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Research Article
Attenuating Effect of Some Antioxidants on Caffeine Induced Oxidative Stress and Hepatotoxicity in Male Albino Rats

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Abstract

Background and Objective: Oxidative stress and liver diseases are major health issues in life and have been linked to several factors, therefore this study investigated the attenuating potential of some antioxidants: Cellgevity, MaxOne, purslane and vitamin C on a caffeine-induced oxidative stress and hepatotoxicity in male albino rats. **Materials and Methods:** Sixty Albino rats were divided into ten groups of two rats in three replicates using a Completely Randomized Design (CRD). Group 1 served as control. Groups 2^{-1} 0 were treated orally for 65days sacrificed. After administration using chloroform anaesthesia. Data obtained were analyzed using analysis of variance (ANOVA). **Results:** Results showed that caffeine caused a significant reduction in the serum levels of catalase, glutathione peroxidase, superoxide dismutase and increased malondialdehyde and liver function enzymes: Aspartate aminotransferase, alanine aminotransferase and alanine phosphatase. However, Cellgevity, MaxOne, purslane and vitamin C attenuated the effect of caffeine in all the parameters evaluated. **Conclusion:** Results show that Cellgevity, MaxOne, purslane and vitamin C have the potential to attenuate oxidative stress and hepatotoxicity induced by caffeine in Albino rats.

Key words: Caffeine, Cellgevity, MaxOne, purslane, vitamin C, attenuating potential, oxidative stress, hepatotoxicity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reactive oxygen species produced by exogenous and endogenous factors are highly reactive oxygen derivatives with a half-life in the nano to milliseconds range. These molecules have been shown to play a vital role in altering various physiological functions. Lifestyle modifications, technological growth, increasing proportions of pollution, consumption of alcohol, cigarette smoking, physical stress and dangers associated with toxic substances constitute the primary external sources of production of free radicals. Also, several other pathways which involve metabolic activities in the cell membrane, mitochondria, peroxisomes and endoplasmic reticulum can result in the manufacture of ROS[1-4].

Glutathione is an endogenous antioxidant present in almost every cell in the body, playing a role in the detoxification of drugs and xenobiotics. It is a coenzyme that mediates the protection of the cell against the free radicals generated during cellular oxidative mechanisms. Whole glutathione consumption cannot be effective because it would be destroyed in the digestion process before reaching the cells. The active ingredient in riboceine supplements (Cellgevity and MaxOne) is D-Ribose-L-Cysteine. The ribose component of the riboceine solves the challenges of effectively protecting and delivering the fragile cysteine molecule, enabling the cells to produce glutathione when the cells need it[5].

L-cysteine is called semi-essential amino acid because humans can synthesize it from the amino acid, methionine along with a host of proteins. It is a precursor of glutathione which is considered very important for the detoxification of cellular oxidative stress. Elevated levels of oxidative stress can potentially impair cellular glucose metabolism through a variety of mechanisms including redox imbalance, insulin resistance and reproductive dysfunction[5].

Purslane (*Portulaca oleracea*, William Darlington, 1859: Portulacaceae: Caryophyllales) also commonly called *mmong mmong ikong mbakara* in Efik. Purslane is listed by the World Health Organization as one of the most used medicinal plants and has even been termed as a "global panacea" for several diseases[6]. The plant has muscle relaxant, anticonvulsive, analgesic and anti-inflammatory properties and also a potential anti-anxiety effect. It has further been shown to exhibit hepatoprotective activity in rats with hepatic injuries[7]. Research indicated that purslane offers better nourishment than the major cultivated vegetables due to its shoot is a rich source of omega-3-fatty acids, a-tocopherols, ascorbic acid, b-carotene and glutathione. Its seeds also contain a high percentage of a-linolenic acid. These organic compounds contribute to the anti-oxidative properties of purslane[8].

Antioxidants reduce oxidative stress by scavenging free radical species and since the majority of antioxidants are phenolic compounds, they are known to be responsible for the antioxidant activity of plants[9]. Experimental evidence reveals purslane to be effective as an antioxidant agent as well as providing nourishment for the liver, kidneys, testes and heart tissues[10].

Vitamin C is needed for many physiological functions. It is a natural antioxidant that prevents increased production of free radicals due to oxidative damage against lipids and lipoproteins in cellular compartments and tissues. The protective role of vitamin C against oxidative stress has been well reported[11].

Caffeine constitutes one of the most constantly consumed psychoactive substances globally and is present in several foods, drugs and beverage products such as energy drinks, coffee and tea. Invariance to most other psychoactive substances, it is legalized and unregulated in the majority of the countries of the world with an estimated 80% of the world's population consuming a caffeine-containing substance daily[12]. Caffeine and other methylxanthines are used in clinical medicines as diuretics, analgesics, muscle relaxants and can aid in the treatment of brain disorders such as headache and Parkinson's diseases[13]. In humans, low and average doses of caffeine produce increase alertness and positive effects on the myocardium, while high doses cause caffeine dependency with a wide range of unpleasant physical and mental conditions such as nervousness, irritability, restlessness, insomnia, headache and heart palpitations[14]. Consumption of caffeine has also been linked with delayed conception, reproductive and developmental toxicities, liver diseases and an increase in the frequency of sperm abnormalities[15-18]. This study was aimed at investigating the attenuating potential of some antioxidants: Cellgevity, MaxOne, purslane and vitamin C on caffeine-induced oxidative stress and hepatotoxicity in male Albino rats.

MATERIALS AND METHODS

Location of the study: This research was conducted in the Animal House of the Department of Genetics and Biotechnology, University of Calabar, Calabar, Cross River State. The study lasted for 6 months (September, 2020-February, 2021).

Collection of materials: Caffeine was acquired from Sigma-Aldrich (St. Louis, USA). The antioxidant agents: MaxOne and

Cellgevity were purchased from Max International, LLC, (Salt Lake City, USA). Vitamin C was purchased from Emzor Pharmaceutical Industries Limited, Lagos. Purslane leaves were obtained from the University of Calabar Botanical Garden and its environs. The leaves were authenticated by Mr. Effa Anobeja of the Herbarium Unit, Department of Plant and Ecological Studies, University of Calabar. The voucher specimen was deposited with the Resource Room of the Department of Genetics and Biotechnology, University of Calabar, Calabar with the number GBT/PLT/20/098. The leaves were processed into crude extracts.

Experimental animals: Sixty male Albino rats, twelve weeks old weighing between 160-200 g were purchased from the Department of Zoology and Environmental Biology, University of Calabar, Nigeria. The animals were kept in steel cages covered with wire mesh under a standard laboratory environment. They were given water and commercial feed from Top feed limited (crude protein: 18%, metabolizable energy: 2800 kcal kg^{-1}) *ad libitum* during the study. Animals were allowed to adapt to their environment for 1 week before treatment. Animals were handled in line with the Helsinki protocol for care of experimental animals and the local ethical committee (Approval number: CRS/MH/CGS and EH/Vol.1/102).

Experimental design and procedure: The 60 Albino rats were randomly divided into ten groups consisting of two rats in three replicates using the Completely Randomized Design (CRD). The treatment protocol was as shown in Table 1 and lasted for 65 days. Chloroform fume was used to anaesthetize the rats 24 hrs after administering the last dose. Blood samples of the male rats were obtained and processed for oxidative stress markers and liver function enzymes assay.

Superoxide dismutase: Packed erythrocytes were obtained from the blood sample and washed four times with 5 mL of 0.9% saline solution and centrifuged at 3500 rpm for 10 min. The cells were lysed with ice-cold distilled water and centrifuged twice to obtain erythrocyte membrane and hemolysate. The cells were further treated with chloroform and ethanol and used to determine SOD enzyme activity which was expressed in pg mL^{-1} [19].

Catalase: Catalase activity was determined according to the method of Djondjevic[20]. The method is based on the decomposition of H_2O_2 by catalase. Sample containing catalase was incubated in H_2O_2 and then mixed with 4-aminophenazone and 3, 5-dichloro-2-hydroxybenzene sulfonic acid and catalysed by horseradish peroxidase. The resulting quinone imine dye was measured at 510 nm and expressed in pg mL^{-1}.

Glutathione peroxidase: Glutathione peroxidase (GPx) was assessed according to the method of Lu[21], using the Fortress diagnostic kit (Fortress Diagnostic, Technology Park, Antrim, United Kingdom). GPx catalyses the oxidation of glutathione and then the oxidized glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH. The NADPH is oxidized to NADP and a decrease in absorbance at 340 nm is measured and expressed in IU mL^{-1}.

Malondialdehyde: Malondialdehyde (MDA) is the end product of lipid peroxidation. MDA was determined by the method of Wozniak *et al.*[22]. The principle of this method is based on fluorometric measurement of the colour produced during the reaction of thiobarbituric acid (TBA) with MDA. Absorbance was measured using a spectrophotometer (Agilent, Santa Clara, CA, USA) at 532 nm. The concentration of MDA was calculated and expressed in nmol mL^{-1}.

Determination of serum AST activity: AST activity was determined using the colourimetric method of Huang *et al.*[23].

Determination of serum ALT activity: ALT was estimated according to the method of Huang *et al.*[23].

Table 1: Protocol for treatment of experimental animal

Treatment groups	Description of treatments
Control	1 mL of physiological saline, no caffeine, purslane, vitamin C, MaxOne and Cellgevity
C	Caffeine in 1 mL of physiological saline, 200 mg kg^{-1} b.wt., orally by gavage
P	Purslane, 200 mg kg^{-1} b.wt., orally
Vc	Vitamin C, 100 mg kg^{-1} b.wt., orally
Mx	MaxOne, 200 mg kg^{-1} b.wt., orally
Cg	Cellgevity, 200 mg kg^{-1} b.wt., orally
C+P	Caffeine, 200 mg kg^{-1} b.wt. and purslane, 200 mg kg^{-1} b.wt., both orally
C+Vc	Caffeine, 200 mg kg^{-1} b.wt. and vitamin C, 100 mg kg^{-1} b.wt., both orally
C+Mx	Caffeine, 200 mg kg^{-1} b.wt. and MaxOne, 200 mg kg^{-1} b.wt., both orally
C+CG	Caffeine, 200 mg kg^{-1} b.wt. and Cellgevity, 200 mg kg^{-1} b.wt., both orally

Determination of serum ALP activity: ALP was assayed according to the method as described by Al-Hadithy *et al*.[24].

Statistical analysis: Data obtained on oxidative stress markers and liver function tests were analyzed using Analysis of Variance (ANOVA) on SPSS version 20. The least significant difference was utilized to compare means at $p<0.05$.

RESULTS

Table 2 shows a significant reduction in GPx in the caffeine group (142.90±3.25 IU mL^{-1}) when compared with the control (166.78±2.85 IU mL^{-1}) and other treatment groups. Cg, Mx, Vc and Pu groups had 163.47±2.03, 157.38±1.92, 177.85±15.86 and 163.98±3.24 IU mL^{-1}, respectively. The level of GPx increased significantly ($p<0.05$) in C+Vc, C+Cg, C+Mx and C+Pu groups (156.15±3.41, 156.60±1.02, 175.17±2.08 and 175.73±1.37 IU mL^{-1}, respectively) indicating attenuating effect of the antioxidants with Pu having the best effect. The serum level of SOD also significantly decreased in animals treated with caffeine only when compared with the control group (890.67±15.19 and 998.67±0.21 pg mL^{-1}, respectively). The Cg, Mx, Vc and Pu groups had 998.50±0.22, 949.00±17.61, 949.50±48.10 and 998.83±0.17. When compared with animals treated with the caffeine alone, SOD increased significantly in the C+Pu group to 981.83±1.37 pg mL^{-1}. This indicates that the effect of caffeine was significantly attenuated in the C+Pu group only.

The level of catalase reduced significantly in the caffeine group (12.12±0.58 pg mL^{-1}) when compared with the control (22.72±0.99 pg mL^{-1}). Catalase level significantly increased in the C+Mx group indicating attenuating effect of MaxOne as the best on the effect of caffeine on catalase level in the rats. On the other hand, caffeine caused a significant increase ($p<0.05$) in MDA levels. MDA level was 1.37±0.14 umol mL^{-1} in the caffeine group, which was significantly higher than the control group (0.48±0.03 nMol mL^{-1}). Cg, Mx, Vc and Pu groups had mean values of 0.75±0.03, 0.90±0.08, 0.90±0.08 and 0.55±0.03 µmol mL^{-1}, respectively MDA levels reduced significantly in C+Cg, C+Mx, C+Pu and C+Vc groups (0.83±0.13, 0.93±0.56, 1.00±0.68 and 0.8l±0.16 nMol mL^{-1}, respectively depicting attenuating effect).

Table 3 revealed that ALT increased significantly in the caffeine group (42.50±3.32 IU L^{-1}) in comparison with the control group (26.00±0.78 IU L^{-1}). Cg, Mx, Vc and Pu groups had 17.33±0.56, 26.00±2.94, 21.33±1.50 and 29.33±0.92, respectively. ALT levels decreased significantly in C+Cg, C+Mx, C+Pu and C+VC groups indicative of attenuating

Table 2: Effect of some antioxidants on oxidative stress markers of male rats treated with caffeine

Parameters	Control	Cg	Mx	Vc	Caffeine	Pu	C+Cg	C+Mx	C+Pu	C+Vc	LSD
Gpx (IU mL^{-1})	166.78±2.85	163.47±2.03	157.38±1.92	177.85±15.86	142.90±3.25	163.98±3.24	156.60±1.02	175.17±2.08	175.73±1.37	156.15±3.41	3.98
SOD (Pg mL^{-1})	998.67±0.21	998.50±0.22	949.00±17.61	949.50±48.10	890.67±15.19	998.83±0.17	939.33±59.24	952.17±34.66	981.83±1.37	949.00±10.27	4.12
CAT (Pg mL^{-1})	22.72±0.99	75.73±1.17	27.63±0.86	24.00±2.45	12.12±0.58	48.62±7.62	17.40±1.17	37.16±5.52	10.10±1.17	13.79±0.56	1.65
MDA (nMol mL^{-1})	0.48±0.03[d]	0.75±0.03	0.90±0.08	0.90±0.08	1.37±0.14	0.55±0.03	0.83±0.13	0.93±0.56	1.00±0.68	0.81±0.16	0.13

CG: Cellgevity, Mx: MaxOne, Vc: Vitamin C, C+CG: Caffeine and cellgevity, C+Mx: Caffeine and MaxOne, C+Pu: Caffeine and purslane, C+Vc: Caffeine and vitamin C, GPx: Glutathione peroxidase, SOD: Superoxide dismutase, CAT: Catalase and MDA: Malondialdehyde

Table 3: Effect of some antioxidants on liver function enzymes of rats treated with caffeine

Parameters	Control	Cg	Mx	Vc	Caffeine	Pu	C+Cg	C+Mx	C+Pu	C+Vc	LSD
ALT (IU L^{-1})	26.00±0.78	17.33±0.56	26.00±2.94	21.33±1.50	42.50±3.32	29.33±0.92	31.67±3.07	26.00±2.94	38.67±2.23	24.50±1.57	4.91
AST (IU L^{-1})	28.00±0.97	28.00±0.97	29.83±0.40	23.50±2.17	75.50±6.40	52.50±3.91	46.50±3.52	30.33±2.14	63.67±3.90	38.00±1.07	5.08
ALP (IU L^{-1})	50.83±12.32	42.67±1.80	46.17±8.28	39.50±3.54	91.17±0.54	60.83±14.46	70.00±0.68	84.33±2.75	85.17±1.68	70.17±7.99	5.32

CG: Cellgevity, Mx: MaxOne, Vc: Vitamin C, C+CG: Caffeine and cellgevity, C+Mx: Caffeine and MaxOne, C+Pu: Caffeine and purslane, C+Vc: Caffeine and vitamin C, ALT: Alanine aminotransferase, AST: Aspartate Aminotransferase and ALP: Alanine phosphatase

effect. Pu had the best attenuating effect on ALT. Caffeine also causes an increase in serum AST level when compared with the control group. The caffeine group had 75.50±6.40 IU L^{-1} while the control group had 28.00±0.97 IU L^{-1}. The value reduced in the combination groups which imply attenuating effect (46.50±3.52, 30.33±2.14, 63.67±3.90 and 38.00±1.07 IU L^{-1} for C+Cg, C+Mx, C+Pu and C+Vc, respectively). Pu also had the best attenuating effect on AST.

The level of ALP increased significantly in animals treated with caffeine alone (91.17±0.54 IU L^{-1}) when compared with the control group (50.83±12.32 IU L^{-1}). Cg, Mx, Vc and Pu groups recorded 42.67±1.80, 46.17±8.28, 39.50±3.54 and 60.83±14.46 IU L^{-1}, respectively. The effect of caffeine on ALP was attenuated in the combination groups with Pu having the best attenuating effect.

DISCUSSION

Results obtained revealed that the levels of SOD, GPx and CAT were significantly reduced in the caffeine group which is suggestive of oxidative stress. Oxidative stress entails the presence of ROS as well as free radicals generated in normal conditions of cells but become deleterious when they cannot be eliminated by the antioxidant defense system[25]. The reduction in serum level of SOD, GPx and CAT observed in this study agreed with the findings of Hatice *et al.*[26] and Ekaluo *et al.*[27]. Previous studies also showed that SOD, GPx and CAT were vital antioxidants that remove harmful ROS from male reproductive systems[28].

On the other hand, MDA significantly ($p<0.05$) increased in animals treated with caffeine which agreed with the findings of Shirwaikar *et al.*[29], who reported a significant reduction in antioxidants and elevation in serum MDA in caffeine treated animals. An increase in serum MDA as also observed in this study, is indicative of lipid peroxidation activity which is a crucial biological consequence of oxidative cellular damage, hence, the increase in the concentration of MDA reflects oxidative stress[25,30].

An increase in lipid peroxidation also inhibits the activities of antioxidative enzymes such as SOD, GPx and CAT as well as total antioxidant status[31]. Lipid peroxidation and oxidative stress have been reported to be an autocatalytic chemical process that enhances itself and is linked with anomalous fertilization[32]. This, therefore, implies that an increase in ROS and oxidative stress can interfere with the fertility of male animals. Results obtained also indicated that SOD, GPx and CAT increased significantly in groups C+CG, C+Mx, C+Pu and C+Vc which denotes attenuating effect of Cellgevity, MaxOne, purslane and vitamin C. This may be due to their antioxidant precursory and enhancing potentials[5]. This also implied that the antioxidant treatments might have engineered the increase of SOD, GPx and CAT which are reported to provide the main defense against free radicals generated by caffeine in the treated animals[33].

The effect of caffeine on the concentration of MDA was also attenuated in the C+Cg, C+Mx, C+Pu and C+Vc groups which indicated that the antioxidants might have played a major role in scavenging free radicals that accumulate to cause lipid peroxidation, oxidative damage to DNA and proteins as well as its concomitant effect on reproductive functions[34].

The serum level of ALP, AST and ALT increased significantly in the caffeine group when compared to other treatment groups which were in line with the observations of Emmanuel *et al.*[35] and Dungubat *et al.*[36], who reported the toxic effect of caffeine on hepatocytes. However, the findings of this study contradicted those of Ruhl and Jeverhart[37], Umoh and Jimmy[38] and Modi *et al.*[39], who revealed the protective effect of caffeine on hepatocytes when mostly taken in coffee. studies of liver function enzymes are adopted as markers of biochemical variations in the liver due to different toxicants and diseases[40]. The most widely adopted enzymes are the amino-transferases (ALT and AST) and Alkaline Phosphatase (ALP). Whereas, an abnormal increase in amino-transferases, ALT in particular as observed in this study, generally reflect liver cell damage (hepatotoxicity), that of ALP is more specific for cholestasis-hepatobiliary defects[35].

The increase in the liver function enzymes may be attributed to caffeine-induced ROS and oxidative stress in the liver cells. This was in line with the report of Muriel and Gordilo[41], who reported that oxidative stress adds to a wide range of hepatic dysfunction with a wide range of aetiology. Increasing oxidative stress in hepatocytes is one of the main mechanisms of liver disease and dysfunction[42]. This, therefore, implied that the decrease in the antioxidative enzymes: SOD, GPx and CAT and increase in MDA in the caffeine group observed in this study may have given rise to the liver dysfunction and resultant increase in the liver function enzymes.

The effect of caffeine was attenuated in C+CG, C+Mx, C+Pu and C+Vc groups as evident in the significant decrease in the serum level of the enzymes when compared with what was observed in the caffeine group indicating the attenuating potential of Cellgevity, MaxOne, purslane and vitamin C. This may also not be unconnected with their antioxidant properties which protect the cells and tissues from free radical

injury and facilitate repair of damaged tissues[40]. This study implies that the deleterious effects of caffeine occasioned by its wide and daily consumption can be ameliorated by consuming Cellgevity, MaxOne, purslane and vitamin C. Therefore, based on the findings of this study, it is recommended that arbitrary intake of caffeine-containing substances should be curtailed and the antioxidants studied should be utilized by persons exposed to caffeine because of their attenuating potentials. This study is limited since it did not cover the attenuating effect of the antioxidants on every organ and system to have more comprehensive data. However, other areas should be considered in further research.

CONCLUSION

The present study revealed the significant oxidative stress and hepatotoxic effects of caffeine. However, the findings of this study provided substantial evidence that Cellgevity, MaxOne, purslane and vitamin C can attenuate caffeine-induced oxidative stress and hepatotoxicity in male rats.

SIGNIFICANCE STATEMENT

This study discovered that Cellgevity, MaxOne, vitamin C and purslane can be beneficial for attenuating the harmful effect of caffeine consumption on oxidative stress markers and liver function enzymes. Findings of the study also revealed that purslane competed favourably with other standard antioxidants thereby presenting a cheap and readily available alternative for attenuating caffeine-induced oxidative stress and hepatotoxicity. The findings of this study will help the researchers to uncover the critical areas of attenuating oxidative stress and hepatotoxicity using readily available and affordable antioxidants enhancement that many researchers were not able to explore. Thus a new theory on oxidative stress and hepatotoxicity as well as ROS-antioxidants milieu as well as attenuating potential of many commonly used plants may be arrived at.

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