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Research Article

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Effect of Caffeine on Body Weight and Hippocampal Cells in a Murine Model

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Abstract Caffeine is a psycho-stimulant and mild diuretic consumed around the world as natural components of chocolate, coffee and tea and as added components to soda and energy drinks. It's used clinically and recreationally, motivated this study on its effects on the histomorphology of the hippocampus in a murine model. 24 albino mice was grouped (n = 6) as control, 25 mg/kg, 30 mg/kg and 40 mg/kg caffeine groups. They were administered intraperitoneally for 14 days. On day 15, the animals were sacrificed after chloroform anaesthesia and perfusion-fixed with 10% neutral buffered formalin. Their entire brains were removed and post-fixed, and their extracted hippocampa were routinely processed for Cresyl fast violet staining. Cellular population was determined using ImageJ software. Results showed that there was no difference in body weight change in the test groups compared with the control group. The histological appearance of the hippocampus showed no apparent histopathology, but the hippocampal cell populations were significantly ($P < 0.05$) lower in the 30 and 40 mg/kg group. In conclusion, the consumption of the given low and high doses of caffeine did not affect body weight, but caused hippocampal cell population loss, which was dose dependent.

Keywords Caffeine, Hippocampus, Nissl, Body weight, Murine

Introduction

Caffeine is a psycho-stimulant and mild diuretic consumed around the world as natural components of chocolate, coffee and tea and as added components to soda and energy drinks [1-4]. It is also a common ingredient in diet pills and some other drugs [5]. As a food ingredient, caffeine is generally considered safe based on a long established history of use and on extensive research conducted over more than a century. It is also known as coffeine, theine, mateine, guaranine, methyltheobromine, and is the common name for 1,3,7-trimethylxanthine or 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione, with the formula $C_8H_{10}N_4O_2$ [6]. It is a white crystalline solid with molar mass, 194.19 g/mol, and density, 1.23 g/ml [7-9]. There is no difference in its chemical structure or characteristics whether sourced naturally or synthesized [10].

Caffeine is rapidly and completely absorbed from the gastrointestinal tract [11-12], with 99% of caffeine being absorbed within 45 minutes of ingestion [13] and is metabolized by the liver to form dimethyl- and mono-methyl-xanthines, di-methyl and mono-methyl uric acids, tri-methyl- and di-methyl-allantoin, and uracil derivatives [14]. Caffeine metabolism via the cytochrome P_{450} pathway is considered the primary step in the metabolic pathway accounting for greater than 95% of the primary caffeine metabolism, although a large number of enzymes and intermediate products are noted in its complete metabolism [13]. In rats, C-8-hydroxylation of caffeine is the major metabolic reaction in rat liver microsomes (approx.70%) and liver slices compared to 1-N- and 7-N-demethylation and 3-N-demethylation [15]. In the brain it acts through several mechanisms, but majorly by counteracting adenosine which it does by blocking its receptors [16-18].

Clinically, caffeine is used for the management of asthma, gall bladder disease, attention deficit-hyperactivity disorder, shortness of breath in newborns, and low blood pressure [19]. It has also use for treating different forms of migraine, obesity and in the management of diabetes type 2 and seizures [20-24]. Long-term consumption of caffeine is associated with a lower risk of cardiovascular disease [25-26], and has been reported to increase the metabolic rate of digestion [27]. It is also reported to protect against Parkinson-like features [28], improve performance parameters of rapid information processing tasks [29]. It was earlier posited that caffeine has positive effects on working memory using the tip of the tongue effect [30] but this finding has been refuted [31].

There is conflicting reports on the effect of this psychostimulant on long term memory. Improvement of long-term memory with caffeine intake was observed by performing cognitive tasks such as word recall [32-33] and water maze [34]. However, a negative effect (impaired long term memory) was observed in a step-through passive-avoidance task using mice [35]. Alternatively, other studies have shown that caffeine intake has no effect on long-term memory and other cognitive processes [36-38]. Borota *et al*. [39] posited that caffeine enhance memory consolidation in a post study involving behavioral discrimination task.

Neurotoxicity of caffeine has been reported in different species [6, 40-41], and can present as a spectrum of clinical symptoms. Most of these originate in the central nervous and circulatory systems following ingestion of 1g or more of caffeine [42]. These effects include insomnia, restlessness, muscle twitching and excitement progressing to mild delirium among others [40-46].

According to Han *et al* [47], long-term consumption of caffeine could inhibit hippocampus-dependent learning and memory partially through inhibition of hippocampal neurogenesis. However acute 1-day administration of caffeine had no effect on neuronal precursors in the adult hippocampus but at supraphysiological levels of caffeine there was lower survival rate than control cells and increased proliferation which did not yield an increase in long-term neurogenesis [48]. Another research suggest that the effects of caffeine on hippocampal neurons may be mediated by a decrease of one or more potassium conductance(s), and that adenosine and caffeine may compete for the same electrophysiologically active receptor site on these cells [49]. An acute overdose of caffeine usually in excess of about 300 mg/kg can over-stimulate the central nervous system [40]. Preclinical studies further indicate that caffeine may potentiate the effects of motor in coordination induced by ethanol consumption [50]; antagonized the psychomotor-impairing effects of alcohol [51], dose-dependently decrease locomotion and learning, but increase anxiety in mice [52].

Though literatures abound on the activity of caffeine in the body and particularly the brain, little is reported on its role in brain morphology. It is against this background that this research was carried out to investigate if this psycho-stimulant has effect on the histomorphology of the hippocampus and frontal cortex.

Materials and Methods

Animal Handling

Twenty four (24) young female Albino mice of average weight 25g were used for the experiment. They were obtained and kept in the Faculty of Basic Medical Science Animal House, University of Uyo and were handled in accordance with international guidelines for the care and use of animals for research purpose. The animals were housed in wooden cages and were fed with standard feed pellets (Vital Feed Company Ltd., Nigeria) and clean drinking water. They were allowed 12:12 hours' light and dark condition and room temperature of 27^{o}C was maintained throughout. The animals were allowed a week of acclimatization before the beginning of the experiment.

Caffeine preparation and animal groupings/administration

0.5 g of pure caffeine (101187527, Sigma-Aldrich, England) with molecular weight 194.19 g/mol was dissolved in 100 ml of distilled water in a glass jar forming the caffeine stock solution and the dose for administration was calculated for the groups. The animals were divided into four (4) groups of six (6) animals each. Group 1 was the control and the animals were administered 8 ml/kg body weight of distilled water (i.p.), while groups 2-4 received 25 mg/kg, 30 mg/kg and 40 mg/kg body weight of caffeine (i.p.), respectively for 14 days.

On day 15 of the experiment, the animals were sacrificed after chloroform anaesthesia and perfusion-fixed with 10% neutral buffered formalin. The whole brain was then excised and post-fixed in 10% neutral buffered formalin. On complete fixation at 7 days, the hippocampal region was processed through paraffin and sectioned for staining with Cresyl fast violet. Stained sections were viewed under the bright field microscope and photomicrographs were obtained with the aid of a computer-assisted digital camera attached to the microscope.

Cellular population estimates were determined with ImageJ™ software (version 1.77c, National Institutes of Health, USA). Briefly, live images (at the predetermined area) of the sections were captured using the ImageJ™ software through the light microscope at ×400 magnification. They were converted to 8-bit images and threshold to 200 at the scale of 1 µm while ensuring that the scale was in the global mode. Microscopic scale was then set for camera binning of 1 × 1 at ×10 objectives. Nuclei of the cells were then quantified at this magnification.

One way analysis of variance was applied to analyze data, and post hoc Tukey's test was used to compare individual groups. Data with probability level $p < 0.05$ was regarded as significant. Data are presented as Mean ± standard error of mean

Results

Body Weight Change

There was no difference in body weight change in the 25 mg/kg, 30 mg/kg and 40 mg/kg caffeine groups compared with the control group. There was also no difference in body weight change among the test groups (Table 1).

Histomorphological (Hippocampus; Cresyl Fast Violet)

The histological appearance of the hippocampus of the control group animals showed stained Nissl substance marking out the soma of both glia and neurons throughout the layers (Fig. 1a). The histological appearance of the hippocampus of the 20 mg/kg caffeine group animals showed no apparent changes in Nissl substance compared with the control group (Fig. 1b). The histological appearance of the hippocampus of the 30 mg/kg group animals showed no apparent changes in Nissl substance compared with the control group (Fig. 1c). The histological appearance of the hippocampus of the 40 mg/kg group animals showed no apparent changes in Nissl substance compared with the control group (Fig. 1d).

There were significantly (0.05) lower cellular population in the 30 mg/kg and 40 mg/kg caffeine groups compared with the control group. No difference existed in the 25 mg/kg caffeine group compared with the control group, and also among the test groups (Fig. 2).

Table 1: The body weight change in the experimental animals

Group (n=6)	**Day 1 F=13.10 P=0.0001**	**Day 8 F=11.93 P=0.0001**	**Body weight change (g)**	**Body weight change (%)**
Control	21.33±1.36	21.83±1.20	0.50	2.34
25 mg/kg caffeine	20.80±0.86^{NS}	21.00±0.71^{NS}	0.20	0.96
30 mg/kg caffeine	23.00±0.41	24.00±1.24^{NS}	1.00	4.35
40 mg/kg caffeine	28.00±0.52*^{,b,c}	27.67±0.49*^{,b}	-0.33	1.18

Data are presented as mean ± standard error of mean

* - Significantly different from Control group at $P < 0.05$

b - Significantly different from group B at $P < 0.05$

c - Significantly different from group C at $P < 0.05$

NS - Not significantly different from Control group at $P < 0.05$

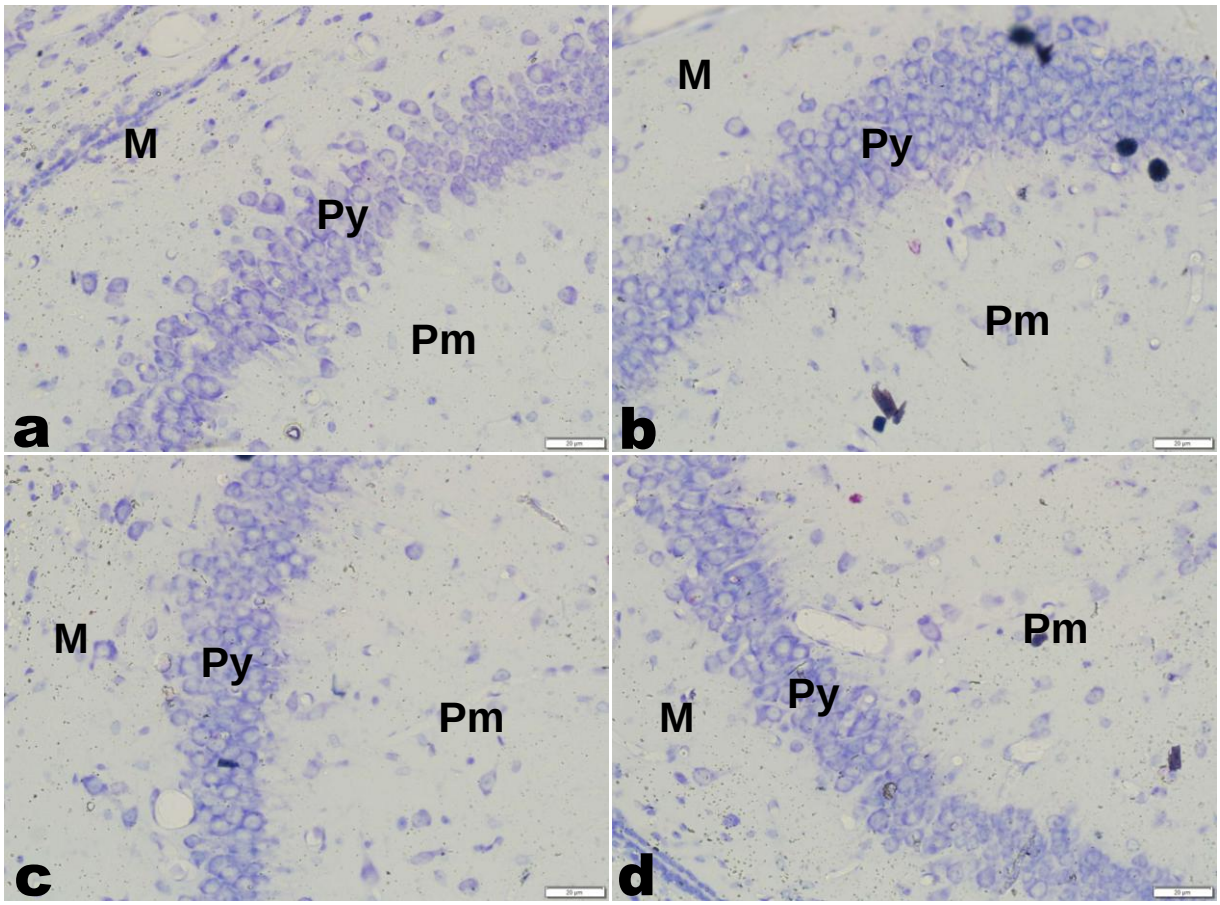

Figure 1: Sections of the hippocampus of the control (a), 25 mg/kg (b), 30 mg/kg (c) and 40 mg/kg (d) groups. There is no apparent adverse histopathology in all the test groups compared with the control group. (cresyl fast violet, CFV, Mag. ×200)

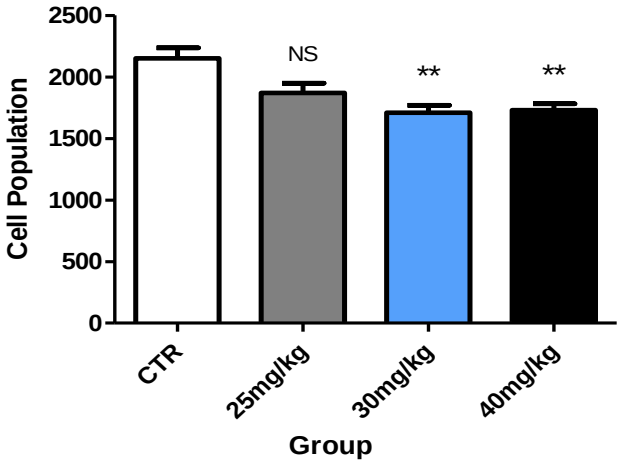

Figure 2: Hippocampal Cellular population of the control, 25 mg/kg, 30 mg/kg and 40 mg/kg groups
*Mean ± standard error of mean; ** - Significantly different from Control group at P < 0.01; NS - Not significantly different from Control group at P < 0.05; CTR - Control*

Discussion

Caffeine is reported to cause diverse effects in the body. It's used clinically and recreationally warranted this study to ascertain its effects on the histomorphology of the hippocampus in a murine model. There was no difference in body weight in the 25 mg/kg, 30 mg/kg and 40 mg/kg caffeine groups compared with the control. These results indicate that caffeine, at the given doses did not affect body weight. The result of the present study on body weight is at variance with previous reports were body weight loss were reported with high caffeine consumption [53-54]. It is reported that caffeine elicits body weight loss through thermogenesis by inhibiting the phosphodiesterase-induced degradation of intracellular cyclic adenosine mono-phosphate [55], and this may not be applicable in the present study. However, increase body weight even with low caffeine consumption has also been reported [38, 54.

Caffeine has been reported to stimulate gliosis in the straitum, hippocampus, substantia nigra pars-compacta and cerebral cortex [9, 56] which usually account for cell density increase. The present histological study showed no apparent difference in hippocampal appearance, but less cellular population especially in the high doses groups (30 and 40 mg/kg), indicating that caffeine administration resulted in hippocampal cell loss, which may have arisen from degeneration. The present result maight influence the overall activity of the hippocampus.

Caffeine is reported to increase and decrease all horizontal and vertical motor activity in different caffeine combinations [16, 17, 57]. However, no difference was reported in the dark and light maze test [38]. It is reported that caffeine inhibits neurulation and adult neurogenesis [47, 48, 58], which may a reason for the less cell population in the present study.

The hippocampus influence memory associated with emotions [59]. A decrease cellular population may lead to functional impairment, which may have even more far reaching implications.

Conclusion

Consumption of the given low and high doses of caffeine did not affect body weight, but caused hippocampal cell population loss, which was dose dependent.

References

1. Zoumas BL, Kreiser WR and Martin R (1980). Theobromine and caffeine content of chocolate products. Journal of Food Science. 45(2): 314-316.
2. Nehlig A1, Daval JL, Debry G. (1992). Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Rev. 1992 May-Aug;17(2):139-70.
3. Wanyika H. N., Gatebe, E. G., Gitu L. M. , Ngumba E. K. and Maritim C. W. (2010). Determination of caffeine content of tea and instant coffee brands found in the Kenyan market. Afr. J. Food Sci. 4(6): 353-358.
4. Seifert SM, Schaechter JL, Hershorin ER, Lipshultz SE (2011). Health Effects of Energy Drinks on Children, Adolescents, and Young Adults. Pediatrics 2011;127(3):511–528.
5. Derry CJ1, Derry S, Moore RA. (2012). Caffeine as an analgesic adjuvant for acute pain in adults. Cochrane Database Syst Rev. 2012 Mar 14;3: :CD009281. doi: 10.1002/14651858.CD009281.pub2.
6. Peters JM (1967). Factors affecting caffeine toxicity: a review of the literature. The Journal of Clinical Pharmacology and the Journal of New Drugs 7 (3): 131–141.
7. Gennaro MC and Abrigo C. (1992) Caffeine and theobromine in coffee, tea and cola-beverages. Simultaneous determination by reversed-phase ion interaction HPLC, Fresenius J. Anal. Chem. 343, 523–525.
8. Zhao Y, Lunte CE. (1997). Determination of caffeine and its metabolites by micellar electrokinetic capillary electrophoresis, J. Chromatogr. B, 688, 1997, 265–74.
9. Chen QC, Wang J, Simultaneous determination of artificial sweeteners, preservatives, caffeine, theobromine and theophylline in food and pharmaceutical preparations by ion chromatography, J. Chromatogr, A 937,2002, 57–64.

10. IOM (2004) Caffeine for the Sustainment of Mental task Performance. Formulations for Military Operations. Institute of Medicine (IOM). National Academy Press, Washington, DC. p. 1- 149.
11. Newton, R; Broughton LJ, Lind MJ, Morrison PJ, Rogers HJ, Bradbrook ID (1981). Plasma and salivary pharmacokinetics of caffeine in man. European Journal of Clinical Pharmacology 21 (1): 45–52.
12. Magkos F1, Kavouras SA. (2005). Caffeine use in sports, pharmacokinetics in man, and cellular mechanisms of action. Crit Rev Food Sci Nutr. 2005;45(7-8):535-62.
13. Carrillo, J. A. and Benitez, J. (2000). Clinically significant pharmacokinetic interactions between dietary caffeine and medications. Clinical pharmacokinetics 39: 127- 153.
14. Arnaud MJ. (1993). Metabolism of caffeine and other components of coffee. in Caffeine, Coffee and Health, ed Garattini S (Raven Press, New York), pp 43–95.
15. Kot, M. and Daniel, W. A. (2008) Caffeine as a marker substrate for testing cytochrome P450 activity in human and rat. Pharmacological Reports 60:789-797.
16. Choi OH, Shamim MT, Padgett WL, Daly JW. (1988). Caffeine and theophylline analogues: correlation of behavioral effects with activity as adenosine receptor antagonists and as phosphodiesterase inhibitors. Life Sci. 43(5):387-398.
17. Kaplan GB, Greenblatt DJ, Kent MA, Cotreau-Bibbo MM. (1993). Caffeine treatment and withdrawal in mice: relationships between dosage, concentrations, locomotor activity and A1 adenosine receptor binding. J Pharmacol Exp Ther. 266(3):1563-1572.
18. Fredholm BB. (1995). Astra Award Lecture. Adenosine, adenosine receptors and the actions of caffeine. Pharmacol Toxicol 76:93–101.
19. Jahanfar S, Sharifah H (2009). Effects of restricted caffeine intake by mother on fetal, neonatal and pregnancy outcome. In Jahanfar S. Cochrane Database System Revise (2): CD006965.
20. Lee S, Hudson R, Kilpatrick K, Graham TE, Ross R (2005). Caffeine Ingestion Is Associated With Reductions in Glucose Uptake Independent of Obesity and Type 2 Diabetes Before and After Exercise Training. Diabetes Care March 2005 vol. 28 no. 3 566-572.
21. Goldstein J, Silberstein SD, Saper JR, Ryan RE Jr, Lipton RB. (2006). Acetaminophen, aspirin, and caffeine in combination versus ibuprofen for acute migraine: results from a multicenter, double-blind, randomized, parallel-group, single-dose, placebo-controlled study. Headache. 2006 Mar;46(3):444-453.
22. Diepvens K, Westerterp KR, Westerterp-Plantenga MS. (2006). Obesity and thermogenesis related to the consumption of caffeine, ephedrine, capsaicin, and green tea. Am J Physiol Regul Integr Comp Physiol. 2007 Jan;292(1):R77-85. Epub 2006 Jul 13.
23. Gilmore B, Michael M (2011). Treatment of Acute Migraine Headache. Am Fam Physician. 2011 Feb 1;83(3):271-280.
24. Dobson NR, Hunt CE (2013). Pharmacology Review: Caffeine Use in Neonates: Indications, Pharmacokinetics, Clinical Effects, Outcomes. NeoReviews. 14(11): e540-e550.
25. Thompson R, Keene K (2004). The pros and cons of caffeine. The Psychologist. 17 (12): 698–701.
26. Dam RM (2008). Coffee consumption and risk of type 2 diabetes, cardiovascular diseases, and cancer. Applied Physiology, Nutrition and Metabolism. 33(6): 1269–1283.
27. Koot P, Deurenberg P (1995). Comparison of changes in energy expenditure and body temperatures after caffeine consumption. Annals of Nutrition and Metabolism 39(3): 135-142.
28. Chen X, Lan X, Roche I, Liu R, Geiger JD. (2008). Caffeine protects against MPTP-induced blood-brain barrier dysfunction in mouse striatum. J Neurochem. 2008 Nov;107(4):1147-57. doi: 10.1111/j.1471-4159.2008.05697.x. Epub 2008 Sep 20.
29. Hasenfratz, M., Bunge, A., Dal Pra, G. and Battig, K. (1993) Antagonistic effects of caffeine and alcohol on mental performance parameters. Pharmacology Biochemistry and Behavior 46:463-465.
30. Terry, W and Phifer, B. (1986). Caffeine and memory performance on the AVLT. Journal of clinical psychology. 42 (6): 860–863.

31. Lesk, V, E and Womble, S. P. (2004). Caffeine, priming, and tip of the tongue: Evidence for Plasticity in the Phonological System. Behavioural Neuroscience. 118 (3): 453–461.
32. Warburton, D. M.; Bersellinni, E.; Sweeney, E. (2001). "An evaluation of a caffeinated taurine drink on mood, memory and information processing in healthy volunteers without caffeine abstinence". Psychopharmacology. **158** (3): 322–328.
33. Hameleers P, Van Boxtel M, Hogervorst E, Riedel W, Houx P, Buntinx F and Jolles J. (2000). Habitual Caffeine Consumption and its Relation to Memory, Attention, Planning Capacity and Psychomotor Performance across Multiple Age Groups. Human Psychopharmacology. 15 (8): 573–581.
34. Angelucci MEM, Cesário C, Hiroi RH, Rosalen PL and Da Cunha C. (2002). Effects of caffeine on learning and memory in rats tested in the Morris water maze. Brazilian Journal of Medical and Biological Research. 35 (10): 1201–1208.
35. Luszczki JJ, Zuchora M, Kozinska J and Ozog AA. (2006). Caffeine impairs long-term memory in the step-through passive avoidance task in mice. Annales Universitatis Mariae Curie-Sklodowska. 61: 792–796.
36. Herz, R. (1999). "Caffeine effects on mood and memory". Behaviour Research and Therapy. 37 (9): 869–879.
37. Warburton DM (1995). Effects of caffeine on cognition and mood without caffeine abstinence. Psychopharmacology. 119 (1): 66–70.
38. Ekong MB, Akpan EH. Nwakanma AA (2016). Murine's lateral frontal cortical histomorphology and its behavior after caffeine administration. Ann Bioanthropol. 4: 84-89.
39. Borota D, Murray E, Keceli G, Chang A, Watabe JM, Ly M, Toscano JP and Yassa MA. (2014). Post-study caffeine administration enhances memory consolidation in humans. Nature Neuroscience 17, 201–203.
40. Benowitz NL (1990). Clinical pharmacology of caffeine. Annual Review of Medicine. 41: 277-288.
41. Klosterman L (2006). The facts about caffeine (Drugs). New York: Benchmark Books: p 43.
42. Synder SH (1981). Adenosine receptors and the actions of methylxanthines. Trends in Neurosciences. 4: 242-244.
43. Christian, M. S. and Brent, R. L. (2001). Teratogen update: evaluation of the reproductive and developmental risks of caffeine. Teratology 64(5): 1-78.
44. Nolan, L. K. (2001). The world's favorite beverage- coffee- and health. Journal of Herbs, Spices and Medicinal Plants 8:119- 159.
45. Johnson-Kozlow M, Kritz-Silverstein D, Barrett-Connor E, Morton D (2002). Coffee consumption and cognitive function among older adults. American Journal of Epidemiology. 156 (9): 842–850.
46. Smith A (2002). Effects of caffeine on human behavior. Food Chemical Toxicology. 40 (9): 1243–1255.
47. Han M-E, Park K-H, Baek S-Y, Kim B-S, Kim J-B, Kim H-J, Oh S-O. Inhibitory effects of caffeine on hippocampal neurogenesis and function. Biochemical and Biophysical Research Communications 356 (2007) 976–980.
48. Wentz CT and Magavi S. S.P. (2009). Caffeine alters proliferation of neuronal precursors in the adult hippocampus. Neuropharmacology, 56(6-7): 994-1000.
49. Greene RW, Haas HL and Hermann A. (1985). Effects of caffeine on hippocampal pyramidal cells in vitro. Br J Pharmacol. 85(1):163-9.
50. Dar, M. S. and Wooles, W. R. (1986) Effect of chronically administered methylxanthines on ethanol-induced motor incoordination in mice. Life Sciences 39: 1429-1437.
51. Fillmore, M. T. (2003) Alcohol tolerance in humans is enhanced by prior caffeine antagonism of alcohol-induced impairment. Experimental and Clinical Psychopharmacology 11:9- 17.
52. Gulick, D. and Gould, T. J. (2009) Effects of ethanol and caffeine on behavior in C57BL/6 mice in the plus-maze discriminative avoidance task. Behavioral Neuroscience 12(3): 1271 -1278.
53. Kovacs EM, Lejeune MP, Nijs I, Westerterp-Plantenga MS. (2004). Effects of green tea on weight maintenance after body-weight loss. Br J Nutr. 2004 Mar;91(3):431-7.

54. Westerterp-Plantenga MS, Lejeune MP, Kovacs EM. (2005). Body weight loss and weight maintenance in relation to habitual caffeine intake and green tea supplementation. Obes Res. 2005 Jul;13(7):1195-204.
55. Dulloo AG. Ephedrine, xanthines and prostaglandin-inhibitors: actions and interactions in the stimulation of thermogenesis. Int J Obes Relat Metab Disord 17 Suppl 1: S35-S40, 1993.
56. Khairnar A, Plumitallo A, Frau L, Schintu N, Morelli M (2010). Caffeine Enhances Astroglia and Microglia Reactivity Induced by 3,4-Methylenedioxymethamphetamine ('Ecstasy') in Mouse Brain. Neurotox Res (2010) 17:435–439.
57. Kaplan GB, Greenblatt DJ, Kent MA, Cotreau MM, Arcelin G, Shader RI. (1992). Caffeine-induced behavioral stimulation is dose-dependent and associated with A1 adenosine receptor occupancy. Neuropsychopharmacology. 6(3):145-153.
58. Marret S, Gressens P, Van-Maele-Fabry G, Picard J, Evrard P (1997). Caffeine-induced disturbances of early neurogenesis in whole mouse embryo cultures. Brain Research 773 1997 213–216.
59. Durani SK, Ford R, Sajjad SH (1991). Capgras syndrome associated with a frontal lobe tumour. Irish Journal of Psychological Medicine. 8(2): 135-136.