups.

Caffeine is endowed with anti-inflammatory effects, which coincide with previous study (32).The finding of the study aligns with investigations conducted by (33), that found caffeine administration triggers an inflammatory response linked to hyperalgesia, inhibit the production of inflammatory cytokines, specifically TNF-α, which can alter the effect of PGE2 on liver injury possibly explaining the significant reduction of PGE2 in Group C when compared to Group B. The finding supported by the research (29) that high caffeine doses can alleviate acute pain, but they don't

stop inflammation development. This is because high caffeine concentrations increase but do not reduce basal mechanic sensitivity in the central systems. This may explain the insignificant differences observed in groups receiving larger doses of caffeine co-administrated with nefopam compared to the control group.

Finally, there are a lot of options for reducing the amount of nefopam used orally to alleviate discomfort. When used in tiny doses, caffeine amplifies the analgesic benefits of nefopam, making it an even more effective pain reliever. By influencing prostaglandin E2 (PGE2), caffeine has shown anti-inflammatory properties when administered in conjunction with nefopam.

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N/A

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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فعالية الكافيين مع النيفوبام لتسكين الالم والمضادة للالتهابات عند الفئران

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فرع الفسلجة والكيمياء الحياتية والادوية، كلية الطب البيطري، جامعة بغداد، العراق

الخلاصة

ان الهدف من هذه الدراسة هو فحص الخصائص المسكنة للالم للنيفوبام والتاثيرات المساعده المحتمله عند اضافه للتناول المتزامن للنيفوبام والكافيين معا وكذلك هدفت هذه الدراسه الى التاكد من فعاليه التاثير المضاد للالتهابات على PGE2وشملت الدراسة ٥٦ فأراً من إناث الألبينو مقسمة إلى سبع مجموعات، ثمانية فئران لكل مجموعة. وتم تقسيم الحيوانات عشوائيا إلى سبع مجموعات، ثمانية فئران في كل مجموعة، (المجموعة أ) وهي كانت مجموعة السيطرة التي تم اعطائها الماء المقطر، (المجموعة ب) التي تلقت جرعة من نيفوبام فقط، وتحديدا ٠,٧٥ ملغ لكل وزن الجسم الفار، تعطى عن طريق أنبوب المعدة. (المجموعة ج) تلقت نيفوبام بجرعة ٠,٧١ ملغ لكل وزن الجسم الفار والكافيين بجرعة ٠,١٢٥ ملغ لكل وزن الجسم الفار، أيضا تعطى عن طريق أنبوب المعدة. (المجموعة د) تلقت نيفوبام بجرعة ٠,٦٧ ملغ لكل وزن الجسم الفار والكافيين بجرعة ٠,٢٥ ملغ لكل وزن الجسم الفارعن طريق أنبوب المعدة. (المجموعة هـ) تلقت نيفوبام بجرعة ٠,٦٣ ملغ لكل وزن الجسم الفار والكافيين بجرعة ٠,٣٧ ملغ لكل وزن الجسم الفارعن طريق أنبوب المعدة. (المجموعة و) تلقت نيفوبام بجرعة ٠,٦ ملغ لكل وزن الجسم الفار والكافيين بجرعة ٠,٥ ملغ لكل وزن الجسم الفار عن طريق أنبوب المعدة. وأخيرا، (المجموعة ز) تلقت نيفوبام بجرعة ٠,٥٦ ملغ لكل وزن الجسم الفار والكافيين بجرعة ٠,٦٢ ملغ لكل وزن الجسم الفارعن طريق أنبوب المعدة. تم استخدام نماذج تجريبية لفحص الفوائد المسكنة والمضادة للالتهابات. يقيم اختبار الفورمالين الاستجابة الحساسة للألم؛ كان هناك انخفاض ملحوظ في الاستجابة الحساسة للألم، التي تشمل كلا من الألم الحاد والمزمن. وقد أظهرت النتائج في المرحلة ١ (٠-٥ دقائق) في الجفل واللعق كانت الأدنى في (المجموعة E) التي تعاملت مع نيفوبام عند جرعة ٠,٦٣ ملغم لكل وزن الجسم الفار والكافيين بجرعة ٠,٣٧ ملغ لكل وزن جسم، في حين كانت الاستجابة المسبب للألم في المرحلة الثانية (٢٠-٦٠ دقيقة) في الجفل واللعق هي الأدنى في (المجموعة د) التي عولجت بالنيفوبام بجرعة ٠,٦٧ ملغ لكل جسم. الوزن والكافيين بجرعة ٠,٢٥ ملغ لكل وزن الجسم.وكذلك اظهرت النتيجه انخفاضًا ملحوظًا في البروستاجلاندين في (المجموعة ج) التي تلقت جرعة من نيفوبام ٠,٧١ ملغ لكل وزن الجسم الفار والكافيين بجرعة ٠,١٢٥ ملغ لكل وزن جسم الفار). أن إضافة جرعات قليله من الكافيين إلى جرعة تقليدية من المسكنات المستخدمة بشكل شائع قد لوحظ أنها تعزز تخفيف الألم بكميات أقل مقارنة بإعطاء المسكن وحده. يتمتع الكافيين بالتزامن مع نيفوبام بخصائص مضادة للالتهابات تظهر مع البروستاجلاندين E2) PGE2).

الكلمات المفاحية: مسكن للألم، نيفوبام، كافيين، الفئران، اختبار الفورمالين، بروستاجلاندين