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Evaluation of Anti-Hyperglycaemic Potential of Allium Cepa, Coffee and Oxidative Stress

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Abstract

Allium cepa Linn, botanicals, Liliaceae (family) commonly known as onion, and coffee are special delicacies, and conventional as functional foods that contain physiologically active ingredients that for centuries have been exploited as folk remedies for the treatment of diseases. The present study was conducted to evaluate the therapeutic efficacies and the possible participation of oxidative stress of Nigerian *Allium cepa* and coffee such as antioxidant, antihyperglycaemic, hypoglycaemic, hyperglycaemic in normal experimental wistar rats.

Materials and Methods: qualitative phytochemical screening of hydromethanolic extract of Allium cepa was carried out for the identifications of the phytoconstituents. Hydromethanolic extract of Allium cepa 100mg kg -1, vitamin E 400mg kg -1, and coffee 500mg kg-1, combinations of Allium cepa and coffee and vitamin E and coffee respectively, administered orally to each group of rats daily. During the experimental periods glucose concentrations were estimated at baseline 0 (time of treatment ingestion), age 21 and 42 days respectively, with corresponded oxidative stress markers - superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and malondialdehyde (MDA) analyzed at age 42 days.

Results: Result ranked time-dependent (in days) glucose lowering potential of treatments as: - *Allium cepa* + coffee > *Allium cepa* > vitamin E > coffee + vitamin E; with corresponded up regulated of SOD, CAT, GPx and GSH and reduced MDA. Conversely, coffee and control group (normal rats) significantly in time – dependent, raised blood glucose level with corresponded increase in MDA with respect to other biomarkers, suggesting lipid peroxidation oxidative stress induced impairment of cellular antioxidant protective system.

Conclusion: It can be concluded that synergistic effect, presence of the additive bioactive phytoconstituents, and/or antioxidant efficacies or both, and potentiation action of several compounds in Allium cepa and coffee combined , over time, offer the intrinsic antioxidant defence system much better defensive mechanism to protect against free radicals, and alleviate the toxic effects of accumulation of reactive oxygen species (ROS); thence providing protective mechanisms against the development of elevated blood sugar levels. Evidently, Allium cepa and coffee combined is

efficacious and potent hypoglycemic agent, which could be beneficial as a dietary supplement for prevention, treatment or management of raised blood glucose levels or diabetes.

Keywords: *Allium cepa*; Hydromethanolic extract; Oxidative stress; Coffee; Antioxidant; Hypoglycaemia; Normal rats

Abbreviations: SOD: Superoxide Dismutase; CAT: Catalase; GPx: Glutathione Peroxidase; GSH: Glutathione; MDA: Malondialdehyde.

Introduction

There is growing interest in increasing prevalence of diabetes worldwide [1]. The prevalence of diabetes (type 2 diabetes and type 1 diabetes) for all age group worldwide has been estimated as 2.8% in 2000 and 4.4% in 2030 [2]. The number of people globally with diabetes mellitus is projected to rise from 285 million in 2010 to 439 million by 2030, a 54% increase; and 54% to more than about 54.9% million Americans between 2015 and 2030 [3,4]. More so, it has been projected that diabetes will be the seventh leading cause of death in 2030, and underscore the need for immediate and aggressive action to help prevent diabetes whenever possible in those at risk, and interventions which will be more effective in addressing the needs of people with diabetes [1].

Medicinal plants with physiologically active ingredients that for centuries have been exploited as folk remedies for treatment of diseases are gaining much importance in light of lesser side effects and cost savings opposed to orthodox methods [4-7]. Accordingly, although the minimum intake required reducing diabetes remains to be determined, *Allium cepa* and coffee are of physiological interest, and the use of these as therapeutic agents seems promising as their efficacy and safety is reflected by their consumption globally as vegetables and beverages.

Allium cepa, commonly known as onion, is a worldwide culinary and belonging to the family Liliaceae. It has also been used as a folk remedy for a verity of ailments/ disorders. Allium cepa has been found as a source of a complex mixture of sulphur compounds that possess bioactive properties such as quercetin, fructose, quercetin-3-glucoside, isorhamnetin-4-glucoside, xylose, galactose, glucose, mannose, organosulfur compounds, allylsulfides, flavonoids, flavenols, S-alk(en)yl cysteine sulfoxides, cycloalliin, selenium, thiosulfinates, sulfur, seleno, vitamins and some minerals [8-14]. Allium cepa has potential as an anticancer, antidiabetic, antimicrobial, antihypertensive, antioxidant [15-19].

Coffee is a common beverage used worldwide, and more recently, the potential physiological effects has been reported. Coffee contains over 1000 chemical compounds and recent studies have shown to be rich in caffeine, chlorogenic acids, diterpenes, polyphenols molecules with antioxidant properties and phyto-components [20-22]. Coffee in the short-term has been suggested to raise blood glucose levels, and regular coffee drinking is linked to reducing the risk of diabetes [22-26]. Such information on the mechanism of coffee activity on the short-term and the long-term on blood sugar are not clear. Paucity of literature on Allium cepa, coffee or the combination of both and the association of oxidative stress whether or not could have synergistic, potentiative, antagonistic, physio-pharmacological or therapeutic efficacies, formed the basis of this study.

Oxidative stress results when the accumulation of generation of excessive oxygen-free radicals (ROS) or reactive forms of oxygen are produced faster than they can be safely neutralized by *in vivo* antioxidant defence mechanisms- non-enzymatic antioxidant systems, such as glutathione (GSH), malondialdehyde (MDA) and defense-related enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and /or from a weakened or impaired intrinsic antioxidant defense, with resultant formation of pathological conditions [27-29]. The aim of present study was to evaluate oxidative stress level, antihypergylcaemic activity of *Allium cepa* and coffee in normal experimental animal models.

Materials and Methods

Plant Material

Fresh and healthy large and pungent *Allium cepa* bulbs (red onion) were purchased and extracted by maceration process for 72 h using hydromenthanol procedure [30,31]. Qualitative or preliminary phytochemical studies revealed the presence of flavonoids, glycoside, carbohydrates, tannins, amino acid and sterols etc in *Allium cepa* hydromnthanolic extract.

Experimental Animal and Drug Treatment

Thirty adult wistar rats, weighing 160 to 300g were obtained from the animal house, Department of Physiology. The animals were randomly divided into six (6) groups containing 5 rats per group.

Hydromethanolic extract of *Allium cepa* 100mg kg^{-1}, Vitamin E (a known antioxidant) 400mg kg^{-1}, and Coffee 500mg kg^{-1}, combinations of *Allium cepa* and coffee, and vitamin E and coffee respectively were administered orally to each group of rats once daily for 42 days. The control group received normal saline. Animals had free access to standard pellets as a basal diet and water *ad libitum.*

Estimation of Blood Glucose

Blood samples from the tail of the animals were collected at baseline 0 (time of treatment ingestion), age 21 and 42 days respectively and analyzed based on glucose oxidase-perioxidase principles using digital glucometer (ACC-CHEK Aviva glucometer) [32].

Estimation of Oxidative Stress Markers

Estimation of liver enzymatic antioxidants activity - catalase (CAT), superoxide dismutase (SOD), and selenium-dependent glutathione peroxidase (GPx) and non-enzymatic antioxidants concentration - reduced glutathione (GSH) and malondialdehyde (MDA) were measured by adopting the methods described and analyzed at age 42 days [33-38].

Ethical Approval

The study was approved by the Institutional animal ethics committee and all experimental procedures were carried out in strict accordance with guidelines prescribed by the committee for the purpose of control and supervision on experimentation animals. Anaesthetics were applied according to the standards given by the guide of EC Directives 86/609/EEC. Animals were anaesthetized with the administration of diethyl ether.

Statistical Analysis

Data are presented in mean ±SEM and percentage. SPSS 20.0 are used for statistical analysis such as mean comparisons, and values are significant at $p \leq 0.05$ or at confidence interval of 95%.

Results

The physical inactive and sedentary control groups on super nutritional dietary intake produced in a time – dependent significant ($p < 0.05$) increased higher blood glucose levels with percentage increment of 32.9% at age 21 days and 33.7% at age 42 days in comparison with basal levels (Table 1).

Treatments (mg/kg/body weight)	day 0 (mmol/l)	day 21 (mmol/l)	% Relative Change	day 42 (mmol/l)	% Relative Change		%overall Increase/decrease in blood glucose
			0→21		0→42	21→42	
Control (Normal saline)	5.16±0.77	6.86±0.10	32.9	6.90±0.22	33.7	0.58	0.8
Vitamin E400	5.90±0.70	5.80±0.20*	-1.69	5.22±0.22*	-11.52	-10	-9.83
***Allium cepa*100**	5.96±0.30	5.30±0.10*	-11.0	4.60±0.20*	-22.8	-13.2	-11.8
Coffee 500	6.12±0.40	7.04±0.31*	15.0	7.14±0.20*	16.6	1.42	1.6
***Allium cepa*100+Coffee500**	5.92±0.19	6.30±0.12*	6.41	4.80±0.23*	-18.91	-23.8	-25.32
Vitamin E400+ Coffee 500	5.96±0.12	5.38±0.10*	-9.73	5.34±0.16*	-10.4	-0.74	-0.67

Table 1: Time-dependent effect of *Allium cepa*, coffee and vitamin E on blood glucose level.

Correspondingly, at age 42 days, control groups produced significant decreased enzymatic antioxidant activities of supraoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and non-enzymatic antioxidant reduced glutathione (GSH) by 90.8%, 9.82%, 7.8% and 49.1% respectively, and increased malondialdehyde (MDA), a marker of lipid peroxidation due to oxidative stress, by 21.5%, in comparison with positive control (vitamin E)(Tables 2 & 3, role 2). Table 2 column 3 also gives supraoxide dismutase (SOD) ratio, R, to control group (normal rats). Table 1 (columns 7 and 8) also gives percentage increment in blood glucose level between ages 21 to 42 days and the overall increment after age 42 days

The treatment with combination of *Allium cepa* and coffee, *Allium cepa and* Vitamin E, and combination of vitamin E and *Allium cepa* produced significant time – dependent overall percentage decreased blood glucose concentration of 25.32, 11.8, 9.83, and 0.67 after age 42 days respectively (Table 1 column 8), with corresponded increased SOD, CAT, GPx, and GSH and decreased MDA in comparison with control group (normal rats) or positive control (vitamin E) (Tables 2 & 3).

Treatments (mg/kg /body weight)	Supraoxide Dismutase (u/ml)	Ratio of SOD relative to control	%Relative Change to	Catalase (u/g)	%Relative Change to		Glutathione Peroxidase (µg/ml)	%Relative Change to	
			Vitamin E		Control	Vitamin E		Control	Vitamin E
Control	21.7±0.32	1	-90.8	202.0±15.93	0	-9.82	29.4±5.04	0	-7.83
Vitamin E 400	237.2±20.13*	1:11	0	224.0±32.75*	10.8	0	31.9±4.43*	8.5	0
***Allium cepa* 100**	337.3±23.20*	1:16	42.2	230.0±37.70*	13.8	2.67	38.9±7.50*	32.3	21.9
Coffee500	121.5±32.0*	1:06	-48.7	91.8±11.14*	-54.5	-59	20.2±15.7*	-31.2	-36.6
***Allium cepa* 100+ coffee 500**	487.6±46.22*	1:23	105.5	220.0±19.40*	8.91	-1.78	42.0±5.04*	42.8	31.6
Vitamin E 400 + coffee 500	457.6±32.8*	1:21	92.9	206.0±29.80*	1.98	-8.03	32.9±6.44*	11.9	3.13

Table 2: *Allium cepa*, coffee and vitamin E effects on enzymic markers

Treatments	Malondialdehyde	% Relative change to		Reduced Glutathione (µg/ml)	% Relative change to	
		Control	Vitamin E		Control	Vitamin E
Control (Normal saline)	**97.51±6.88**	**0**	**21.5**	**11.9±0.84**	**0**	**-49.1**
Vitamin E 400	**80.2±18.46***	**-17.7**	**0**	**23.4±1.33***	**96.6**	**0**
***Allium cepa* 100**	**73.9±16.80***	**-24.2**	**-7.85**	**28.0±15.6***	**135.2**	**19.6**
Coffee 500	**156.1±21.0***	**60.08**	**94.6**	**9.1±1.90***	**-23.5**	**-61.1**
***Allium cepa*100 + coffee 500**	**74.7±3.70***	**-23.7**	**-7.23**	**19.2±1.33***	**61.3**	**-17.9**
Vitamin E 400+ coffee 500	**86.4±10.80***	**-11.3**	**7.7**	**10.6±0.90***	**-10.9**	**-54.7**

Table 3: *Allium cepa*, coffee and vitamin E effects on non- enzymic markers

Table 1(column 7) also depicts percentage increment in blood glucose level between ages 21 to 42 days for the various treatments.

Coffee treatment similar to control (normal rats) provoked gradual increase in blood glucose level over time (in days) (Table 1, role 5), with corresponded significant decreased SOD, CAT, GPx, and GSH and increased MDA (Tables 2 and 3 columns 5). Table 1 (role 5 columns 7 & 8) also depicts percentage increment in blood glucose level between ages 21 to 42 days and after 42 days for the treatment.

Table 4 compared time-dependent inter group percentage relationship change in blood glucose modulation by the various treatments in comparison with control group (normal rats) and vitamin E. The table shows that coffee group and normal rats produced significant increased blood glucose levels while *Allium cepa* lowers. Meanwhile, administration of the combination of *Allium cepa* and coffee produced raised blood glucose levels after day 21 which was significantly reduced to a point below that produced in the positive control group after 42 days; while coffee and vitamin E similarly produced raised blood glucose levels after day 42 (Table 4 role 6 and 7).

Treatment groups	Time-dependent (in days) percentage change in blood glucose level relative to:			
	Control		Vitamin E	
	Day 21	Day 42	Day 21	Day 42
Control (normal saline)	**0**	**0**	**18.2**	**32.1**
Vitamin E	**-15.4**	**-24.3**	**0**	**0**
Allium cepa	**-22.7**	**-33.3**	**-8.6**	**-11.8**
Coffee	**2.62**	**3.47**	**21.3**	**36.7**
***Allium cepa* + Coffee**	**-18.1**	**-30.4**	**8.62**	**-8.04**
***Allium cepa*+ Vitamin E**	**-21.7**	**-22.6**	**-7.24**	**2.29**

Table 4: Time-dependent Inter group percentage relationship change in blood glucose level

Discussion

The present study was conducted to investigated time - dependent (in days) hypoglycaemic potential of *Allium cepa,* coffee, vitamin E (which also acts as antioxidant) as well as the combination of coffee and *Allium cepa* or vitamin E and *Allium cepa* on blood sugar level, with corresponded levels of antioxidant defence mechanisms/oxidative stress markers (SOD, MDA, CAT, GPx, GSH) in normal experimental rats. The results were compared with control group (normal rats) and positive control group (vitamin E) respectively; however, the changes in the parameters studied were within physiological normal range.

Physical inactive and sedentary lifestyles can be viewed as stressors which can lead to oxidative cellular damage, likely due to enhanced production of ROS - induced release of toxic non-enzymatic metabolite, MDA, a marker of end products of the lipid peroxidation process associated with pathological conditions [27-29]. In the present study, the control group (normal rats) produced significant ($p<0.05$) hypergylcaemic activity (33.7%) by raising blood glucose levels above the basal levels (day 0) at age 42 days, with corresponded decreased SOD, CAT, GPx and GPH and increased MDA, with respect to positive control (vitamin E). Such effects were also evidently noted during coffee administration (see below) where there was the development and progression of raised blood glucose levels precisely in agreement with the view of an increased association of MDA, a marker of lipid peroxidation with oxidative vascular injury [39-47]. Our observation is also in support of the view that lack of regular physical activity predisposes to raised blood sugar level or onset of type 2 diabetes [1]. Furthermore, the observed molecular perturbation perhaps may also cause impairment of TRPM2 (transport receptor potential cation channel melastatin-related subfamily member 2) channel which plays a critical role in mediating oxidative-stress-induced cell death, thereby, contributing to the pathogenesis of higher blood glucose level. In contrast to the present findings, some studies have reported that plasma postprandial glucose level of control group (normal rats) showed a negligible change during the experimental period [8,48]. However, these were largely studies at age 15 days. Other studies have suggested that exercise of sufficient intensity or duration can promote increase in free radical production which may overwhelm antioxidant defence. Post-intensive bout of physical exercise resulted in an up regulation in MDA, CAT and GPx than at rest or at pre-physical exercise; which CAT and GPx possibly offered protection from ROS tissue damage [49]. This is in contrast to the findings of this study in that dynamic equilibrium did not exist between oxygen consumption and ROS production.

As the results have shown, *Allium cepa* (onion) gradually and significantly ($p <0.05$) reduced the blood glucose levels to a point below that produced in the negative control group after 42 days (Table 1) with corresponded up regulation of GSH, CAT, SOD, and GPx and reduction in MDA (Tables 2 and 3). This is evident that the association probably reflects a possible mechanistic links with bioactive constituents and antioxidant potential of *Allium cepa* capable of modulating biomarkers of lowered blood glucose levels. Precisely, our observation supports the report in which was stated that *Allium cepa* acts as a hypoglycemic agent by mechanisms rather than increasing insulin levels having extra pancreatic effects; acting directly on tissues as liver, muscles etc. and alter the activities of the regulatory enzymes of glycolysis, gluconeogenesis and other pathways [7,50,51]. Other studies reported that antihyperglycaemic efficacy of *Allium cepa* is brought about by the antioxidant properties of its essential oil components through suppression of oxidative stress, thereby preventing hyperglycaemia [52]. Nevertheless, our findings that *Allium cepa* have glucose lowering properties, and may be beneficial to those with diabetes in view of its safety as reflected by its worldwide use as vegetable collaborate with others [7,53-56].

Coffee, in the short- term, has been reported to produce increased blood sugar level; and some studies showed that over time, drinking coffee actually led to a decrease in blood sugar levels, including a reduced risk of type 2 diabetes [23,57-62]. Meanwhile, the exact mechanism that governs the physiological regulation of coffee induced short-term production of raised blood sugar level, but significantly lower in the long term is still unclear. The result of the present study showed that coffee produced a time – dependent raised blood glucose level with significant ($p < 0.05$) hyperglycaemic activity (15% and 16.6%) at age 21 and 42 days respectively; with corresponded decreased SOD, CAT, GPx, and GSH and increased MDA, in comparison to control group (normal rats) or positive control (vitamin E). We also established decreased supraoxide dismutase (SOD) ratio, R, to control group (normal rats) and ranked as: coffee < vitamin E < *Allium cepa* < vitamin E + coffee < *Allium cepa* + coffee (Table 2 column 3). Interrelationship between decreased ratio, *R* = SOD/control group (1:6) versus the lipid peroxidation oxidative stress biomarker, MDA (1:2) was observed. The levels of MDA were significantly elevated suggesting possibly adverse association of coffee with biomarkers for hyperglycaemic induced oxidative cellular damage. Nonetheless, the observation that coffee produces raised blood glucose level support the findings by others in which it was stated that the hormone adrenaline plays an important role in short-term coffee induced raised blood glucose levels [62].

We have also demonstrated that *Allium cepa* and coffee combined showed gradual and significant decrease in blood glucose levels (from 6.41% at age 21 days to 18.91% at age 42 days) with corresponding decrease in MDA (23.7%) compared to normal rats, suggesting that the combination of *Allium cepa* and coffee also did not provoke oxidative stress. Combination of *Allium cepa* and coffee showed significant antihyperglycaemic activity (23.8% or 25.32%) by bringing down blood glucose level at age between 21 to 42 days or at age 42 day in comparison to Allium cepa alone. This was evident that the different components present in *Allium cepa* and coffee probably have additive mechanistic actions that may exact synergetic effects and potentiate suppression of oxidative stress, thereby lower blood glucose level. Thus, the combination of coffee and Allium cepa possibly offers the intrinsic antioxidant defence system much better defensive mechanism to protect against free radicals and alleviate the toxic effects of accumulation of reactive oxygen species (ROS) than *Allium cepa* alone. Our result is in support that the combinations of antioxidants are capable of improving intrinsic antioxidant status and can help in the prevention and postponing the onset of the pathogenesis of vascular damage [47].

The effect of vitamin E, a non-enzymatic antioxidant, which protects the tissues from the ill effects of oxidative stress, on blood glucose in the animal models, was also studied. Similar to the effect of *Allium cepa* alone, vitamin E group or the groups administer the combination of vitamin E and coffee did not also seem over time, to make blood sugar levels higher in comparison with control group (normal rats). Combination of *Allium cepa* and coffee (blood glucose level 25.32% and MDA 23.7%) and *Allium cepa* alone (blood glucose level 11.8% and MDA 24.2%) produced much better blood sugar lowering effects as well as the oxidative stress biomarkers, in comparison with the combination of vitamin E and coffee (blood glucose level 0.67%; and both MDA 11.3% and GSH, 10.9%) and vitamin E alone (blood glucose level 9.83%; and MDA 17.7%) at age 42days. These discrepancies may plausibly be due to antagonist association of the bioactive constituents and antioxidant potential of coffee and vitamin E and/or to hydrophobic characteristics of vitamin E being contrary to hydrophilic enzyme antioxidant system or both, thereby, suppressing the development of antihyperglycaemic activities.

Our results further showed an increase in blood glucose level following administration of the combination of *Allium cepa* and coffee (8.62%) after the first 21 days, and in the combination of vitamin E and coffee (2.29%) after 42 days, in comparison with positive control (vitamin E) (Table 4). These may be a reflection of the glucogenic effects of the combinations of coffee and *Allium cepa* or coffee and vitamin E, which perhaps may counteract the common side effects, hypoglycemia of many anti-diabetic agents. Previous studies have reported an increase in blood glucose level after the first 4 hour following *Allium cepa* administration, and which was attributed to the glucogenic effects of *Allium cepa* [7].

Conclusion

It can be concluded that the combination of *Allium cepa and* coffee, over time, significantly lowers blood sugar level possibly due to synergistic effect, the presence of the additive bioactive phytoconstituents, and/or antioxidant efficacies or both, and potentiation action of several compounds over *Allium cepa* alone, combinations of Allium cepa and vitamin E or coffee and vitamin E. This mechanistic links offer the intrinsic antioxidant defence system much better defensive mechanism to protect against free radicals, and alleviate the toxic effects of accumulation of reactive oxygen species (ROS), thereby, providing protective mechanisms against the development of raised blood glucose levels. It is evident that *Allium cepa* and coffee combined produced significant hypoglycaemic effects which could be beneficial as a dietary supplement for the prevention and/or care of diabetes.

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