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Effect of aqueous solution of caffeine on serum level of superoxide dismutase in male Wister rats

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Abstract

Caffeine is an active ingredient of coffee (Nescafe) as one of the major sources of dietary antioxidant compounds which counteract the action of reactive oxygen species (ROS) which are the main contributors to oxidative stress. This research is carried out to investigate the role of caffeine in modulating superoxide dismutase and caffeine's contribution to aging. Forty five sexually matured albino rats with weights ranging from 120-200g were purchased from the animal house of the Department of Anatomy, University of Port Harcourt. These animals were divided into five groups according to their weights and were housed in fifteen well ventilated cages containing sawdust which serves as beddings for the comfort of the animals and for easy removal of their feaces. They were fed daily with animal feed, water *ad libitum* and allowed to acclimatize for a period of one week, after which their weights were taken and caffeine administration commenced.The results obtained showed a significant ($p<0.05$) increase in the body weight of the animals on days 14 and 21 following the administration of 20mg/kg of the extract when compared with day 0 and a significant ($p<0.05$) increase in serum superoxide dismutase activity for 100mg/kg on day 14 when compared to the control. The findings of this study suggests that caffeine (NESCAFE) may have little or no significant effect on the activity of superoxide dismutase.

Keywords: Aqueous; Effect; Caffeine; Serum; Superoxide Dismutase; Male Wister rats

1. Introduction

Caffeine is one of the world's widely consumed psychoactive drugs. Because of its stimulant activity, caffeine has been believed to have a beneficial impact and such benefits include mental alertness. In the morning a cup of coffee or tea removes the last trace of sleepiness and keeps us awake in the evening, this is because caffeine is found naturally occurring in coffee plants, tea plants, cola nuts and over 63 species of known plants. Caffeine is a central nervous system stimulant of the methylxanthine class chemically known as 1, 3, 7-trimethylxanthine. At room temperature, caffeine is a white, odorless powder with a bitter taste.

Just like most natural products, caffeine as a secondary plant metabolite acts as a natural pesticide and a defense mechanism which paralysis and kills predator insects as well as inhibits the growth of nearby caffeine containing plants. The benefits of caffeine has made it an important compound to manufacturers in the field of food and medicine whereas beverages containing caffeine such as chocolate, green tea, coffee, soft drinks and energy drinks enjoy great popularity.The use of coffee and it's beneficial effect on health dates back many centuries, although further studies are linked with the scientific revolution despite the fact that drinking of coffee has been invoked for many decades, the actual role of it has been long debated mainly because some potential negative aspects have been hypothesized (Daniela *et al.* 2016). In 1991, the International Agency for Research on Cancer (IARC) classified coffee as "possibly carcinogenic to humans" which could be related to a weak positive relationship between coffee consumption and the occurrence of

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bladder, pancreatic and ovarian cancer (WHO, 1991). Further studies (Daniela *et al*2016) described coffee as one of the most sources of dietary antioxidant compounds which counteract the action of reactive oxygen species (ROS) which are the main contributors to oxidative stress which occurs when the cellular production of oxidants exceeds the availability of antioxidants.Recent studies (Simone *et al*. 2015) explained the effect of caffeine at cellular level in three mechanisms of action: the antagonism of adenosine receptors, especially in the central nervous system; the mobilization of intracellular calcium storage, and the inhibition of phosphodiesterases.Caffeine blocks adenosine receptors, mainly A and A subtypes, competitively antagonizing their action and causing an increased release of dopamine, noradrenalin, and glutamate. Caffeine is able to reduce cerebral blood flow. It is also able to reduce myocardial blood flow, by inhibiting A_1, A_{2A} and A_{2B}adenosine receptors in blood vessels and limiting adenosine-mediated vasodilation. The function of adenosine is to regulate various biological processes throughout the body, including the cardiovascular and nervous systems. Adenosine is associated with decreased activity in all of the following functions: blood pressure, central nervous system activity, respiration, urine output, and intestinal peristalsis. By blocking adenosine receptors, caffeine prevents adenosine from having its depressing effect on all of the processes previously listed. The ability of caffeine to block adenosine receptors can be observed also at low doses, such as those contained in a single cup of coffee (Ruxton *et al* 2008). Other mechanisms of action, such as the mobilization of intracellular calcium and the inhibition of phosphodiesterases, require higher doses of caffeine, unlikely to be obtained with the common daily dietary sources of caffeine. Regular caffeine intake is associated with lowered risk of type 2 diabetes and Parkinson's disease, and is thought to preserve long term cognitive functioning by reducing damage caused by lowered glucose and oxygen supplies to the brain through its action in blocking adenosine receptors (Rogers, 2007).More recent studies (Rogers, 2007 and Rogers *et al.* 2010) suggest that Caffeine induces calcium release from the sarcoplasmic reticulum and can also inhibit its reuptake. Through these mechanisms, caffeine can increase contractility during sub-maximal contractions in habitual and non-habitual caffeine users. Intracellular calcium determines the activation of endothelial nitric oxide synthase (eNOS), with the production of higher quantities of nitric oxide.Caffeine acts as a nonselective competitive inhibitor of phosphodiesterases. These enzymes hydrolyze the phosphodiester linkages in molecules, such as cyclic adenosine monophosphate (cAMP), inhibiting their degradation. cAMP stimulates lipolysis by triggering the activity of the hormone-sensitive lipase (HSL) and has a vital role in the adrenaline cascade. It also activates protein kinase A, which in turn phosphorylates several enzymes implicated in glucose and lipid metabolism. These mechanisms of action require very high doses of caffeine, unlikely to be present in the standard diet, which contains moderate amounts of caffeine.Superoxide dismutase is an antioxidant enzyme that catalyzes the dismutation of the highly reactive superoxide radical to molecular oxygen (O_2) and to a less reactive hydrogen peroxide H_2O_2. It has the enzyme classification number of EC 1.15.1.1 and catalyzes the rapid removal of superoxide radicals as shown in the half reactions below (Abreu, *et a*, 2009)

$$M^{(n+1)+}\text{---SOD} + O^{2.-} \rightarrow M^{n+}\text{---SOD} + O^2$$

$$M^{n+}\text{---SOD} + O^{2.-} + 2H+ \rightarrow M^{(n+1)+}\text{---SOD} + H_2O_2$$

Where M= Cu, n= 1, M=Mn, n =2, M =Ni, n= 2

2. Material and methods

A coffee product "NESCAFE" in particular was purchased from a supermarket at Choba, Port Harcourt, Nigeria. The caffeine concentrations were prepared with distilled water in the laboratory of the Department of Biochemistry, University of Port Harcourt, Nigeria These concentrations were prepared according to the weight of the rats in each groups arranged; such that the concentration of caffeine of the group 1 weighing 120g was 0.28g of coffee in 144ml of water; group 2 weighing 140g was 1g of coffee in 168 ml of distilled water; group 3 weighing 160g was 1.92g of coffee in 192 ml of water, and group 4 weighing 180g was 3.36g of coffee in 240ml of distilled water, respectively. This process of caffeine preparations was carried out weekly, three times throughout the research work.Forty five sexually matured albino rats with weights ranging from 120-180g were purchased from the animal house of the Department of Anatomy, University of Port Harcourt, Nigeria. These animals were divided into five groups according to their weights and were housed in fifteen well ventilated cages containing sawdust which serves as beddings for the comfort of the animals and for easy removal of their feaces. They were fed daily with animal feed, water *ad libitum* and allowed to acclimatize for a period of one week after which their weights were taken and caffeine administration commenced.

1000g = 1kg

$$\text{Dosage in mg} = \frac{\text{Body Weight of Animal (g)}}{1000\text{g}} \text{X Dose (mg)} \ldots\ldots (\text{ Erhirhie} et\ al. 2014)$$

Table 1 Group, number of animals, no of cages, weight of animals, concentration of caffeine administered and volume of caffeine solution administered

Group	No Of Animals	No Of Cages	Weight Of Albino Rats	Sex Of Albino Rats	Concentration Of Caffeine Administered	Vol.(ml)
Group 1	12	3	120g	Male	20mg/kg body weight	1.2
Group 2	12	3	140g	Male	60mg/kg body weight	1.4
Group 3	12	3	160g	Male	100mg/kg body weight	1.6
Group 4	12	3	180g	Male	140mg/kg body weight	1.8
Control	9	3	120g	Male	NIL	NIL

At the end of 7 days administration of caffeine, 3 rats from each group was sacrificed, this was repeated for day 14 and day 21using diethyl ether as narcotics.Blood samples were collected from the rats via heart puncture during sacrificing. The blood samples were put in plain sample bottles. The blood samples were allowed to clot and taken to the laboratory where they were centrifuged and the serum separated and stored properly awaiting superoxide dismutase analysis. The activity of superoxide dismutase was determined by method of Misra and Fridovich (1972).Data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 20.0. Data were expressed as mean ± standard error of mean. Analysis of Variance (ANOVA) was done using Least Significant Difference (LSD) to determine the significant difference in mean at 95 percent confidence interval ($p<0.05$).

3. Results and discussion

Table 2 shows effects of the aqueous solution of Caffeine on animals' body weight following administration of graded doses of the extract. There was a significant ($p<0.05$) increase in the body weight of the animals on days 14 and 21 following the administration of 20mg/kg of the extract when compared with day 0. However, an increase was observed for day 7 which is not statistically significant when compared with day 0.A significant ($p<0.05$) increase in the body weight of the animals was observed on day 14 following the administration of 60mg/kg of the aqueous solution of Caffeine when compared with day 0. However, an increase was observed for days 7 and 21 which is not statistically significant when compared with day 0.There was an increase in the body weight of the animals on days 7, 14 and 21 following the administration of 100mg/kg of the aqueous solution of Caffeine which is not significant when compared with day 0.There was a significant ($p<0.05$) increase in the body weight of the animals on days 14 and 21 following the administration of 140mg/kg of the aqueous solution of Caffeine when compared with day 0.

Table 2 Effect of aqueous solution of Caffeine on body weight following administration of graded doses in wistar rats

Groups	Body Weight (g)			
	Day 0	Day 7	Day 14	Day 21
Control	120.00 ± 0.00	160.00 ± 0.00	200.00 ± 0.00	173.33 ± 23.09
20mg/kg	120.00 ± 0.00	156.67 ± 5.77	200.00 ± 0.00*	180.00 ± 0.00*
60mg/kg	140.00 ± 0.00	176.67 ± 25.17	193.33 ± 11.55*	186.67 ± 11.55
100mg/kg	160.00 ± 0.00	193.33 ± 11.55	186.67 ± 23.09	186.67 ± 23.09
140mg/kg	200.00 ± 0.00	200.00 ± 0.00	196.67 ± 5.77	223.33 ± 15.28*

Values are expressed as mean ± SEM; n=3; *Significant at $p<0.05$ when compared with day 0

In this study, there was a significant ($p<0.05$) increase in the body weight of the animals on days 14 and 21 following the administration of 20mg/kg of the aqueous solution of Caffeine when compared with day 0. However, the findings of this study is opposed to the findings of researchers Bahlool*et al.* (2014);Malmooona (2017) and Kazoo *et al* (2005) whose research findings states that caffeine intake is associated with weight loss. The results of this study showed an increase in body weight which may be as a result of the 0.1g protein content of the coffee product used (NESCAFE) which may have been utilized for theprimary purpose ofbody building thereby increasing the size of the experimental

animal and consequently its weight. On the other hand, the increase in body weight may be as a result of the duration of study owing to the fact that longer exposure to caffeine may yield a significant decrease in body weight (Bohlool*et al.* 2014) since purely isolated caffeine was not used in this study.

Table 3 shows the effect of aqueous solution of Caffeine on the serum superoxide dismutase in Wistar rats. There was a non-significant ($p>0.05$) decrease in the serum level of superoxide dismutase following administration of aqueous solution of Caffeine at doses of 20mg/kg, 60mg/kg, 100mg/kg and 140mg/kg on day 7 when compared withthecontrol.Alternately, a significant ($p<0.05$) increase was recorded for 100mg/kg on day 14 when compared with the control. Administration of aqueous solution of Caffeine at doses of 20mg/kg, 60mg/kg, 100mg/kg and 140mg/kg on day 21 revealed a non-significant increase in the serum level of superoxide dismutase compared with the control.There was a decrease in the serum level of superoxide dismutase following administration of the aqueous solution of Caffeine at doses of 20mg/kg, 60mg/kg, 100mg/kg and 140mg/kg on day 7 which is not statistically significant when compared with the control.A significant ($p<0.05$) increase was recorded for 100mg/kg on day 14 when compared with the control. Administration of aqueous solution of Caffeine at doses of 20mg/kg, 60mg/kg, 100mg/kg and 140mg/kg on day 21 revealed an increase in the serum level of superoxide dismutase that is not statistically significant when compared with the control. The results of this study are not in agreement with the findings of Corea*et al.* (2012) and kotyczka*et al.* (2011), but are in excellent agreement with the findings of Daniela *et al.* (2016) in a research carried out on coffee consumption and oxidative stress which did not provide adequate evidence about the role of coffee in modulation of antioxidant enzymes.

Table 3 Effect of aqueous solution of Caffeine on superoxide dismutase level in wistar rats

Conc. (mg/kg)	Time (days)		
	Day 7 (mean ±S.E.M)	**Day 14 (mean±S.E.M)**	**Day 21 (mean ± S.E.M)**
Control	0.21 ± 0.07	0.16 ± 0.06	0.22 ± 0.06
20.00	0.31 ± 0.05	0.20 ± 0.05	0.23 ± 0.07
60.00	0.36 ± 0.11	0.29 ± 0.02	0.27 ± 0.06
100.00	0.29 ± 0.09	0.31 ± 0.05*	0.29 ± 0.05
140.00	0.14 ± 0.04	0.21 ± 0.02	0.35 ± 0.02

Values are expressed as mean ± SEM; n=3; *Significant at $p<0.05$ when compared with the control.

4. Conclusion

Superoxide dismutase has an anti-inflammatory activity and is used to alleviate many physiological conditions. It is effective in the treatment of urinary tract inflammatory disease and is responsible for the disproportion of the superoxide anion a major contributor to aging. The findings of this study suggest that caffeine (NESCAFE) may have little or no significant effect on the activity of superoxide dismutase. However, the results of this study showed an increase in body weight which may be as a result of the 0.1g protein content of the coffee product used (NESCAFE) which may have been utilized for the primary purpose of body building thereby increasing the size of the experimental animal and consequently its weight.

Compliance with ethical standards

Acknowledgment

John, G.N

Disclosure of conflict of interest

No conflict of interest to disclosed.

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