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EFFECTS OF CO-ADMINISTRATION OF TOBACCO LEAF OR CIGARETTE EXTRACTS WITH CAFFEINE ON OXIDATIVE STRESS MARKERS IN BRAIN AND PLASMA OF WISTAR RATS
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EFFECTS OF CO-ADMINISTRATION OF TOBACCO LEAF OR CIGARETTE EXTRACTS WITH CAFFEINE ON OXIDATIVE STRESS MARKERS IN BRAIN AND PLASMA OF WISTAR RATS

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ABSTRACT

Background: **Oxidative stress has been linked with diseases associated with brain damage resulting from overconsumption of psycho-active neurotoxicants such as nicotine and caffeine. This study investigated the effect of administration of extracts of *Nicotiana tabacum* (tobacco leaf used for production of nicotine/cigarettes) and cigarette with caffeine on biomarkers of oxidative stress in brain and plasma of Wistar rats. The outcome of this study is expected to unveil the adverse oxidative effect of the pysho-active agents.**

Design: **Fifty-five rats were allotted randomly into eleven groups (n=5/group). Group one (control) received the vehicle, other groups were administered the following; group 2 (caffeine 10 mg/kg), group 3 (caffeine 20 mg/kg), group 4 & 5 (tobacco extracts 170 & 340 mg/kg respectively), group 6 & 7 (cigarette extracts 170 & 340 mg/kg respectively), group 8 (caffeine 10 mg/kg + tobacco extract 170 mg/kg), group 9 (caffeine 20 mg/kg + tobacco extract 340 mg/kg), group 10 (caffeine 10 mg/kg + cigarette extract 170 mg/kg), and group 11 (caffeine 20 mg/kg + cigarette extract 340 mg/kg).**

Results: **In this study, administration of caffeine, and extracts of tobacco/cigarette alone or in combination to the rats markedly increased malondialdehyde (MDA) level in the brain and plasma while marked reduction in catalase activity was observed when compared to control.**

Conclusion: **In conclusion, the present study showed that co-administration or use of nicotine and tobacco/cigarette extracts, or lone use may induce oxidative stress by modulation of the antioxidant system via increased level of malondialdehyde and decreased catalase activity.**

INTRODUCTION

Oxidative stress is a major mechanism for cellular damage associated with diverse neurotoxicants. Its high content of oxidisable polyunsaturated fatty acids combined with characteristic high oxygen consumption places the brain at the pinnacle of one of the most vulnerable organs to oxidative stress and neurotoxicants effects (1). The protective mechanisms in the brain against neurotoxicants and oxidative damage include glia which offers protection to neurone against oxidative molecules, glutathione and other protective enzymes. These protective mechanisms are more developed in the adult brain compared to the developing brain. This coupled with the heightened metabolic demands as a result of rapid growth make the developing brain more vulnerable to neurotoxicants and oxidative stress compared to the adult brain.

Caffeine (1, 3, 7-trimethylxanthine) is present in several food and beverage products, such as coffee, kola nut (*Kola nitida*) and tea. It is widely consumed, primarily for its stimulating effect on the CNS. Methylxanthines, including caffeine, have found use in therapy of brain disorders like Parkinson's disease. Others include use as analgesics, muscle relaxants and diuretics. Caffeine is also used as an ergogenic aid; moderate dosage has been reported to improve performance of athletes. The reduction in fatigue and drowsiness, and increased mental alertness produced by small quantity of caffeine stems from cortical stimulation. Reports showed protective cellular damage effects of caffeine (2, 3) coupled with beneficial antioxidant effects linked to its metabolites, 1-methyluric acid and 1- methylxanthine. These metabolites have been shown to be highly effective antioxidants capable of preventing LDL oxidation (4). However, in contrast to the aforementioned benefit, chronic administration and high dosage of caffeine may induce tremors, insomnia, nervousness, and associated effects (5). Caffeine-derived effects could favour overproduction of free radicals in cells (6) which subsequently attack macromolecules (carbohydrate, lipid protein and nucleic acid) in a chain reaction processes (7).

Nicotine [3-(1-methyl-2-pyrrolidinyl) pyridine], a naturally occurring alkaloid is a widely used psycho-stimulants in the world. Cigarettes are the readily available and common source of nicotine. Aside the nicotine content, some detrimental chemical substances, that can affect the respiratory system adversely, gain access to the system through smoked tobacco. In order to circumvent global restrictions on smoking and keep the line of patronage, a variety of smokeless alternatives have been developed by the tobacco industry. The alternatives, however, tend to contain and deliver higher concentrations of nicotine when compared with the regular cigarettes. Of interest is pulverised tobacco leaves (snuff) which when inhaled (snuffed) or chewed delivers higher nicotine concentration to the system. Worthy of mention is the habit in some individuals of chewing cigarette; another way of circumventing the policy on smoking restriction. Nicotine has been shown to deplete the body's mechanisms of antioxidant defence while generating reactive oxygen species and contributing substantially to the overall oxidative stress caused by tobacco (8). Clinical and animal model studies have shown that nicotine significantly increases level of malondialdehyde, conjugated dienes, hydroperoxides, and free fatty acids in the liver and serum of smokers (9,10). Smokers incur a sustained free radical load that increases their ascorbic acid and α-tocopherol requirements. Kola nut (*Kola nitida*) a major source of caffeine and coffee beverage are noted to be consumed by individuals who also take the different sources of nicotine. Hence, the aim of this

study was to determine the effects of exposure to caffeine and nicotine on oxidative markers in the brain and plasma of Wistar rats with specific interest on first line antioxidants defence mechanism and lipid peroxidation products (malondialdehyde).

MATERIALS AND METHODS

Drugs, Reagents and Chemicals: Thiobarbituric acid (TBA) and Trichloroacetic acid (TCA) were purchased from BDH, India Acetic acid, bicarbonate and Caffeine were purchased from Sigma-Aldrich, St. Louis, MO, USA. Other chemicals and reagents were of analytical grade. Popular brand of cigarette was purchased locally.

Sample Preparation: Tobacco leaves was procured locally and, identified and authenticated at the Department of Botany, Delta State University, Nigeria (IDV/TBC/2348). The tobacco leaf was rinsed with distilled water and was air dried. Thereafter, it was pulverised to powder with a warring blender. Extraction from cigarette and tobacco pulverised leaf was by Soxhlet extraction. The extracts were concentrated using Rotary evaporator. Estimated doses of nicotine and caffeine were adopted from previous studies (11, 12).

Animal Care and Ethics: A total of fifty-five (55) adult male albino rats weighing between 180 – 200g were procured from the Animal House, Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria. They were acclimatized for fourteen days prior to the commencement of study with unrestricted access to water and rat chow. Animals care and handling were in compliance with the ethical standard of the Institutional Animal Ethics Committee (IAEC) of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria (FBMS/EAS/7102/008).

Experimental Design: Animals were randomly distributed into seven (11) groups (n=5/group). Caffeine (10mg/kg and 20mg/kg) and extracts from tobacco/cigarette (170mg/kg and 340mg/kg), either as single or in combination were administered orally once daily for 2 weeks (14 days). The groups and nomenclature are presented in table 1 below:

Table 1

Grouping/Dose administered and Nomenclature of the Groups

Groups	Dose administered	Nomenclature
Group 1	Control	NC
Group 2	Caffeine (10mg/kg)	CF1
Group 3	Caffeine (20mg/kg)	CF2
Group 4	Tobacco extract 1 (170mg/kg)	NICT1
Group 5	Tobacco extract 2 (340mg/kg)	NICT2
Group 6	Cigarette extract 1 (170mg/kg)	NICC1
Group 7	Cigarette extract 2 (340mg/kg)	NICC2
Group 8	Caffeine (10mg/kg) + Tobacco extract 1 (170mg/kg)	CNICT1
Group 9	Caffeine (20mg/kg) + Tobacco extract 2 (340mg/kg)	CNICT2
Group 10	Caffeine (10mg/kg) + Cigarette extract 1 (170mg/kg)	CNICC1
Group 11	Caffeine (20mg/kg) + Cigarette extract 2 (340mg/kg)	CNICC2

***Blood and Tissue Collection*:** After the agents were administered to the rats for 14 days, animals were fasted overnight (12 hour). Blood samples were collected from the animas for clinical chemistry (antioxidant assay). The rats were thereafter sacrificed and the brain harvested for clinical chemistry assay. The preparation and storage of the brain tissues are as

described by Stocks et al. (13) and Prabhakara et al. (14).

***Catalase Activity Assay*:** Using the method of Sinha (24) with little modification, 75 uL of each sample was placed in two different test tubes labelled 1 and 2 and 750 uL of phosphate buffer (0.01 M, pH 7.0) was added. 300 uL of H_2O_2 (0.2 M) was added to each of the test tubes (1 and 2) and mixed. Then 500 uL of 5% potassium dichromate in of glacial acetic acid was added to the reaction mixture at one minute and 3 minutes (to stop the reaction). The mixture was boiled at 100°C for 10 minutes and allowed to cool The absorbance was measured at 570 nm using spectrophotometer. Catalase activity was calculated using the relationship below:

$$\Delta OD\ (R) = \frac{OD_1 - OD_2}{t}$$

***Superoxide Dismutase (SOD) Activity Assay*:** In this assay, 80 uL of each sample was placed in labelled test tubes and 80 ul of distilled water was placed in a labelled test tube for blank. 1000 uL of carbonate buffer (0.05 M, pH 10.2) was added to each sample and blank. The mixture was allowed to equilibrate at 37°C for 5 minutes. Then 120 uL of freshly prepared epinephrine (0.3 mM) was added and read at 30 second and after 180 seconds (3 minutes) at 480 nm using spectrophotometer.

***Malondialdehyde (MDA) Assay*:** In this assay, 100 uL of each sample was placed in labelled test tubes and 100 uL of distilled water was placed in a test tube labelled "blank". The 500 ul of 1% TBA in 20% NaOH was added to the labelled test tubes and 500 uL of glacial acetic acid was added, mixed and incubated in boiling water for 15 minutes. The mixture was allowed to cool and read at 532 nm using a spectrophotometer.

***Statistical Analysis*:** Results of each parameter were determined in triplicate. Results were presented as mean ± standard deviation of five rats in a group. Test groups were compared to control ANOVA with Tukey (HSD) test. $p< 0.05$ was considered to be significant.

RESULTS

***Effects of co-administration of Extracts and Caffeine on malondialdehyde level in the brain and serum of Wistar rats*:** There were increased concentrations of malondialdehyde in the brain and serum of animals from groups that received extracts from tobacco leaf (340mg/kg), caffeine (10mg/kg) + extract from cigarette (170mg/kg), caffeine (10mg/kg) + extract from tobacco leaf (170mg/kg) and caffeine (20mg/kg) + extract from tobacco leaf (340mg/kg) when compared with rats in group 1 (control) (Figure 1).

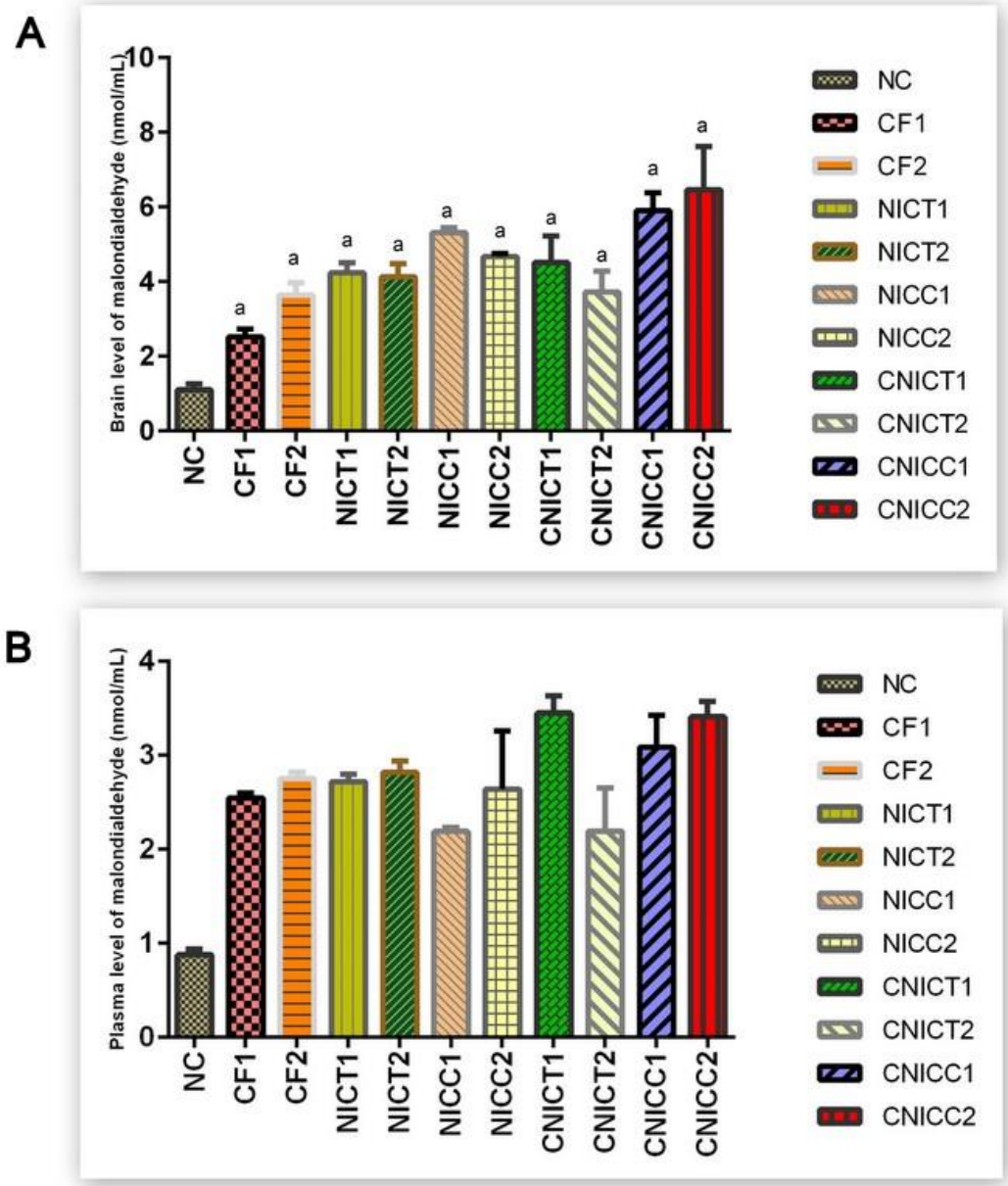

Figure 1: Effects of co-administration of Caffeine and extracts on malondialdehyde concentration in brain (A) and serum (B) of Wistar rats.

Effects of single or co-administration of Extracts and Caffeine on superoxide dismutase (SOD) activities in the brain and serum of Wistar rats: From the results there was no significant difference in superoxide dismutase activity in the brain and serum of Wistar rats treated with lone or co-administration of extracts and caffeine when compared to rats in the control group.

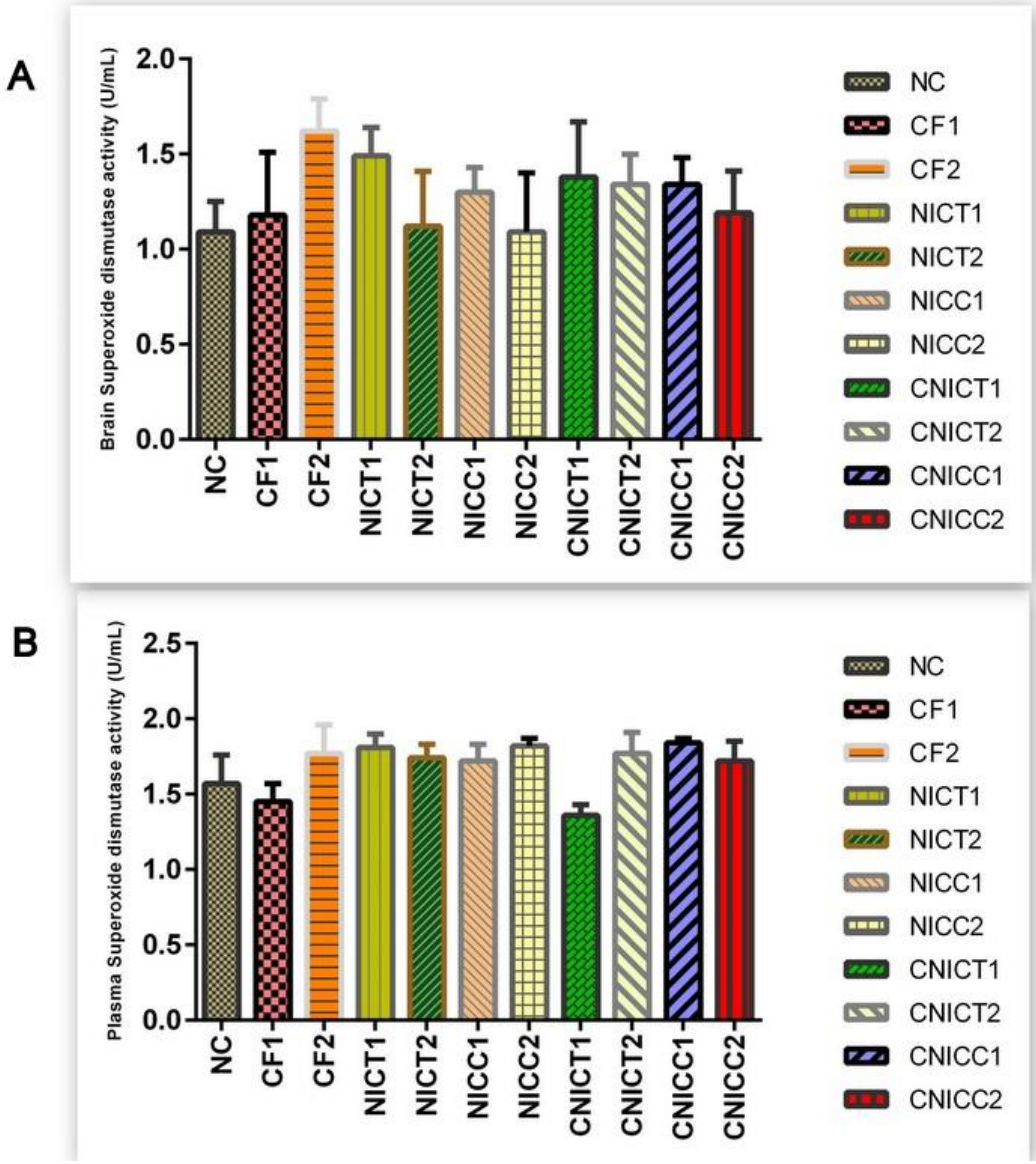

Figure 2: Effects of single or co-administration of extracts and caffeine on superoxide dismutase activity in the brain (A) and the serum (B) of Wistar rats.

***Effects of single or co-administration of extracts and caffeine on catalase activities in the brain and serum of Wistar rats*:** Marked decrease in the activity of catalase was observed in the brain and serum of rats that were administered extracts alone, caffeine alone and combination of both at selected low and high doses. Illustration about catalase activity can be found in figure 3 below.

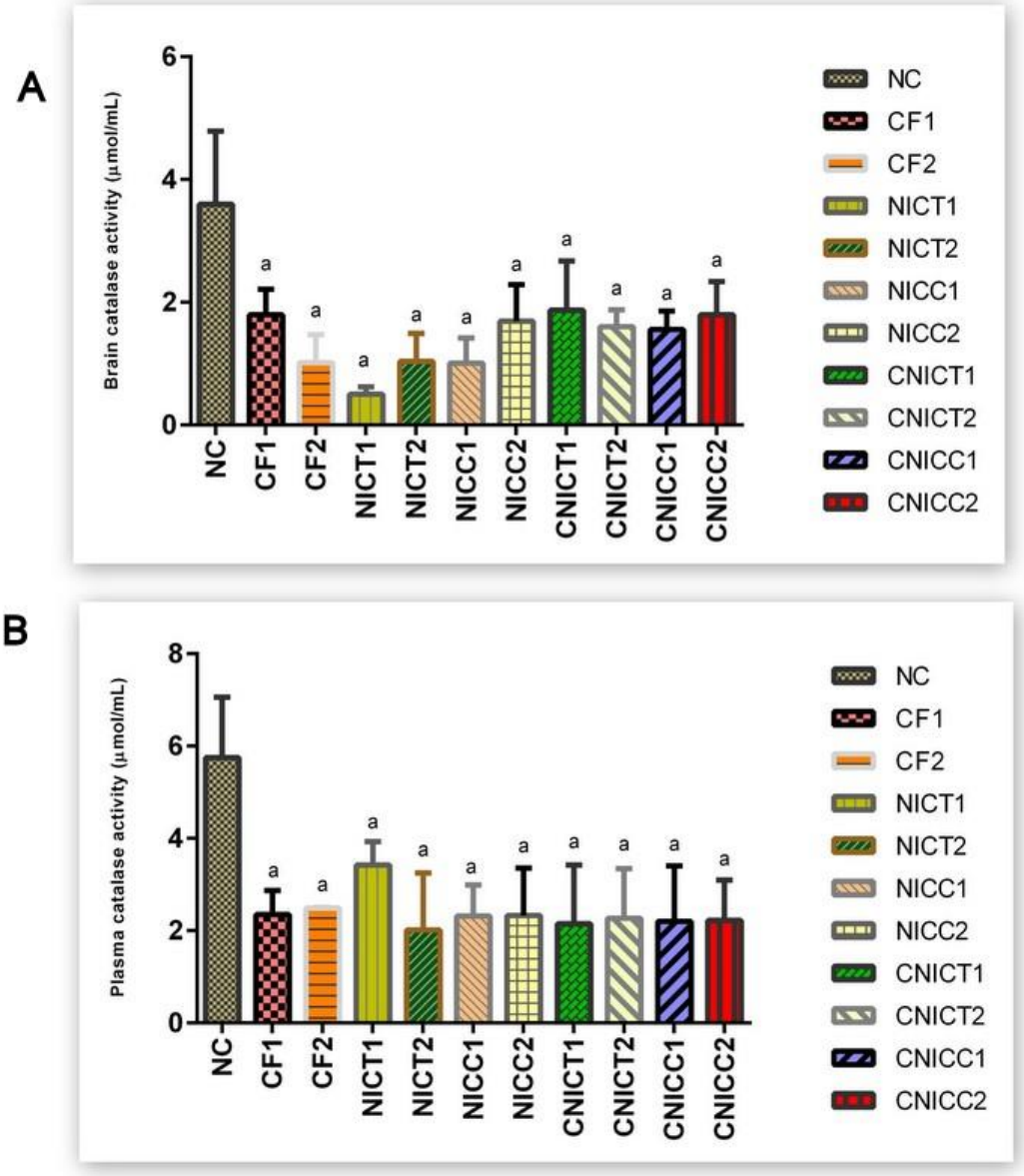

Figure 3: Effects of co-administration of extracts and caffeine on catalase activity in the brain (A) and catalase activity in the serum (B) of Wistar rats

DISCUSSION

Environmental factors and genetic susceptibility coupled with changes by oxidative stress are contributory to the progression of depressive disorders. These stem from cell death or cell injury as a result of the oxidative stress. Since tobacco is essentially nicotine and cigarette is made from tobacco leaf it could be inferred that the extracts contained nicotine. The present study demonstrated that extracts of tobacco/cigarette and caffeine used either alone or in combination are capable of increasing oxidative stress in physiological rats. The results corroborates the clinical studies on smokers evidenced association between depressed smokers who consumed

high level of nicotine and high level of oxidative damage to protein (15), lipid peroxidation (16), C-reactive protein, fibrinogen, (17) and reduced concentration of total reactive antioxidant potential (TRAP) (18).

Although some studies claimed low dose caffeine protects against cellular damage by producing beneficial antioxidant effects (6), findings from the present study revealed that co-administrations of caffeine (10 and 20 mg/kg, p.o.) with cigarette extracts (170 and 340 mg/kg, p.o.) and tobacco leaf extracts (170 and 340 mg/kg, p.o.) produced marked increase in level of MDA in brain and plasma of experimental rats. This finding is in agreement with some studies that suggested caffeine as potent substance capable of inducing oxidative damage by increasing lipid peroxidation (19). Some of the effects of caffeine may favour the production of free radicals and lead to a subsequent increase of lipid peroxidation which invariably increases chain reactions of oxidative stress (19). This coupled with reduced catalase may be an indication of oxidative stress. In addition, increased effect of co-administration may be as a result of sum potential of individual substance to induce oxidative damage. An epidemiological study has shown that low dose nicotine smoking induces oxidative stress, inflammation response and apoptosis in tissues (20).

CONCLUSION

In conclusion, results of the present study showed that single use or co-administration of caffeine and sources of nicotine (cigarette and tobacco leaf) at low dose or high dose may induce oxidative stress which may lead to brain or neurodegenerative disorders by modulating the antioxidant system via increased level of malondialdehyde and decreased catalase activity in brain and plasma of experimental rats.

REFERENCES

1. Gupta RC. Brain regional heterogeneity and toxicological mechanisms of organophosphates and carbamates. *Toxicology Mechanisms and Methods* 2004; 14 (3):103-143.
2. Kamat P. Boloor KK, Devasagayam TPA, Jayashree B. Kesavan JPC. Differential modification by caffeine of oxygen-dependent and independent effects of γ-irradiation on rat liver mitochondria. *International journal of radiation biology* 2000; 76 (9):1281-1288.
3. Kriško A, Marina K, Greta P. Effect of caffeine on oxidation susceptibility of human plasma low density lipoproteins. *Clinica chimica acta* 2005; 355 (1-2):47-53.
4. Lee C. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. *Clinica Chimica Acta* 2000; 295 (1-2):141-154.
5. Fredholm BB, Karl B, Janet H, Astrid N, Edwin E. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews* 1999; 51 (1):83-133.
6. Thompson DCW, Michael K, Nicholas CW, Lakomy HKA, McArdle F, Jackson MJ. 2001. Muscle soreness and damage parameters after prolonged intermittent shuttle-running following acute vitamin C supplementation. *International journal of sports medicine* 2001; 22 (01):68-75.
7. Çakatay U, Ayşegül T, Refik K, Fatma T, Tülay A, Ahmet S. Relation of aging with oxidative protein damage parameters in the rat skeletal muscle. *Clinical biochemistry* 2003; 36 (1):51-55.
8. Guan Z, Wen-Feng Y, Agneta N. Dual effects of nicotine on oxidative stress and neuroprotection in PC12 cells. *Neurochemistry international* 2--3; 43 (3):243-249.
9. Ashakumary L, Vijayammal PL. Additive effect of alcohol and nicotine on lipid peroxidation and antioxidant defence mechanism in rats. *Journal of Applied Toxicology* 1996; 16 (4):305-308.

10. Zhang J, Shuguang J, Ronald RW. Antioxidant supplementation prevents oxidation and inflammatory responses induced by sidestream cigarette smoke in old mice. *Environmental Health Perspectives* 2001; 109 (10):1007.
11. Raisi AEA, Manouchehri S. Quantitative analysis of nicotine in several cigarette brands available in Iran. Cromatographia 1986; 21:711–717.
12. Celik E, Uzbay T, Karakas S. Caffeine and amphetamine produce cross-sensitization to nicotine-induced locomotor activity in mice. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2006; 30 (1): 50-55.
13. Stocks J, Gutteridge JMC, Rosemary JS, Dormandy TL. Assay using brain homogenate for measuring the antioxidant activity of biological fluids. Clinical Science and Molecular Medicine 1974; 47:215-222.
14. Prabhakara PV, Utkarsh A, Reddya SP, Singha A. Balasubramanyama M, Rahmana S, Indu Kumaria SB, Agawaneb USN, Murtya PG, Mohammed M. Oxidative stress induced by aluminum oxide nanomaterials after acute oral treatment in Wistar rats. J. Appl. Toxicol. 2011; 32: 436–445
15. Carnevale R, Sebastiano S, Francesco V, Cristina N, Lorenzo L, Ludovica P, Mariangela P, Antonino GMM, Elena DF, Isotta C. Acute impact of tobacco vs electronic cigarette smoking on oxidative stress and vascular function. Chest 2016; 150 (3):606-612.
16. Bhagwat SV, Vijayasarathy C, Haider R, Jayati M, Narayan G Avadhani. "Preferential effects of nicotine and 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone on mitochondrial glutathione S-transferase A4-4 induction and increased oxidative stress in the rat brain." Biochemical pharmacology 1998; 56 (7):831-839.
17. Zahn D, Frank P, Idun U, Georg J, Horst N, Anna-Karolina H, Jens W, Stephan H. New pathways of increased cardiovascular risk indepression: a pilot study on the association of high-sensitivity C-reactive protein with pro-atherosclerotic markers in patients with depression. Journal of Affective Disorders 2013; 146:420–425.
18. Vargas HO, Sandra OVN, Márcia RPC, Mateus MV, Décio SB, Chiara CB, Kamalesh V, Seetal D, Michael B. Oxidative stress and inflammatory markers are associated with depression and nicotine dependence. Neuroscience letters 2013; 544:136-140.
19. Vistisen K, Henrik EP, Steffen L. Foreign compound metabolism capacity in man measured from metabolites of dietary caffeine. Carcinogenesis 1992; 13 (9):1561-1568.
20. Crowley-Weber C, Katerina D, Cheray C, Harris B, Carol B, Harinder G, Claire MP. Nicotine increases oxidative stress, activates NF-κB and GRP78, induces apoptosis and sensitizes cells to genotoxic/xenobiotic stresses by a multiple stress inducer, deoxycholate: relevance to colon carcinogenesis. Chemico-biological interactions 2003; 145 (1):53-66.