Afr. J. Med. med. Sci. (2015) 44, 229-236

Effect of taurine and caffeine on plasma c-reactive protein and calcium in Wistar rats

BV Owoyele[1], AL Oyewole[1], SA Biliaminu[2] and Y Alashi [1]

Neuroscience and Inflammation Unit, Departments of Physiology[1], and Chemical Pathology and Immunology[2], Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria

Abstract

Background: Caffeine is a component of several beverages such as coffee and tea. It has been shown to possess psychoactive properties because it increases alertness, energy and ability to concentrate at moderate doses. Taurine on the other hand, is an amino acid which has the capacity to promote neural development, osmoregulation and neuroprotection. There is paucity of information on the effect of the combined administration of taurine and caffeine on C-reactive protein (CRP) – a marker of inflammation and plasma calcium level in rats.

Aim: The present study was designed to investigate the effects of combined taurine and caffeine on the plasma level of CRP, Ca^{2+} as well as the effect of nifedipine on calcium level.

Method: Fifty four rats weighing 120-140 g were used for these studies. The animals were divided into nine groups consisting of six animals each. Group 1 was treated with 10 ml/kg of normal saline, Groups 2 and 3 were given 100 mg/kg and 200 mg/kg of taurine respectively, groups 4 and 5 received 7.5 mg/kg and 15 mg/kg of caffeine respectively while group 6 was administered taurine (200 mg/kg) and caffeine (15 mg/kg), groups 7 and 8 were treated with taurine (200 mg/kg) *plus* nifedipine (10 mg/kg) and taurine (200 mg/kg) *plus* furosemide (20 mg/kg) respectively while grup 9 was given taurine plu caffeine plus nifdipine plus furosemide. Treatment was done once daily for 21 days and blood was finally collected via cardiac puncture for the assay of CRP and calcium while the animals were under anaesthesia.

Results: The results showed that CRP was significantly decreased in five of the treated groups compared with the control with the exception of the group treated with taurine alone (Group 2), and that treated with combined taurine and caffeine (Group 6). The Ca^{2+} level of groups treated with caffeine (11.70 ± 0.29 mg/dL) and taurine with caffeine (11.64 ± 0.15 mg/dL) were significantly ($p<0.05$) increased compared with the control (10.70 ± 0.29 mg/dL). However, treatment with taurine and nifedipine (Group 7) led to significant ($p<0.05$) reduction in plasma Ca^{2+} level.

Conclusion: The results have shown that combined caffeine and taurine can boost plasma calcium level and decrease plasma CRP level. Moreover, taurine combined with nifedipine but not furosemide can act synergistically to lower both plasma Ca^{2+} and CRP levels, a result which may have implication for the treatment of hypertension.

Keywords: *CRP, Caffeine, Calcium blocker, Taurine, Inflammation marker*

Résumé

Contexte: La caféine est un composant de plusieurs boissons telles que le café et le thé. Elle a été montrée pour posséder des propriétés psycho-actives, car elle augmente la vigilance, l'énergie et la capacité de se concentrer à doses modérées. Le taurine d'autre part est un acide aminé qui a la capacité de promouvoir le développement neuronal, la régulation de l'osmose et la protection du neurone. Il ya manque d'information sur l'effet de l'administration combinée de la taurine et de la caféine sur la protéine C-réactive (PCR) - un marqueur d'inflammation et du niveau de calcium dans le plasma dans les rats.

Objectif: La présente étude a été conçue pour étudier les effets combinées de la taurine et de la caféine sur le niveau de PCR, Ca2 + ainsi que l'effet de la nifédipine sur le niveau de calcium du plasma.

Méthode: Quarante-huit rats pesant 120-140 g ont été utilisés pour ces études. Les animaux ont été divisés en huit groupes de six animaux. Le groupe 1 a été traitée avec 10 ml / kg de solution saline normale, les groupes 2 et 3 ont reçu 100 mg / kg et 200 mg / kg de taurine respectivement, les groupes 4 et 5 a reçu 7,5 mg / kg et 15 mg / kg de caféine respectivement, tandis que le groupe 6 a été administré la taurine (200 mg / kg) et la caféine (15 mg / kg), les groupes 7 et 8 ont été traités avec la taurine (200 mg / kg) plus la nifédipine (10 mg / kg), et la taurine (200 mg / kg) plus le furosémide (20 mg / kg), respectivement. Le traitement a été effectué une fois par jour pendant 21 jours et le sang a été recueilli enfin par ponction cardiaque pour le dosage de PCR et de calcium, tandis que les animaux étaient sous anesthésie.

Correspondence: Mr. A.L. Oyewole, Neuroscience and Inflammation Unit, Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria. E-mail: oyewoleal@unilorin.edu.ng.

Résultats: Les résultats ont montré que la PCR était significativement diminuée dans cinq des groupes traités par rapport au témoin, à l'exception du groupe traité seulement avec de la taurine (groupe 2), et celui traité avec la taurine et la caféine combiné (groupe 6). Le niveau de Ca2+ des groupes traités avec la caféine (11,70 ± 0,29 mg / dL) et taurine avec caféine (11,64 ± 0,15 mg / dL) étaient significativement ($p < 0,05$) augmentés par rapport au groupe témoin (10,70 ± 0,29 mg / dL).
Cependant, le traitement avec la taurine et la nifédipine (Group7) a conduit à une réduction significative ($p < 0,05$) dans le niveau Ca2+ du plasma.
Conclusion: Les résultats ont montré que la caféine et la taurine combinée peut augmenter le niveau de calcium dans le plasma et de diminuer le niveau de PCR plasmatique. En outre, la taurine combinée avec la nifédipine, mais pas avec le furosémide peut agir de manière synergique pour diminuer, en même temps, les niveaux de Ca2+ et de PCR plasmatique, un résultat qui peut avoir d'incidence sur le traitement de l'hypertension.

Mots-clés: *PCR, Caféine, calciques, taurine, marqueur d'inflammation*

Introduction

Caffeinated foods and beverages are widely consumed to meet energy needs and for variety of medical purposes [1]. Caffeine, one of the most consumed psychoactive substances in the world is believed to have gained this popularity globally because of its documented ability to enhance wakefulness, mood and cognition [1-3]. As a psychoactive substance, the effects of caffeine are known to be dose dependent [3,4]. At low doses for instance, mild euphoria, alertness, and enhanced cognitive performance have all been identified with caffeine [3]. At high doses however, it produces nausea, anxiety, trembling, and jitteriness [4]. The effects seen in either of the above doses are connected with the ability of caffeine to increase dopamine action by blockade of A2A receptors. Consequently, locomotor activities are increased due to reduction in adenosine's inhibition of striatal dopaminergic activity [5]. While a previous study claimed that even at an extremely high level of intake, caffeine has no detectable effect on the balance or tissue concentrations of calcium, fluoride or phosphorus in rats[6], another study reported that caffeine increases calcium absorption and excretion and raises parathyroid hormone (iPTH) levels[7]. Related reports also documented caffeine to cause increase loss of calcium, magnesium and zinc [8-10]. In another study, it was observed that caffeine had no significant effect on CRP in human placed on clozapine medication [11] but in culture medium, it can potentiate synthesis and secretion of CRP as well as increased accumulation of CRP mRNA [12].

This earlier work showed that caffeine acts at a pretranslational level and does not simply facilitate release of newly synthesized CRP from intracellular storage pools or decrease intracellular CRP degradation.

Unlike caffeine, taurine is a naturally occurring amino acid with a similar structure to the neurotransmitters glycine and gamma amino butyric acid [13]. Despite this structural similarity, the effects of taurine cannot be mimicked by either glycine or GABA [14-16]. Apart from glutamine, taurine is the most abundant free amino acid in the brain [17]. Therefore, there are obvious expressions of taurine transporters in various neurons and astroglial cells [18]. Studies indicate that taurine plays a major role in neural development, osmoregulation and neural protection. Also, it was reported that taurine might have protective effects in the retinal neurons against glutamate toxicity in certain disease [13]. Taurine deficiency is linked with epilepsy, anxiety, depression and hyperactivity, while taurine supplementation has been shown to relieve these symptoms [19]. Taurine is also believed to help the cardiovascular system through a variety of mechanisms including an improved lipid profile, modulation of $(Ca^{2+})i$, antioxidant effects and antagonism of angiotensin II action [20]. Several studies had implicated taurine in the maintenance of homeostasis of intracellular Ca^{2+} concentrations $(Ca^{2+})i$ and intracellular Na^{+}, and in the balance of neurotransmitters [21-23]. Furthermore, literature shows that taurine is able to increase adiponectin levels, and to decrease high-sensitivity CRP and lipid peroxidation in obese women [24].

Calcium (Ca^{2+}) is also a naturally occurring element that acts as second messenger in the regulation of various cellular processes [25]. Ca^{2+} signaling mechanisms had been proved to be necessary for fertilization, cell differentiation and proliferation [26]. The importance of Ca^{2+} has also been established in intercellular signaling, transcription factor activation and in various death programme including necrosis and apoptosis [27]. Calcium enters the cytoplasm by an influx via plasmalemmal voltage-operated and via receptor-operated Ca^{2+} channels that can be shut by blockers thus prevent any possible influx into the cytoplasm. Voltage Gated Calcium Channels Blocker (VGCC) can be a non-specific blocker or a selective calcium channel blocker. The non-specific VGCC blockers such as cadmium can block L, N, P, Q, R, and T-type calcium channels while a selective calcium blocker

can only block a particular VGCC [28-30]. Both nifedipine and furosemide selectively block L-type calcium channels [31,32].

C-reactive (CRP) protein is an established typical marker of inflammation that acts as an indicator for various pathological processes and tissue damage in both humans and animals [33]. Plasma CRP concentrations may rise within 24 – 48h in an acute inflammation and continues to circulate equally in the vascular compartment [34]. Measurement of plasma CRP is a good marker of disease activity and it is sometime targeted for diagnosis and management of infectious diseases and other inflammatory conditions [34-36]. Recently, there is an increased investigations into the effects of CRP because studies have implicated it as a marker of inflammation in cardiovascular diseases especially atherosclerosis [34, 37-39].

Because calcium participates directly or indirectly in many physiological functions and CRP is an established maker for inflammation which in turn is an underlying genesis for many diseases, we believe that any substance that can balance body Ca^{2+} and CRP levels positively will be beneficial to healthy living. Thus, the present study was designed to establish possible beneficial or harmful effects of caffeine and taurine intake (separately and combined) on plasma $Ca^{2+}$and CRP levels in normal rats and rats pretreated with Ca^{2+} blockers.

Materials and method

Animal preparation

The study was carried out using fifty four adult Wistar rats weighing 120-140g. The rats were obtained from the breeder stock of Biological Sciences, University of Ilorin, Nigeria. Nine groups of six animals were formed and the animals were housed in well ventilated standard cages. The animals were kept throughout the study, in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin, Nigeria. They had free access to rat pellet (feeds) and clean water. Rules guiding animal studies as stipulated by the Ethical Committee of University of Ilorin, Nigeria were strictly followed. These rules are similar to international guidelines on animal handling [40].

Drug preparation

The taurine and caffeine used for these studies were produced by Titan Biotech LTD (BHIWADI -301 019, Raj.) and Sigma-Aldrich Co. USA (NO. 63103) respectively. Nifedipine and furosemide were the Ca^{2+} blockers used for these investigations and were both produced by Jiangxi Xier Kangtai Pharmaceuticals Co Ltd (Batch No: 120820) and Tianjin Pharmaceutical Group Xinzheng Co Ltd (Batch No: 1211081) respectively. Each drug was dissolved separately in normal saline to form the stock solution. Drugs were administered intraperitoneally except nifedipine which was administered orally. Animals in the control group received normal saline intraperitoneally. Where blockers were used, animals were given the blockers thirty (30) minutes before the treatment with caffeine, taurine or both. All treatments were done every morning, once daily for a period of twenty-one (21) days with an inter-treatment time interval of 24 ± 2 hours.

Animal grouping

Animals were grouped into nine consisting of six rats each and treated as follows: Group 1, the Control group was given 10 ml/kg of normal saline, Group 2 was treated with 100 mg/kg of taurine, Group 3 was given 200 mg/kg taurine, Group 4 was given 7.5 mg/kg of caffeine, Group 5 was treated with 15 mg/kg caffeine, Group 6 took taurine (200 mg/kg) *plus* caffeine (15 mg/kg), Group 7 took taurine (200 mg/kg) *plus* nifedipine (10 mg/kg), Group 8 was administered taurine (200 mg/kg) *plus* furosemide (20 mg/kg), while Group 9 was administered taurine (200 mg/kg) *plus* caffeine (15 mg/kg) *plus* nifedipine (10 mg/kg) *plus* furosemide (20 mg/kg).

Measurements of CRP

Animals were given 50mg/kg of ketamine that was produced by Rotexmedica, Trittau, Germany. Under the influence of this anaesthetic agent, thoracicoabdominal dissection was done and blood (2.5-3.5ml) was collected through cardiac puncture from each rat into a lithium heparinized sample bottle. The blood was then centrifuged in a centrifuging machine and plasma was extracted from the sample for CRP assay. Analysis of CRP was carried out immediately after collection of samples using C-Reactive Protein (CRP) ELISA Kit produced by the Cell Biolabs, INC. (USA). The detection limit was 1ng/mL

Measurement of calcium

Part of the plasma collected from the blood samples was also used to assay calcium levels. The samples were momentarily stored at -4°C till CRP quantifications were fully done. Calcium concentration was determined by using calcium Colorimetric Assay Kit produced by BioVision, INC. (USA). The kit detection limit was 0.4-100 mg/dL.

Statistical analysis

The results were analyzed using one-way analysis of variance of SPSS 17 (SPSS Inc., Chicago, IL,USA). Data are presented as means ± standard error of the mean (SEM) for 6 rats per group. Statistically significant difference at $p<0.05$ was accepted.

Results

Effect of taurine and caffeine on plasma calcium level

The results (Table 1) showed that administration of taurine alone at the two doses used did not produce significant effects on plasma calcium level. In contrast, caffeine administration at the dose of 15 mg/kg increased the plasma calcium compared with the control group. Furthermore, coadministration of taurine and caffeine also led to significant ($p<0.05$) increase in plasma calcium compared with the control group.

Table 1: Effect of taurine and caffeine on plasma calcium level in rats

Group	Ca^{2+} (mg/L)
Normal saline 10ml/Kg	10.60 ± 0.37
Taurine 100mg/kg	10.34 ± 0.23
Taurine 200mg/kg	10.58 ± 0.34
Caffeine 7.5mg/kg	10.70 ± 0.29
Caffeine 15mg/kg	11.44 ± 0.33*
Taurine 200mg/kg + Caffeine 15mg/kg	11.64 ± 0.15*

Each value is the mean ± S.E.M. of six rats; Ca^{2+}
**p < 0.05 compared with control*

Effect of taurine and caffeine on plasma CRP

The results of the effect of co-administration of taurine and caffeine on plasma CRP are shown in table 2. The results showed that plasma CRP concentration was significantly ($p<0.05$) decreased in the groups administered taurine (200 mg/kg), caffeine (7.5 mg/kg) and caffeine (15 mg/kg).

Table 2. Effect of taurine and caffeine on plasma C-reactive protein in rats

Group	CRP (mg/L)
Normal saline 10ml/Kg	0.72 ± 0.01
Taurine 100mg/kg	0.58 ± 0.37
Taurine 200mg/kg	0.66 ± 0.01*
Caffeine 7.5mg/kg	0.58 ± 0.04*
Caffeine 15mg/kg	0.62 ± 0.03*
Taurine 200mg/kg + Caffeine 15mg/kg	0.46 ± 0.37

*Each value is the mean ± S.E.M. of six rats; CRP *p < 0.05 compared with control*

Effect of taurine and caffeine on plasma calcium level in calcium blocker pretreated rats

In the calcium blocker pretreated rats, the results (Fig. 1) showed that furosemide did not produce significant effect on plasma calcium level compared with the control. Also, co-administration of taurine and caffeine in the pretreated nifedipine *plus* furosemide rats did not produce significant effect on plasma calcium level. However, when taurine was administered to nifedipine pretreated rats, there was a significant ($p<0.05$) decrease in plasma calcium level compared with the control.

Effect of taurine and caffeine on plasma CRP level in calcium blocker pretreated rats

The results (Fig.2) showed that CRP concentration was significantly ($p<0.05$) decreased in the group administered taurine plus nifedipine (200 mg/kg +10mg/kg) and taurine *plus* caffeine *plus* nifedipine *plus* furosemide compared with the control. There was no significant difference in the group given taurine (200 mg/kg) *plus* furosemide (20mg/kg).

Discussion

The recent surge in production of various caffeinated and taurine added foods and beverages makes the consumption of both substances almost inevitable. Hence, it is important to ascertain whether these caffeinated and taurine added foods and beverages are as safe as claimed by the manufacturers.

In the investigation into plasma calcium level in the treated groups, it was observed that plasma calcium level in the caffeine (15 mg/Kg) group and the taurine (200 mg/kg) *plus* caffeine (15 mg/kg) group were significantly increased when compared with the control group (table 1). Based on each value, plasma calcium level was found to be higher in taurine (200 mg/kg) *plus* caffeine (15mg/kg) group than the caffeine (15 mg/Kg) group (Table 1) though there was no significant difference in between treated groups. Interestingly, this observed action of caffeine is different from what was reported in some previous studies where caffeine administration was reported to decrease the net balance of calcium as a result of increased calcium excretion [41-43]. However, it was concluded in a review that there is no evidence that caffeine has any harmful effect on bone status or on the calcium economy in individuals who ingested daily recommended amounts of calcium [44]. The possible mechanisms for this observed increase in plasma calcium level in the caffeine (15 mg/Kg) treated group is not known. The taurine (200 mg/kg) *plus* caffeine (15 mg/kg) group on the other hand can be

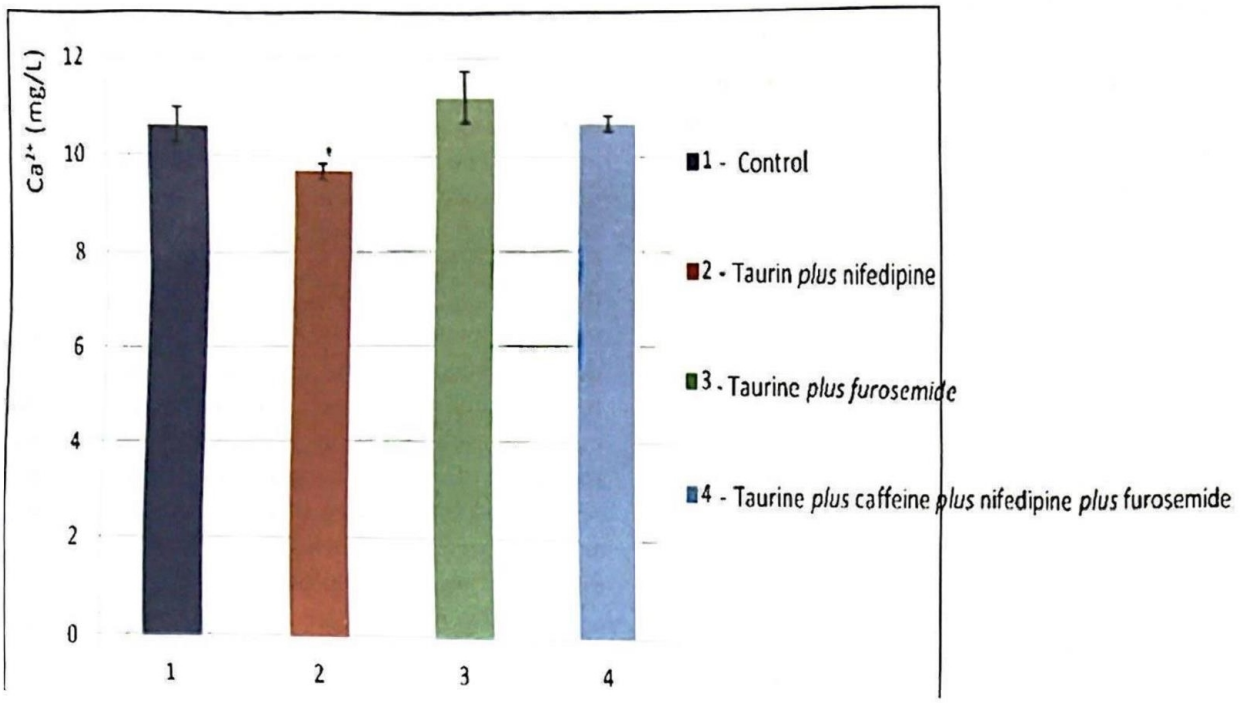

Fig.1. Effect of taurine and caffeine on plasma calcium level in calcium blocker pretreated rats Each value is the mean ± S.E.M. of six rats; Ca^{2+} *p < 0.05 compared with control

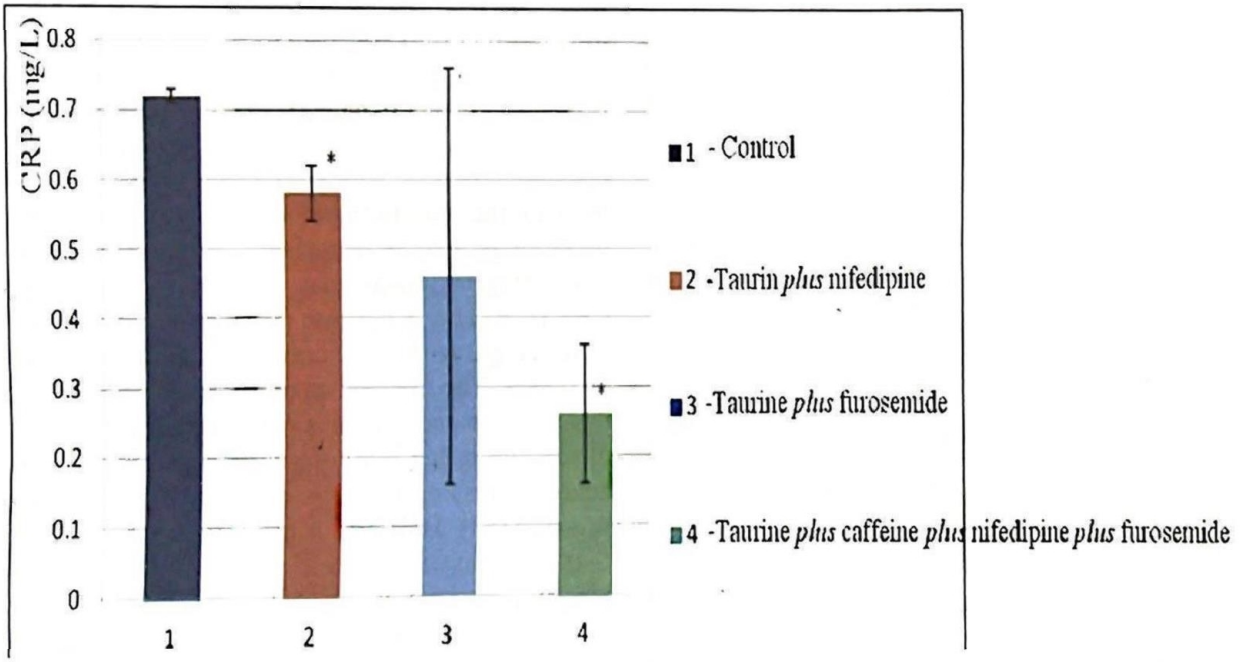

Fig.2 Effect of taurine and caffeine on plasma CRP level in calcium blocker pretreated rats Each value is the mean ± S.E.M. of six rats: CRP *p < 0.05 compared with control

said to show increased plasma calcium level partly due to the effect of the co-administered taurine. Taurine has been documented to be involved in the maintenance of intracellular Ca^{2+} homeostasis and intracellular Na^{+} as well as in the balance of neurotransmitters [21-23]. Observations from this study showed taurine (200 mg/kg) *plus* nifedipine (10 mg/kg) treated group had significant decrease in plasma calcium level compared with the control group (Fig. 1). The result showed that taurine's potency to maintain intracellular Ca^{2+} homeostasis is masked by the nifedipine blocking action on L-type VGCC thus preventing inflow of calcium into the intracellular compartment. This is contrary to what was observed in the other calcium blocker group, the taurine (200 mg/kg) *plus* furosemide (20

mg/kg) treated group where results showed no significant difference compared with the control group at $p<0.05$ (Fig. 1). Since furosemide also blocks L-type Ca^{2+} channel then it can be assumed that the mechanism of action of taurine on Ca^{2+} is not via L-type Ca^{2+} channel.

In the investigation on plasma CRP level, the results showed significant decrease in plasma CRP level in all the treated groups when compared with the control at $p<0.05$ except in taurine (100 mg/kg), taurine (200 mg/kg) *plus* caffeine (15 mg/kg) (table 2) and taurine (200 mg/kg) *plus* furosemide (20 mg/kg) group (Fig. 1) that showed no significant difference compared with the control. The observed significant decrease in plasma CRP level seen in both doses of caffeine used is contrary to previous reports that caffeine neither has effect on CRP [11] nor increased its synthesis and secretion [12]. The significant decrease recorded in taurine (200 mg/kg) group is however similar to a claim herein stated that taurine is able to decrease high-sensitivity CRP and lipid peroxidation [24]. Interestingly, co-administration of taurine (200mg/kg) *plus* caffeine (15mg/kg) did not decrease CRP level (Table 2). This is surprising since both drugs were able to significantly decrease plasma CRP level when administered separately. One would expect that the combined effects of the two drugs would lead to significant decrease in plasma CRP when compared with the control but this was not so. However, the effect of both caffeine and taurine was more obvious in the group that was administered taurine *plus* caffeine *plus* nifedipine *plus* furosemide. This group showed a remarkably low ($p<0.05$) plasma CRP (Fig. 2). Significant decrease in plasma CRP level was also recorded in the taurine (200 mg/kg) *plus* nifedipine (10 mg/kg) treated group (Fig. 2).

In this study, we were able to establish that caffeine at 15 mg/kg in rats is beneficial and hence may be extrapolated in man. At this dose, we observed a significant increase in plasma calcium levels and significant decrease in plasma CRP levels. CRP is an internationally acceptable inflammatory marker. Inflammation processes have been implicated in many diseases [45,46] such as rheumatoid arthritis [47], neural degeneration [48], cardiovascular diseases [49] among others. Interestingly, level of CRP is used clinically to measure the progress of CRP-associated diseases as well as a measure of response to treatment. Thus, substance that reduces level of CRP might be helpful in the treatment of inflammation that underlies many inflammatory diseases. When added to drinks and beverages at range of doses used in this study, caffeine might help to prevent or reduce neural degeneration such as in mesencephalic dopaminergic neurons. The susceptibility to neural degeneration of mesencephalic dopaminergic neurons is associated with the inflammatory markers studied in plasma (including TNF- a, IL-1b, IL-6 and CRP)[48]. On the other hand, the fact that our low dose of caffeine significantly increased plasma Ca^{2+} showed that this dose can enhance neural function. Ca^{2+} homeostasis in nerve cells is associated with many specific activities such as neurite growth and development, regulation of synaptic connections, neurotransmitter release and propagation of nerve impulses, as well as physiological processes during cell metabolism, differentiation and apoptosis [50-52].

Also of important note is the observation in the taurine (200mg/kg) *plus* nifedipine (10mg/kg) group. At the dosages used, we established that co-administration of taurine and nifedipine led to significant decrease in both Ca^{2+} and CRP. People at risk for developing cardiovascular disease [49] frequently have evidence for a systemic inflammatory response, usually marked by elevations of C-reactive protein (CRP) in their blood. Such people and others diagnosed to be hypertensive and are on nifedipine medication might benefit more by combining taurine with their medication. This will help in the management of the elevated blood pressure as well as decreasing various chances of developing cardiovascular diseases by decreasing CRP level. This is because decrease in CRP level is associated with a decrease in inflammatory processes. The mechanism of action of nifedipine in hypertension and angina is partly through inhibition of calcium influx into arterial smooth muscle cells hence decreasing both systolic and diastolic blood pressure [53]. However, it is important to note here that combination of taurine with other calcium blockers may not always yield the same desirable result. Co-administration of taurine and furosemide in this study produced no significant difference in plasma level of calcium and CRP when compared with the control group.

In conclusion, the use of caffeine can boost plasma Ca^{2+} and reduce CRP while combining taurine with nifedipine can decrease both plasma calcium and CRP level. The former might be useful for the treatment of systemic inflammation, hypocalcaemia and skeletal fragility while the later might be useful in enhancing the management of hypertension.

References

1. Yang A, Abraham AP and Harriet de W. Genetics of caffeine consumption and responses to caffeine Psychopharmacology 2010; 211:245–257
2. Haskell CF, Kennedy DO, Wesnes KA and Scholey AB. Cognitive and mood improvements ofcaffeine in habitual consumers and habitual non-consumers of caffeine. Psychopharmacology (Berl) 2005; 179:813–825
3. Lieberman HR, Tharion WJ, Shukitt-Hale B, *et al.* Effects of caffeine, sleep loss, and stress on cognitive performance and mood during U.S. Navy SEAL training. Sea–Air–Land. Psychopharmacology (Berl) 2002; 164:250–261
4. Daly JW and Fredholm BB. Caffeine—an atypical drug of dependeOnce. Drug Alcohol Depend 1998; 51:199–206
5. Fisone A, Borgkvist A and Usiello A. Caffeine as a psychomotor stimulant: mechanism of action. Cell Mol Life Sci 2004;61:857–872.
6. Chen X and Whitford GM. Effects of caffeine on fluoride, calcium and phosphorus metabolism and calcified tissues in the rat. Archives of Oral Biology 1999; 44:33-39
7. Glajchen N, Ismail F, Epstein S, *et al.* The effect of chronic caffeine administration on plasma markers of bone mineral metabolism and bone histomorphometry in the rat. Calcif Tissue Int. 1988; 43(5):277-280.
8. Hasling C, Sondergaard K, Charles P and Mosekilde L. Calcium metabolism in post-menopausal osteoporotic women is determined by dietary calcium and coffee intake. Journal of Nutrition. 1992, 122(5): 1119-1126.
9. Johnson S. The multifaceted and widespread pathology of magnesium deficiency. Medical Hypotheses 2001; 56(2):163-170.
10. Ribeiro-Alves MA, Trugo LC and Donangelo CM. Use of oral contraceptives blunts the calciuric effect of caffeine in young adult women. Journal of Nutrition. 2003; 133: 393- 398.
11. Raaska K, Raitasuo V, Laitila J and Neuvonen PJ. Effect of Caffeine-Containing versus Decaffeinated Coffee on Plasma Clozapine Concentrations in Hospitalised Patients. Basic & Clinical Pharmacology & Toxicology 2004; 94:13–18.
12. Ganapathi MK, Mackiewicz A, Samols D *et al.* Induction of C-reactive protein by cytokines in human hepatoma cell lines is potentiated by caffeine Biochem. J. 1990; 269, 41-46
13. Simon B and Wen S. Reciprocal regulation between taurine and glutamate response via Ca2+- dependent pathways in retinal third-order neurons. Journal of Biomedical Science 2010; 17(Suppl 1):S5
14. Alexandrov AA and Batuev AS. Intracellular studies of GABA and taurine action of the neurons of the cat sensorimotor cortex. J Neurosci Res 1979; 4:59-64.
15. Okamoto K and Saki Y. Inhibitory actions of taurocyamine, hypotaurine, homotaurine, taurine and GABA on spike discharges of Purkinje cells, and localization of sensitive sites, in guinea pig cerebellar slices. Brain Res. 1981; 206:371-386.
16. Iwata H, Nakayama K, Matsuda T and Baba A: Effect of taurine on a benzodiazepine-GABA-chloride ionophore receptor complex in rat brain membranes. Neurochem Res. 1984; 9:535-544.
17. Sturman JA: Taurine in development. J Nutr 1988; 118:1169-1176.
18. Pow DV, Sullivan R, Reye P and Hermanussen S: Localization of taurine transporters, taurine, and (3)H taurine accumulation in the rat retina, pituitary, and brain. Glia. 2002; 37:153- 168.
19. Kong WX, Chen SW, Li YL, *et al.* Effects of taurine on rat behaviours in three anxiety models. Pharmacol Biochem Behav 2006; 83:271-276.
20. Xu Y-J, Arneja AS, Tappia PS and Dhalla NS. The potential health benefits of taurine in cardiovascular disease. Exp Clin Cardiol 2008; Vol 13 No 2.
21. Guizouarn H, Motais R, Garcia-Romeu and Borgese F. Cell volume regulation: The role of taurine loss in maintaining membrane potential and cell pH. J Physiol 2000; 523:147-154.
22. Parcell S. Sulfur in human nutrition and applications in medicine. Altern Med Rev 2002; 7:22-44.
23. Gupta RC, Win T and Bittner S. Taurine analogues; a new class of therapeutics: Retrospect and prospects. Curr Med Chem 2005; 12:2021-2039.
24. Rosa FT, Freitas EC, Deminice R, *et al.* Oxidative stress and inflammation in obesity after taurine supplementation: a double-blind, placebo-controlled study. Eur J Nutr. 2014; 53(3):823-830.
25. De Smedt H, Verkhratsky A and Muallem S. Ca(2 +) signaling mechanisms of cell survival and cell death: an introduction. Cell Calcium 2011; 50:207–210.
26. Munaron L, Antoniotti S and Lovisolo D. Intracellular calcium signals and control of cell

proliferation: how many mechanisms? J Cell Mol Med 2004; 8:161–168.
27. Orrenius S, Zhivotovsky B and Nicotera P. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 2003; 4:552–565.
28. Yamakage M and Namiki A. Calcium channels–basic aspects of their structure, function and gene encoding; anaesthetic action on the channels–a review. Canadian journal of anaesthesia = Journal canadien d'anesthesie 2002; 49: 151–164.
29. Nishikitani M, Yasuoka Y, Kawada H and Kawahara K. L-type Ca2+ channels in the enteric nervous system mediate oscillatory Cl- secretion in guinea pig colon. The Tohoku journal of experimental medicine 2007; 211: 151–160.
30. Engbers JD, Anderson D, Tadayonnejad R, *et al.* Distinct roles for I(T) and I(H) in controlling the frequency and timing of rebound spike responses. The Journal of physiology 2011; 589: 5391–5413.
31. van Mil HG, Geukes Foppen RJ and Siegenbeek van Heukelom J. The influence of bumetanide on the membrane potential of mouse skeletal muscle cells in isotonic andhypertonic media. Br J Pharmacol 1997;120:39-44.
32. Spafford JD, Dunn T, Smit AB, Syed NI and Zamponi GW. In vitro characterization of L-type calcium channels and their contribution to firing behaviour in invertebrate respiratory neurons. Journal of neurophysiology 2006; 95: 42–52.
33. Volanakis JE. Human C-reactive protein: expression, structure, and function. Mol Immunol 2001;38:189 – 197.
34. Ablij HC and Meinders AE. C-reactive protein: history and revival. Eur J Intern Med 2002; 13: 412 – 422.
35. Morley JJ and Kushner I. Plasma C-reactive protein levels in disease. Ann NY Acad Sci 1982; 389: 406 – 418.
36. Young B, Gleeson M and Cripps AW. C-reactive protein: a critical review. Pathology 1991; 23: 118 – 24.
37. Ridker PM and Rifai N. C-reactive protein and cardiovascular disease. St-Laurent, Canada: MediEdition Inc., 2006:393.
38. Ridker PM, Danielson E, Fonseca FA, *et al.* Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359: 2195 – 2207.
39. Pena JM, MacFadyen J, Glynn RJ and Ridker PM. High-sensitivity C-reactive protein, statin therapy, and risks of atrial fibrillation: an exploratory analysis of the JUPITER trail. Eur Heart J 2012;33:531 – 537.
40. Gouirand AM and Matuszewich L. The effects of chronic unpredictable stress on male rats in the water maze. Physiol Behav 2005; 86: 21–31.
41. Heaney RP and Recker RR. Effects of nitrogen,phosphor us, and caffeine on calcium balance in women. Journal of Laboratory and Clinical Medicine 1982; 99:46–55.
42. Yeh JK and Aloia JF. Differential Effect of Caffeine Administration on Calcium and Vitamin D Metabolism in Young and Adult Rats. Journal of Bone And Mineral Research Volume 1, Number 3, 1986.
43. Whiting SJ, Whitney HL. Effect of dietary caff?eine and theophylline on urinary calcium excretion in the adult rat. J. Nutr. 1987; 117:1224-1228.
44. Heaney RP. Effects of caffeine on bone and the calcium economy. Food and Chemical Toxicology 2002; 40:1263–1270.
45. Ross R. Atherosclerosis - an inflammatory disease. N Engl J Med. 1999; 340:115–126 46.
46. Libby P., Ridker, PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002; 105, 1135–1143
47. Christensen AW, Rifbjerg-Madsen S, Christensen R, *et al.* Temporal summation of pain and ultrasound Doppler activity as predictors of treatment response in patients with rheumatoid arthritis: protocol for the Frederiksberg hospitals Rheumatoid Arthritis, pain assessment and Medical Evaluation (FRAME-cohort) study. BMJ Open 2014; 4:e004313.
48. Hernandez-Romero MC, Delgado-Cortes MJ, Sarmiento M, *et al.* Peripheral inflammation increases the deleterious effect of CNS inflammation on the nigrostriatal dopaminergic system. NeuroToxicology 2012; 33:347–360.
49. Ridker PM. High-sensitivity C-reactive protein: Potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation 2001;* 103: 1813–1818.
50. Kirichok Y, Krapivinsky G and Clapham DE. The mitochondrial calcium uniporter is a highly selective ion channel. Nature 2004; 427:360–364.
51. Yano S, Brown EM and Chattopadhyay N. Calcium-sensing receptor in the brain. Cell Calcium 2004; 35:257–264.
52. Hagenston AM and Bading H. Calcium signaling in synapse-to-nucleus communication. Cold Spring Harb Perspect Biol 2011; 3(11):a004564.
53. Bertel O, Conen D, Radu EW, *et al.* Nifedipine in hypertensive emergencies British Medical Journal Volume 286 1 January 1983