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Evaluation of the Antioxidant Potentials of Caffeine on Pain Hypersensitivity in Wistar Rats Following Repetitive Pain Stimulation

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Abstract **Original Research Article**

The study evaluated the antioxidant potentials of caffeine on pain hypersensitivity in Wistar rats following repetitive pain stimulation. The study design consist of three phases of drug administration, short term 14days, intermediate phase 63 days and long term 105 days, with the treatment groups as follows caffeine (low 10mg/kg, high 15mg/kg). Morphine (low 5mg/kg, high 10kg/kg) and their combination. Pain hypersensitivity was measured using tail immersion and analgesy-meter tests. The brain tissues of the rats was harvested and analyzed for antioxidant potentials using glutathione peroxidase 1, catalase, glutathione and superoxide dismutase levels were assayed. Using sigma-aldrich kits. The biochemical assays were conducted in the biochemistry department of the University of Port Harcourt. The results from the study demonstrated pain attenuating potential of caffeine in a dose depended manner. And also the study demonstrated a consistent increase in antioxidant effect in both the caffeine and morphine groups in a dose depended manner with the high dose having the highest effect. And also the coadministration groups there were a consistent antioxidant effects. The therapeutic effect of caffeine and morphine is linked to their antioxidant potentials and as such caffeine is a good adjuvant to opioid in the management of pain.

Keywords: Caffeine, Antioxidant, Pain Hypersensitivity, Morphine.

1. INTRODUCTION

Caffeine is an alkaloid named "3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione", (*Alireza Baratloo, 2016)*. Caffeine is a psychoactive and central nervous system stimulant of the methylxanthine class that unlike many other psychoactive is legal all around the world. (*Sri Harsha Boppana et al., 2022)* Caffeine is a naturally occurring substance find in fruits, seeds and leaves of many plants, where it was thought to functions as natural pesticides. (*Christopher J Derry, 2014)* It has a long term history of human consumption dated back to at least 5000 years. It is normally consumed as beverage, such as tea, coffee, and food stuff such as chocolate. The usage of caffeine varies from different individuals and population but can be classified as low, moderated and high with the people falling between low and moderate intake. Caffeine is known to act as a central nervous system stimulant as a methylxanthine, which have various physiological effects on humans. The primary receptor site for caffeine in the body is the adenosine receptor. Adenosine is a neurotransmitter that plays a role in regulating sleep-wake cycles, mood, and other physiological processes. Adenosine receptors are found throughout the body, including in the central nervous system (CNS), cardiovascular system, and other tissues. Caffeine, being a chemical structure similar to adenosine, can bind to and block the adenosine receptors, particularly the A1 and A2A subtypes. By blocking these receptors, caffeine can exert its psychostimulating effect, cardiovascular system, and other organs of the body.

2. MATERIALS AND METHOD

Experimental animals weighing between 120–180g were obtained from the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt were used for this study and they were provided with standard laboratory rat feeds and water ad libitum. The experimental study design was categorized into three phases: Phase 1 (Short term) where drugs were administered for fourteen days, Phase 2 (intermediate phase) with a 63-day administration and Phase 3 (long term) lasting 105 days. The animals were grouped as follows: The experiment was structured into three distinct groups, each subjected to different treatment protocols to evaluate their responses to pain and their

antioxidant effect. Group 1 served as the control group, with subjects in Control 1 administered distilled water and maintained in a stress-free environment throughout the experiment. In Control 2, subjects were also placed under stress-free conditions but were exposed to various tests without any drug treatment, facilitating a comparison for the effects of other treatments. Group 2, the morphine group, received repetitive pain stimulation through the use of electroconvulsive unit and hot plate analgesy-meter and thereafter treated with a low dose of morphine (5 mg/kg) or a high dose (10 mg/kg). Following treatment, subjects were evaluated through various cognitomotor tests. Similarly, Group 3, the Caffeine group, was administered low (10 mg/kg) and high (15 mg/kg) doses of, with the animals undergoing the same set of pain sensitivity tests after treatment.

This structured approach allowed for systematic evaluation of antioxidant potentials across different treatments, providing valuable insight into the efficacy of morphine and caffeine in managing pain. Statistical analysis employed one-way ANOVA with Newman-Keuls post-hoc tests to determine significant differences among treatment groups. Ethical approval for the study was granted by the University of Port Harcourt. In the experimental protocols, we utilized a hot plate from Ugo Basile Srl, set to a pre-determined temperature of 52.5°C, which is suitable for rats (*Mun_Fei_Yam, 2020*), to observe responses such as licking, shaking, or stepping of the hind paws, recording the duration of these responses before removing the rat after a maximum of 60 seconds to prevent skin damage. The tail flick method was employed to assess analgesic activity, where the rat instinctively withdraws its tail from heat, indicating pain perception *(Daniel Le Bars et al., 2002)* The Randall-Selitto test, which evaluates mechanical hyperalgesia, involved applying increasing pressure to the rat's paw or tail until withdrawal or vocalization occurred, using the Ugo Basile Analgesy-Meter.(*Mun_Fei_Yam, 2020*).

3. RESULTS

Table 1: Antioxidant effect of Glutathione peroxidase 1

Groups	14 days	63 days	105 days
Group 1 (Control)	0.17±0.00	0.18b±0.10	0.08b± 0.10
Group 2 (Pain Only)	0.16±0.00	0.16*±0.10	0.06*±0.10
Group 3 (Pain + 2mg/kg Morphine)	0.15*b±0.00	0.16*±0.10	0.07*b±0.10
Group 4 (Pain + 5mg/kg Morphine)	0.18b±0.01	0.18b±0.11	0.08b±0.10
Group 5 (Pain + 10mg/kg Caffeine LD)	0.06*±0.00	0.05*±0.00	0.06*±0.00
Group 6 (Pain + 15mg/kg caffeine HD)	0.07±0.00	0.07±0.00	0.07*±0.00
Group 7 (Pain + 5mg/kg morp+10mg/kgcaff	0.08b±0.01	0.07±0.01	0.07b±0.00

The results from the table 1 shows, the effect of morphine on the antioxidant status of the brain tissue, there was a consistency in the low dose from day 14 to 105. However the high dose has the higher antioxidant effect than the low dose. And also in the caffeine group too, there was consistency in the antioxidant effect in the low dose. The high dose shows higher antioxidant effect.

Table 2: Antioxidant effect of Glutathione

Groups	14 days	63 days	105 days
Group 1 (Control)	3.12b±0.34	3.50b±0.19	2.78b±0.25
Group 2 (Pain Only)	2.64*±68.92	2.19*±0.25	1.87*±0.03
Group 3 (Pain + 2mg/kg Morphine)	1.84b±0.03	1.82*±0.07	2.13*±0.07
Group 4 (Pain + 5mg/kg Morphine)	3.02b±0.36	2.69*b±0.29	2.46b±0.02
Group 5 (Pain + 10mg/kg Caffeine LD)	2.04b±0.13	1.82*±0.13	1.95*±0.07
Group 6 (Pain + 15mg/kg caffeine HD)	2.33b±0.09	2.44*±0.06	2.21*±0.04
Group 7 (Pain + 5mg/kg morp+10mg/kgcaff	2.77b±0.30	2.22*±0.32	2.37*b±0.13

The results from table 2 shows, there was consistency in the antioxidant effect on the morphine group, with the high dose showing higher antioxidant effect. In the same manner the caffeine group showed a consistency in the antioxidant effect in a dose depended manner with the high dose having the higher antioxidant effect. And also the coadministration also showed consistency in the antioxidant effect.

Table 3: Antioxidant effect of Catalase

Groups	14 days	63 days	105 days
Group 1 (Control)	2.00.28b±111.20	3.22±0.16	2.87b±0.16
Group 2 (Pain Only)	3.13*±0.23	2.64±0.13	2.13*±0.15
Group 3 (Pain + 2mg/kg Morphine)	2.22*±0.31	2.27±0.29	1.75*±0.06
Group 4 (Pain + 5mg/kg Morphine)	3.25*±0.08	2.83±0.15	2.15*±0.13
Group 5 (Pain + 10mg/kg Caffeine LD)	2.81*±0.51	2.72±0.05	1.66*b±0.19
Group 6 (Pain + 15mg/kg caffeine HD)	2.13*±0.41	2.22±0.05	2.19*±0.02
Group 7 (Pain + 5mg/kg morp+10mg/kgcaff	2.08*±0.64	1.64±0.15	1.26*b±0.02

The results from table 3 shows, there was consistency in the antioxidant effect on the morphine group, with the high dose showing higher antioxidant effect. In the same manner the caffeine group showed a consistency in the antioxidant effect in a dose depended manner with the high dose having the higher antioxidant effect. And also the coadministration also showed consistency in the antioxidant effect.

Table 4: Antioxidant effect of Super oxide dismutase (SOD)

Groups	14 days	63 days	105 days
Group 1 (Control)	0.23±0.11	0.21±0.06	0.17±0.14
Group 2 (Pain Only)	0.20±0.10	0.18±0.02	0.23±0.11
Group 3 (Pain + 2mg/kg Morphine)	0.25±0.10	0.22±0.02	0.23±0.11
Group 4 (Pain + 5mg/kg Morphine)	0.13*b±0.03	0.13±0.01	0.22±0.12
Group 5 (Pain + 10mg/kg Caffeine LD)	0.28±0.04	0.25±0.02	0.24b±0.00
Group 6 (Pain + 15mg/kg caffeine HD)	0.27*±0.00	0.18*b±0.04	0.19*b±0.01
Group 7 (Pain + 5mg/kg morp+10mg/kgcaff	0.14*b±0.02	0.19*b±0.03	0.14*b±0.02

The results from table 4 shows, there was consistency in the antioxidant effect on the morphine group, with the low dose showing higher antioxidant effect. In the same manner the caffeine group showed a consistency in the antioxidant effect in a dose depended manner with the low dose having the higher antioxidant effect. And also the coadministration also showed consistency in the antioxidant effect.

4. DISCUSSION

This study evaluated the potential antioxidant effects of caffeine on pain hypersensitivity after repetitive pain stimulation in Wistar rats. The study sought to establish the potential antioxidant effects of caffeine and morphine on pain management, as well as their possible synergistic benefits. The results from the study are important in elucidating the antioxidant potentials of caffeine and morphine as well as their synergy in mitigating pain and thereby enhancing our understanding in the antioxidant effect of caffeine and morphine and their therapeutic benefits.

Table 1. The study demonstrated that morphine showed antioxidant effect in the low dose in such a manner that there was significant increase ≤.05 in day 14, and 63, and in day 105 and in the high dose too, there was also a significant increase ≤.05 in day 14, 63 and reduction in day 105. The increase in the glutathione peroxidase 1 in the morphine groups showed that

morphine has antioxidant effect and thereby enhancing its analgesic effect. However, its tolerance effect in prolonged use is also seen. (*Saeed_Samarghandian 2014).*

In the caffeine groups, the study demonstrated a significant consistent increase in the low and high doses in the antioxidant effect of glutathione peroxidase 1, showing that caffeine enhances antioxidant effect and thereby it's mitigating effect on pain. (*Bianca-Eugenia Osz et al., 2022)*

There was a consistency in the coadministration, thereby demonstrating the antioxidant effect of the synergy of caffeine and morphine in mitigating pain and as such enhancing our understanding of the synergic effect of caffeine and morphine.

Table 2. Glutathione: the study demonstrated a consistency in the antioxidant effect of glutathione in the morphine groups and as such morphine does possess antioxidant properties.

The caffeine groups also demonstrated antioxidant effect comparable to morphine which elucidates its pain mitigating effect.

There was also a consistency in the coadministration of caffeine and morphine in their antioxidant effect. The synergic effect of caffeine and morphine buttress our understanding in the management of pain. *(Alexander Yashin, 2013)*

Table 3. Catalase the study demonstrated the antioxidant effect of morphine in the low dose and also in the high dose but there was tolerance in the day 105in the low dose and in the high dose. However the high dose showed higher antioxidant effect, on other hand caffeine showed high antioxidant effect in the low dose than the high dose. And the coadministration there was an antioxidant effect. Caffeine and morphine.

Table 4. super oxide dismutase. The study demonstrated there was consistency in the antioxidant effect of morphine in the low and the high dose but low dose having the high antioxidant effect than the high dose. (*Saeed__Samarghandian 2014);* (*Sandrine Reymond, 2022).*

There was also a consistency in the low and high dose of the antioxidant effect of caffeine but the low dose has the higher antioxidant effect than the high dose. These finding elucidate our understanding of pain attenuating effect of caffeine. There was also an antioxidant effect by the coadministration group. Thereby the coadministration of caffeine and morphine may enhance our understanding in the management of pain. (*˙LHAMI GU¨ LC¸ I˙N, 2008)*

5. CONCLUSION

The results demonstrated that caffeine has antioxidant potentials that is consistent in a dose depended manner and this antioxidant effect is the reason of the high enhancing effect in pain management and other benefits, such as cognitive and motor performance. Morphine is potent in managing pain. And this is attributed to its antioxidant potentials. The synergic effect of caffeine and morphine will serve as better way in enhancing analgesic effect in pain and it is also beneficial in mitigating neurodegeneration. These findings contribute to the emerging evidence of the need for tailored, multi-faceted approaches in the management of chronic pain. This finding could provide concise idea and improve other researchers in the management of pain in the future.

REFERENCES

- Alexander Yashin, Yakov Yashin, Jing Yuan Wang & Boris Nemzer. (2013). Antioxidant and Antiradical Activity of Coffee. *Journal of Antioxidants*: 2, 230-245; doi:10.3390/antiox2040230
- Bianca-Eugenia ″Osz, George Jîtcă, Ruxandra-Emilia S, tefănescu, Amalia Pus, cas, Amelia Tero-Vescan & Camil-Eugen Vari (2022). Caffeine and Its Antioxidant Properties—It Is All about Dose and Source. International Journal of molecular sciences: 23, 13074.https://doi.org/10.3390/ijms232113074
- Daniel Le Bars, M Gozariu & Samuel W Cadden (2002) Animal Models of Nociception. *Journal* Pharmacological Reviews 53(4):597-652
- LHAMI GU¨ LC¸ I˙N (2008). In vitro prooxidant effect of caffeine Journal of Enzyme Inhibition and Medicinal Chemistry: 23(1): 149–152
- Mun Fei Yam,˙ Yean Chun Loh., Chuan Wei Oo., & Rusliza Basir . (2020). Overview of Neurological Mechanism of Pain Profile Used for Animal "Pain-Like" Behavioral Study with Proposed Analgesic Pathways. *International Journal of Molecular sciences*. 21(12): 4355
- Saeed Samarghandian, Reza Afshari & Tahereh Farkhondeh ˙(2014). Effect of long-term treatment of morphine on enzymes, oxidative stress indices and antioxidant status in male rat liver. International journal of clinical and experimental medicine: 15;7(5):1449–1453.
- Sandrine Reymond, Tatjana Vujić, Domitille Schvartz, & Jean-Charles Sanchez. (2022) Morphine-induced modulation of Nrf2-antioxidant response element signaling pathwayin primary human brain microvascular endothelial cells. Journal of Scientific Reports: 12:4588 | https://doi.org/10.1038/s41598-022-08712-0
- Sri Harsha Boppana, Michael Peterson, Austin L. Du, L V Simhachalam Kutikuppala, & Rodney A. Gabriel,(2022). Caffeine: What Is Its Role in Pain Medicine? *Journal of Cureus* 14(6): e25603. DOI