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Original Research Article

Effect of caffeine on capsaicin induced hyperalgesia in mice

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ABSTRACT

Aim & Background: Caffeine is the most widely consumed behaviorally active substance in the world. In the past several pharmaceutical companies used caffeine along with other drugs to get analgesic effect. The present research work was undertaken to investigate the effect of interaction of caffeine and capsaicin on animal model of hyperalgesia in mice. To meet these objectives, effect of drugs was studied using tail immersion test, an animal model of thermal hyperalgesia and tail withdrawal test in mice, an animal model of cold hyperalgesia.

Methods: The efficacy of three active principles alone and in combination of indomethacin, caffeine and prochlorperazine in reverting hyperalgesia was studied. Indomethacin 0.3 mg/ kg, i.p., caffeine 0.1 & 0.3 mg/ kg, i.p. and prochlorperazine 0.1 mg/ kg as well as combination reverted morphine withdrawal induced hyperalgesia.

Result: Initial application of capsaicin was found to be algesic leading to noxious stimulation in peripheral nervous system, which may cause allodynia and hyperalgesia. Thus this mechanism is also being studied in this study.

Discussion: Since most of the centrally acting analgesics act by way of their effect on dopaminergic mechanism and modifying calcium release, further studies on hyperalgesic activity were carried out using caffeine, capsaicin, amlodipine, haloperidol in the tail immersion (hot water of 55°C) and the tail withdrawal test (cold ethanol -14°C).

1. Mechanism of Pain Sensation

Above figure shows the effects of different temperatures on the responses of four types of nerve fibers: (1) a pain fiber stimulated by cold, (2) a cold fiber, (3) a warmth fiber, and (4) a pain fiber stimulated by heat. These fibers respond differently at different levels of temperature. For example, in very cold region, only the cold pain fibers are stimulated (if the skin become even colder, so that it nearly freezes or actually does freeze, these fibers cannot be stimulated). As the temperature rises to +10° to 15°C, the cold-pain impulses cease, but the cold receptors begin to be stimulated, reaching peak stimulation at about 24°C and fading out slightly above 40°C. Above about 30°C, the warmth receptors begin to be stimulated, but these also fade out at about 49°C. Finally, at around 45°C, the heat-pain fibers begin to be stimulated by heat and, oppositely, some of the cold fibers begin to be stimulated again, possibly due to damage to the cold endings caused by the excessive heat. (Guyton 2006) [1–28]

2. Mechanism of Stimulation of Thermal Receptors

It is believed that the cold and warmth receptors are stimulated by changes in their metabolic rates, and those changes result from the fact that temperature alters the

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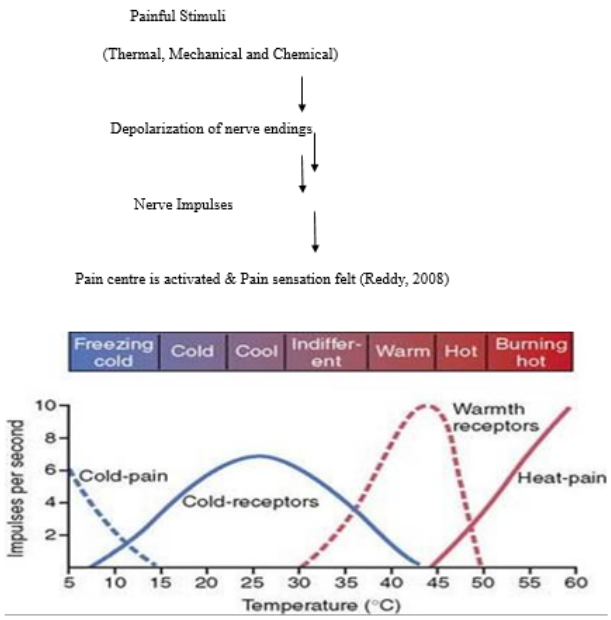

Fig. 1: Thermal receptor stimulation at different temperatures

rate of intracellular chemical reactions more than twofold for each 10°C change. In other words, thermal detection probably results not from direct physical effects of heat or cold on the nerve endings but from chemical stimulation of the endings as modified by temperature. (Guyton 2006)

2.1. Neural mechanism involved in pain transmission

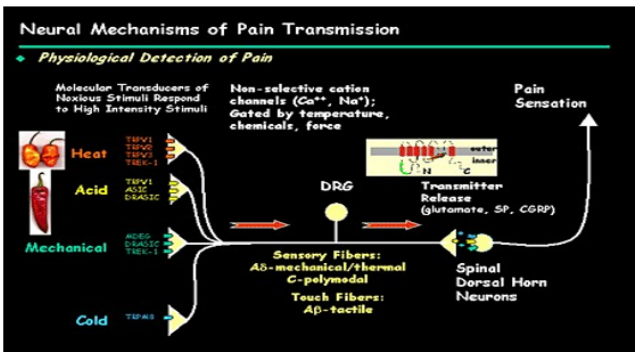

Fig. 2: Neural mechanism of pain transmission. (cme@medscape.net, Frank Porreca et al., 2006)

From above figure in the case of noxious heat, we can use the activation of this molecular transducer called the TRPV1 channel. The TRPV1 channel is major advances in understanding of the neurobiology of pain in several years. It is the channel that can be activated by noxious heat, and it can be activated by the extract of hot chili peppers, by a chemical substance called capsaicin. Capsaicin or the noxious heat can activate the TRPV1 channel, which may result in local membrane depolarization. That may generate action potentials, resulting in the release of the excitatory transmitters from the central terminals of the primary afferent to the first synapse in the spinal dorsal horn. This is then sent to higher centers, where the stimulus applied is later detected by the cortex as a sensation of pain. In the process of transduction, chemical energy from outside world is transduced into the currency of the nervous system, which is a change into an electrical signal that can be understood by the peripheral nociceptive terminals. (Frank Porreca et al., 2006)

3. Objective

Capsaicin a unique alkaloid found primarily in the fruit of the *Capsicum genus* and is what provides it spicy flavor and is generally extracted directly from fruit (Escogido et al., 2011). Initial application of capsaicin was found to be algesic leading to noxious stimulation in peripheral nervous system, which may cause allodynia and hyperalgesia (Winter et al., 1995). Thus, this mechanism is also being studied in this study.

Further there were no previous studies on effect of caffeine and capsaicin (given together) on animal models of hyperalgesia. Hence the present research work was undertaken to investigate the effect of interaction of caffeine and capsaicin on animal model of hyperalgesia in mice. To meet these objectives, effect of drugs was studied using tail immersion test, an animal model of thermal hyperalgesia (Turner, 1971) and tail withdrawal test in mice, an animal model of cold hyperalgesia (Lagerstrom et al. 2011)

Since most of the centrally acting analgesics act by way of their effect on dopaminergic mechanism (Cendan et al., 1995) and modifying calcium release (Koleva & Dimova, 2000), further studies on hyperalgesic activity were carried out using caffeine, capsaicin, amlodipine, haloperidol in the tail immersion (hot water of 55°C) and the tail withdrawal test (cold ethanol -14°C).

4. Materials Animals

Healthy Swiss Albino mice (22-25 g) of either sex, were used for the study.

4.1. Extraction of capsaicin

Dried, ripen fruits of capsicum (capsicum annum) were coarsely powdered for the extraction of oleoresin. The capsicum was extracted with ethanol (90%) in Soxhlet's apparatus. The extract obtained was concentrated and dried. The dried residue was further extracted with cold ethanol (90%) and ethanol was removed by evaporation. Capsicum oleoresin was obtained containing not less than 8% of capsaicin (Rangari et al. 2007).

4.2. Animal models

Tail Immersion Test was used to check the thermal hyperalgesia & Tail withdrawal test was used to check the cold hyperalgesic effect. Mice of either sex with a weight (20-25 g) were used. Swiss albino mice were divided into ten groups (n = 5)

4.3. Tail immersion test: Procedure

Experiments were performed on adult Swiss Albino mice (22-25 gm.) of either sex. Animals were housed in a standard condition of 12-hr light/dark cycle and 25 ± 1°C room temperature and had free access to food and water. Animals were treated and cared according to the ethical guidelines.

The tail was rapidly immersed in water maintained at 55°C. Within few seconds the mice reacted by withdrawing the tail. The reaction time was recorded in seconds by stopwatch. The latency to respond to heat stimulus with vigorous flexion of the tail was measured. Animals were removed immediately after responding and the tail was wipped off with a cloth. The reaction time was determined before and periodically after either intraperitoneal or topical administration of test substance. Cut-off time was set at 15 sec to prevent tissue damage (Turner, 1971). The mice received drug treatment as detailed below.

Table 1: Drug treatment and their doses

Treatment	Dose (ml or mg/kg)
Vehicle	5 ml/kg
Caffeine	10
Amlodipine	5
Haloperidol	0.25
Capsaicin	3.7 mg/kg topically
Combination of Capsaicin and Caffeine	3.7 mg/kg topically + 10
Combination of Capsaicin and Amlodipine	3.7 mg/kg topically + 5
Combination of Capsaicin and Haloperidol	3.7 mg/kg topically + 0.25
Combination of Capsaicin and Amlodipine andCaffeine	3.7 mg/kg topically + 5 + 10
Combination of Capsaicin and Amlodipine and Haloperidol	3.7 mg/kg topically + 5 + 0.25
Combination of Capsaicin and Amlodipine and Haloperidol + Caffeine	3.7 mg/kg topically + 5 + 0.25 + 10

5. Tail Withdrawal Test

5.1. Procedure

Mice of either sex with a weight (20-25 g) were used. Swiss albino mice were divided into ten groups (n = 5).

The mouse were placed in a perplex restrainer and left to settle for 5-10 min. Half of the tail was then immersed in -14°C ethanol and time until the mouse would withdraw/rattle its tail was monitored. The test was repeated 2-3 times. The animal was left to settle for 5 min between the tests. Cut-off was set to 30sec. Data was expressed as mean withdrawal latency for each animal and group ± SEM. (Lagerstrom et al. 2011). The mice received drug treatment as described for the previous experiment.

6. Results

*Pre-formulation Study- Testing of plant actives- a)*Thin layer chromatography

Thin layer chromatography was performed using silica gel-G plate as stationary phase for capsaicin.

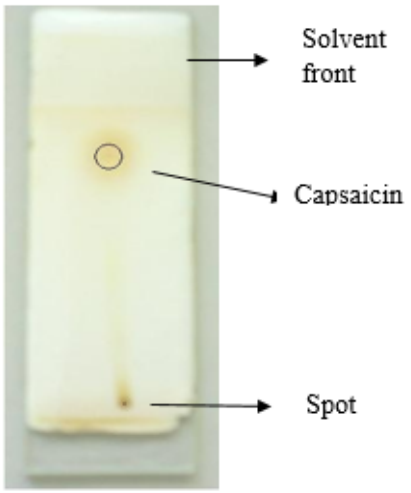

Fig. 3: Thin layer chromatography of *capsicum annum* fruit extract

Capsaicin was eluted with R_f value 0.83. These R_f values of capsaicin complies with standard R_f value of plant actives 0.85 (Table 4). (Wagner et al. 1996).

6.1. UV-spectroscopy

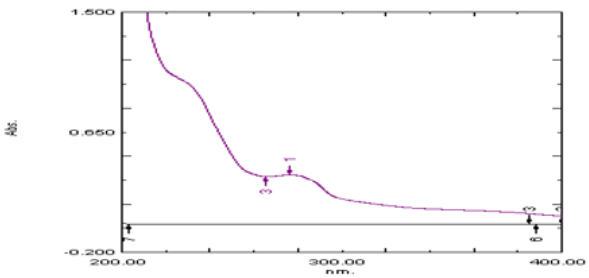

Fig. 4: UV-spectra of capsicum annum fruit extract in chloroform

In present study, capsaicin exhibited absorption maxima at 279nm (in Chloroform) (Figure 3). It concludes that

absorption maxima of capsicum annum fruit extract shifted from 269nm to 279nm.

6.2. Phytochemical screening of capsicum annum fruit extract

The importance of phytochemical study was to identify secondary metabolites responsible for therapeutic effect. Ethanolic extract of Capsicum annum was evaluated for various phyto-constituents. Phytochemical analysis of Capsicum annum fruit showed presence of alkaloids, carbohydrates, phytosterols and volatile oils and therapeutically active phyto-constituents.

Table 2: Identification test of capsaicin

Sr. No.	Tests	Observations	
		Standard	Observed
1.	Thin layer chromatography a) Capsaicin	R_f Value 0.85	R_f Value 0.83
2.	UV- Absorption maxima a) Capsaicin in Chloroform	248nm & 269nm	279nm
3.	Melting point/ Boiling point a) B.P. of Capsaicin	210-220^{o}C	202-204^{o}C

Table 3: Chemical test of capsaicin

Sr. No.	Test	Observation	Inference
1.	Powder crude extract + sulphuric acid.	Crimson colour	Passes the test
2.	The aqueous solution of boric acid + crude extract.	Reddish brown turns to greenish blue addition of alkali	Passes the test
3.	Acetic anhydride +conc. H_2SO_4 + Powder extract & observed under UV light.	Red fluorescence	Passes the test
4.	Test for liquid Capsaicin + pot. permagnate.	Pungency was destroyed	Passes the test

The entire chemical test shows positive result for capsaicin and boiling point of liquid capsaicin was found to be in the range 202-204^o which complies with standard values (Table 4).

Effect of caffeine, amlodipine, haloperidol & capsaicin on tail immersion test in mice Values are expressed as Mean ± SEM, n = 5; p< 0.001 (as compared to control by One-way ANOVA followed by Bonferroni's test)

In vehicle treated group, the reaction time averaged between 2.04 ± 0.37 to 2.63 ± 0.16 sec. Only caffeine increased reaction time significantly till 45 min and thereafter the increase in reaction time was insignificant. Capsaicin (3.7 mg/kg) insignificantly reduced reaction time. Amlodipine and haloperidol (0.25 mg/kg) showed no significant reduction in hyperalgesia. The observations are given in Table and figure.

Tail immersion test in mice(Figure 5)

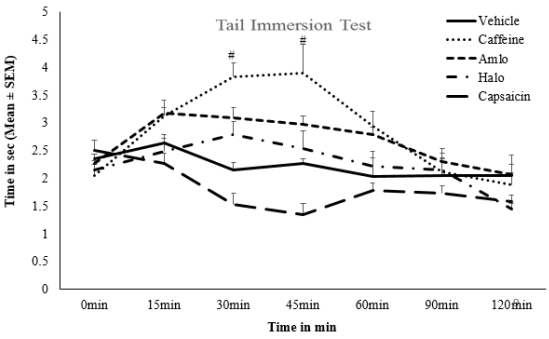

Fig. 5: Effect of caffeine, amlodipine, haloperidol & capsaicin on tail immersion test in mice

6.3. Tail immersion test in mice

Values are expressed as Mean ± SEM, n=5; $^{\#}$p< 0.001 (as compared to control by One-way ANOVA followed by Bonferroni's test)

Treatment with Capsaicin (3.7 mg/kg) + Amlodipine (5 mg/kg) at 30 min and Capsaicin (3.7 mg/kg) + Amlodipine (5 mg/kg) + Caffeine (10 mg/kg) combination at 60 min significantly reduced the hyperalgesia as compared to the vehicle group. Capsaicin (3.7 mg/kg) + Amlodipine (5 mg/kg) + Haloperidol (0.25 mg/kg) at 30 min had antihyperalgesic effects than vehicle group.

Capsaicin (3.7 mg/kg) + Caffeine (10 mg/kg) and Capsaicin (3.7 mg/kg) + Amlodipine (5 mg/kg) + Haloperidol (0.25 mg/kg) + Caffeine (10 mg/kg) did not produce more increase in tail flick latency as compared to other combinations. Observations are given in Table 6 and Figure 6.

6.4. Tail withdrawal test in mice

Values are expressed as Mean ± SEM, n=5; $^{\#}$p< 0.001 (as compared to control by One way ANOVA followed by Bonferroni's test)

Table 4: Effect of caffeine and other combinations on tail immersion test in mice

Groups Mg/kg	0 min	15 min	30 min	45 min	60 min	90 min	120 min
Vehicle	2.35 ± 0.09	2.63 ± 0.16	2.14 ± 0.15	2.26 ± 0.09	2.03 ± 0.34	2.05 ± 0.49	2.04 ± 0.37
Caffeine 10	2.04 ± 0.21	3.12 ± 0.28	3.82 ± 0.26#	3.89 ± 0.53#	2.93 ± 0.28	2.12 ± 0.33	1.88 ± 0.37
Amlodipine 5	2.26 ± 0.17	3.18 ± 0.09	3.09 ± 0.19	2.97 ± 0.15	2.79 ± 0.13	2.3 ± 0.07	2.07 ± 0.02
Haloperidol 0.25	2.15 ± 0.16	2.49 ± 0.23	2.78 ± 0.24	2.53 ± 0.32	2.22 ± 0.26	2.14 ± 0.21	1.44 ± 0.11
Capsaicin 3.7	2.5 ± 0.18	2.27 ± 0.18	1.53 ± 0.2	1.34 ± 0.21	1.78 ± 0.14	1.73 ± 0.13	1.57 ± 0.12
Groups mg/kg	0min	15min	30min	45min	60min	90min	120min
Vehicle	2.35± 0.09	2.63± 0.16	2.14± 0.15	2.26± 0.09	2.03± 0.34	2.05± 0.49	2.04± 0.37
Caf 10 + Cap 3.7	0.96± 0.11#	1.94± 0.26	2.58± 0.34	3.31± 0.30	2.96± 0.30	2.43± 0.32	2.08± 0.28
Cap 3.7 + Amlo 5	3.02± 0.23	3.56± 0.35	4.15 ± 0.29#	3.63± 0.29#	3.39± 0.29#	2.98± 0.25	2.56± 0.16
Cap 3.7 +Halo 0.25	2.20± 0.16	3.13± 0.17	3.17± 0.27	3.62± 0.11#	3.44± 0.13#	2.69± 0.12	2.30± 0.09
Cap 3.7 + Amlo 5 + Caf 10	2.44± 0.49	1.83± 0.26	2.24 ± 0.37	3.52± 0.40#	4.24± 0.38#	3.01± 0.41	1.73± 0.21
Cap 3.7 + Amlo 5 + Halo 0.25	2.60± 0.32	3.37± 0.47	4.03 ± 0.37#	3.54± 0.29#	3.24± 0.20#	2.57± 0.19	2.24± 0.22
Cap 3.7 + Amlo 5 + Halo + Caf 10	2.42± 0.20	3.27± 0.22	3.86 ± 0.33#	3.11± 0.27	2.53± 0.16	2.35± 0.14	1.87± 0.11

Table 5: Comparison of tail flick latency with other combinations

Sr. no.	Groups	Latency to tail flick (Mean ± SEM)
1	Vehicle	10.20 ± 1.35
2	Caffeine (10 mg/kg)	16.25 ± 0.47#
3	Amlodipine (5 mg/kg)	14.60 ± 0.25#
4	Haloperidol (0.25 mg/kg)	12.29 ± 0.20
5	Capsaicin (3.7 mg/kg)	08.26 ± 0.48

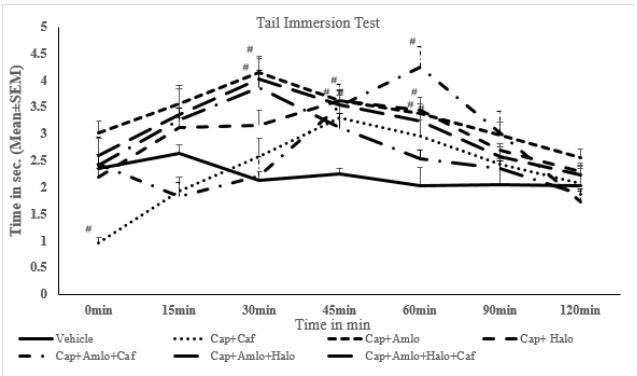

Fig. 6: Effect of Capsaicin, Amlodipine, Haloperidol, and Caffeine on tail withdrawal test in mice

Treatment with caffeine (10 mg/kg) showed significant increase in tail flick latency as compared to the control group, while amlodipine (5 mg/kg) also possessed antihyperalgesic activity as compared to the control. Capsaicin (3.7 mg/kg) totally produced hyperalgesic effect as shown by decrease in tail flick latency in comparison to control and other groups. Haloperidol (0.25 mg/kg) showed no significant reduction in hyperalgesia. Caffeine was found better antihyperalgesic agent compared to other drugs.

7. Discussion

Caffeine is commonly used as adjuvant analgesic in the treatment of acute pain and migraine. The literature indicates loss of analgesic activity of many analgesic agents such as carbamazepine, imipramine, amytriptylline, etc. by caffeine. (Sawynok, 2011)

Table 6: Effect of various drugs combinations on tail withdrawal test in mice

Sr. No.	Groups	Latency to tail flick (Mean ± SEM)
1	Vehicle	10.20 ± 1.35
2	Capsaicin (3.7 mg/kg)	08.26 ± 0.48
3	Combination of Capsaicin (3.7 mg/kg) and Caffeine (10 mg/kg)	13.81 ± 0.67#
4	Combination ofCapsaicin (3.7 mg/kg) and Amlodipine (5 mg/kg)	15.42 ± 0.52#
5	Combination ofCapsaicin (3.7 mg/kg) and Haloperidol (0.25 mg/kg)	13.87± 0.20#
6	Combination of Capsaicin (3.7 mg/kg) and Amlodipine (5 mg/kg) and Caffeine (10 mg/kg)	17.32 ± 0.59#
7	Combination of Capsaicin (3.7 mg/kg) and Amlodipine (5 mg/kg) and Haloperidol (0.25 mg/kg)	14.10 ± 0.22#
8	Combination of Capsaicin (3.7 mg/kg) and Amlodipine (5 mg/kg) and Haloperidol (0.25 mg/kg) and Caffeine (10 mg/kg)	13.87 ± 0.21#

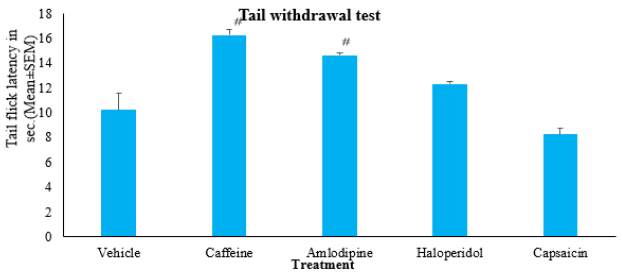

Fig. 7: Effect of capsaicin, amlodipine, haloperidol and caffeine on tail withdrawal test in mice.

There are reports that caffeine+ indomethacin+ trichlorperazine is useful in treatment of migraine. (Galeotti et al., 2002)

Capsaicin cream/gel made from extracts of chilli peppers numbs the nerve fibres that carry pain messages from the tissues around the joints to the brain. Research has shown that it can be effective in reducing pain and tenderness in joints affected by osteoarthritis. There are no major safety problems with capsaicin cream and it can be applied 3-4 times daily to achieve maximum benefits. (myjointpain.org.au, 2013)

Despite of intensive research on pain management the treatment on pain is still grossly inadequate. Activation of transient receptor potential vanilloid 1 channel on C-fibres by capsaicin is used in treatment of inflammatory pain (Porreca et al., 2006). The present study aims at studying the interaction of various drugs on capsaicin induced hyperalgesia in mice.

Tail immersion test is commonly used animal model to study the interactions occurring at spinal cord. (Turner, 1971). The reaction time is a simple yet useful parameter to evaluate antinociceptive agents. Caffeine in a dose of 10 mg/kg produced significant increase in reaction time at 30 and 45 minutes. All the drugs like amlodipine and haloperidol were not effective in the doses used. These doses were selected to see presence or absence of synergism with caffeine and other drugs.In further studies amlodipine, haloperidol significantly increased reaction time at different time periods. (Entrena et al., 2009) The combinations of amlodipine with caffeine and haloperidol significantly increased the reaction time. Thus a potentiation of analgesia was observed.

In the tail withdrawal test (Lagerstrom et al. 2011) both caffeine and amlodipine showed significant analgesic effect as indicated by increased latency to tail flick. Capsaicin showed hyperalgesia in this test. The combination of capsaicin with amlodipine and caffeine produced maximum antinociceptive effect. The analgesic effect produced by capsaicin+ caffeine and that produced by combination of capsaicin+ amlodipine+ haloperidol+ caffeine were almost same. This suggests that various analgesic drugs in this animal model do not function in synergy and capsaicin+ amlodipine+ caffeine can be suitable combination.

7.1. Tail withdrawal test in mice

Value are expressed as Mean ± SEM, n=5; $^{\#}p< 0.001$ (as compared to control by One way ANOVA followed by Bonferroni's test)

Treatment with Capsaicin (3.7 mg/kg) + Amlodipine (5 mg/kg) + Caffeine (10 mg/kg) and Capsaicin (3.7 mg/kg) + Amlodipine (5 mg/kg) combination significantly reduced the hyperalgesia as compared to the vehicle group. Capsaicin (3.7 mg/kg) + Amlodipine (5 mg/kg) + Haloperidol (0.25 mg/kg) had significant antihyperalgesic effects than vehicle group. Capsaicin (3.7 mg/kg) + Caffeine (10 mg/kg) and Capsaicin (3.7 mg/kg) + Amlodipine (5 mg/kg) + Haloperidol (0.25 mg/kg) + Caffeine (10 mg/kg) did not produce significant increase in tail flick latency as compared to other combinations.

7.2. Tail withdrawal test in mice

8. Source of Funding

None.

9. Conflict of Interest

None.

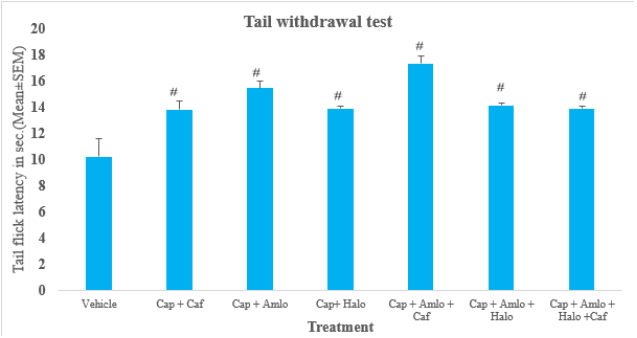

Fig. 8: Effect of caffeine & other combinations on tail withdrawal test in mice

References

1. Guyton AC, Hall JE. Textbook of medical physiology. 13th ed. Philadelphia, Saunders; 2006. p. 607–16.
2. Rojecky LB. Analgesic effect of caffeine and clomipramine: a possible interaction between adenosine and serotonin systems. *Acta Pharm*. 2003;53(1):33–9.
3. Baliki M, Calvo O, Chialvo D, Apkarian A. Spared nerve injury rat's exhibit thermal hyperalgesia on an automated operant dynamic escape task. *Mol Pain*. 2005;1(1):18. doi:10.1186/1744-8069-1-18.
4. Barar F. Essentials of Pharmacotherapeutics. and others, editor; 2007. p. 117–35.
5. Baron R. Peripheral neuropathic pain: from mechanisms to symptoms. *Clin J Pain*. 2000;16(2):12–20.
6. Bennett DJ, Kirby GW. Constitution and biosynthesis of capsaicin. *J Chem Soc C*. 1968;p. 442–6.
7. Bevan S, Szolcsanyi J. Sensory neuron-specific actions of capsaicin: mechanism and application. *Trends Pharm Sci*. 1990;11(8):330–3.
8. The Stationary Office of Medicines and Healthcare products regulatory. In: and others, editor. British Pharmacopeia; 2011. p. 324–72.
9. Campbell E, Bevan S, Dray A. Clinical Applications of Capsaicin and its Analogues. *Wood J Ed*. 1993;21(8):255–72.
10. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science*. 2000;288(5464):306–19.
11. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat activated ion channel in the pain pathway. *Nature*. 1997;389(6653):816–40.
12. Claver AG, Andres MS, Abadia J, Ortega RG, Fernandez AA. Determination of capsaicin and dihydrocapsaicin in capsicum fruits by liquid chromatography-electrospray/Time-of-Flight Mass Spectrometry. *J Agric Food Chem*. 2006;54:9303–9314.
13. Decosterd I, Allchorne A, Woolf CJ. Progressive tactile hypersensitivity after a peripheral nerve crush: non-noxious mechanical stimulus induced neuropathic pain. *Pain*. 2002;100:155–62.
14. Decosterd I, Woolf CJ. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain*. 2000;87:149–58.
15. Fang JY, Wu PC, Huang YB, Tsai YH. In vitro permeation study of capsaicin and its synthetic derivatives from ointment bases using various skin types. *Int J Pharm*. 1995;126:119–147.
16. Ghelardini C, Galeotti N, Bartolini A. Caffeine induces central cholinergic analgesia. *Naunyn Schmiedebergs Arch Pharmacol*. 1997;356:590–95.
17. Gunthorpe MJ, Benham CR, Randall A, Davis JB. The diversity in the vanilloid (TRPV) receptor family of ion channels. *Trends Pharmacol Sci*. 2002;23(4):183–91.
18. Hayes AG, Skingler M, Tyers MB. Effects of single dose of capsaicin on nociceptive thresholds in the rodents. *Neuropharmacology*. 1981;20(5):505–16.
19. Henry CJ, Emery B. Effect of spiced food on metabolic rate. *Hum Nutr Clin Nutr*. 1986;40(2):165–268.
20. Holzer P. Capsaicin: cellular targets, mechanism of action, and selectivity for thin sensory neurons. *Pharmacol Rev*. 1991;43(2):143–201.
21. 2013. Available from: http://physrev.physiology.org/cgi/content/full/89/2/707.1.
22. Molecular Pain. Available from: http://www.molecularpain.com/content/pdf/1744-8069-1-18.pdf.
23. Jancso N, Gabor AJ, Szolcsanyi J. Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. *Brit J Pharmacol*. 1967;31(1):138–51.
24. Jensen TS, Gottrup H, Bach FW, Sindrup SH. The clinical picture of neuropathic pain. *Eur J Pharmacol*. 2001;429(1-3):1–11.
25. Khandelwal K. Handbook of practical Pharmacognosy techniques and experiments. 19th ed. and others, editor. Nirali prakashan; 2008. p. 149–56.
26. Kobata K, Todo T, Yazawa S, Iwai K, Watanabe T. Novel capsaicinoid-like substances, capsiate and dihydrocapsiate, from the fruits of a nonpungent cultivar, CH-19 Sweet, of pepper (Capsicum annuum L.). *J Agric Food Chem*. 1998;46(5):1695–7.
27. Koltzenburg M, Scadding J. Neuropathic pain. *Curr Opin Neurol*. 2001;14:641–88.
28. Kosuge S, Furata M. Studies on the pungent principle of Capsicum. *Agric Biol Chem*. 1970;34(2):248–56.

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