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Effects of Caffeinated Drink on Antioxidant Status In Alloxan-Induced Diabetic Male Albino Rats

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Abstract: *Researchers have shown that diabetes mellitus patients have increased reactive oxygen species leading to oxidative stress as a result of hyperglycemia in their vascular system. There are conflicting reports on the effect of the caffeine content of coffee beans and other caffeinated beverages on oxidative stress in a diabetic state. This study was designed to investigate the effect of caffeinated drink (containing 302.6mg caffeine) on antioxidant status in alloxan-induced diabetic male albino rats. The entire fifty male albino rats were divided into five groups; namely, Negative control, Positive (diabetes) control, along with three groups as test groups. All the experimental rats except those in the negative control group were induced with diabetes using alloxan. Animals in negative (group 1) and positive control (group 2) were fed with standard rat diet and water, while the three experimental test groups were fed with rat diet and diluted caffeinated drink (group 3 with 25% caffeinated drink and group 4 with 50% caffeinated drink) and undiluted caffeinated drink (group 5) respectively for two weeks. At the end of two weeks, malondialdehyde (MDA) level, serum total antioxidant status (TAS), Catalase, Glutathione peroxidase (GPx), and Superoxide dismutase (SOD) activities were determined using the standard spectrophotometric method.Results obtained show remarkable amplification in the activity level of Catalase, glutathione peroxidase and total antioxidant status (TAS), of 2 experimental groups (group 4 &5) compared to the positive and negative control group. Also, there is a remarkable rise in Superoxide dismutase activity in all test groups compared to the two controls ($p<0.05$), MDA level showed a momentous increase ($p < 0.05$) in animals in groups given diluted caffeinated drink and positive control compared to the negative group, although a decreased level was observed only when group 5 was compared to positive control. Therefore, intake of the caffeinated drink promotes activities of primary antioxidant enzymes and may contribute towards ameliorating oxidative stress in rats with diabetes mellitus.*

Keywords: *Diabetes mellitus, caffeinated drink, primary antioxidant enzymes, Malondialdehyde (MDA), Total Antioxidants Status.*

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I. Introduction

Diabetes is a hyperglycemic condition resulting from a group of metabolic disorders due to pancreatic β-cell deficiency and defective secretion of insulin production, inactivity, or a combined effect of both [(1,2)]. The deficient action of insulin on target organs in respect to diabetes mellitus is the basis of abnormalities in fat, protein and carbohydrate metabolism resulting from insulin impairment secretion or diminished insulin response by body tissues in one or more points in the complex pathway of the hormone [(1, 2)]. Diabetes mellitus symptoms range from marked hyperglycemia, polyuria, weight loss, polyphagia, polydipsia to long term complications such as nephropathy and retinopathy [(3, 4)]. Classification of diabetes mellitus depends entirely on the circumstances present at the time of diagnosis, although the majority falls into two broad etiologic categories [(3, 4)]. The two categories are type 1 diabetes mellitus, which has etiology result in Beta-cell destruction of the pancreatic islet due to the immune-mediated mechanism [(5)]. This type 1 diabetes mellitus accounts for 5-10% of diabetes cases worldwide, mostly diagnosed by markers of autoantibodies to GAD65, tyrosine phosphatases IA-2, and IA-2β [(5, 6)]. The second category is the type 2 diabetes mellitus, which results from insulin resistance with relative deficiency to insulin and a defect in insulin secretory cells [(1, 7)]. This category of diabetes mellitus is responsible for 90-95% of cases worldwide, with no known specific etiologies [(7)]. Other types are the genetic defect in insulin action, β-cell function, diseases of the exocrine pancreas, endocrinopathies, infections-mediate, and drug or chemical induced diabetes mellitus [(7)].

Microvascular and macrovascular complications due to diabetes mellitus result in several defects that play a vital role in the damaging tissue effects of hyperglycemia [(2)]. Hyperglycemia is the primary clinical manifestation of diabetes, which is thought to contribute to diabetic complications by altering vascular cellular

metabolism, vascular matrix molecules, and circulatory lipoproteins [8]. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes [7, 8]. There is also an increased formation of free oxygen radicals and decreased antioxidant capacity accompanying diabetes mellitus, which result in oxidative damage of the various cell components [7]. Oxidative stress can be due to an imbalance between a decrease in the activity of antioxidant defense system or increase production of free radicals or a resultant effect of both, which plays a vital role in the pathophysiology of cardiovascular disease and diabetes mellitus [9, 10]. Increased oxidative stress in the tissues and blood is believed to play a significant role in the beginning and progression of microvascular complications and atherosclerosis observed in diabetic patients [10, 11]. Oxidative stress can impact the cellular longevity and survival of the body tissues resulting in apoptosis due to reactive oxygen species generation [9, 114]. This reactive oxygen species results in protein misfolding, mitochondrial dysfunction, DNA damage, neuronal defect, and organelles dysfunction [9]. They are protective pathways that serve to mitigate damages caused by reactive oxygen species, and this involves superoxide dismutase, vitamin B, C, D and K, glutathione peroxidase, and coenzyme Q10 [9,10,12]. Few studies have expatiated on the antioxidant properties of coffee, which is the primary function of its constituents- melanoidins and polyphenols in protection in some chronic disorders [13].

Caffeine is a trimethylxanthine whose primary biological effect is the antagonism of the A1 and A2A subtypes of adenosine receptors [14, 15]. Caffeine is present in many everyday products like coffee, tea, kola nuts, chocolate, soda beverages, drugs, and energy drinks [16]. An average American consumes approximately 200mg of caffeine daily [16]. In Nigeria and other third world countries, the consumption of caffeinated drink has increased possibly as a result of cultural assimilation of red wine [17]. The interest in research about caffeine has increased in recent years, and this has resulted in a surge of publications dealing with a variety of pharmaco-physiological effects of caffeine [18]. Caffeine has been shown to have various pharmacological and cellular responses in a broad spectrum of biological systems [19]. These include stimulation of the central nervous system and cardiac muscle, increased urinary output, and relaxation of smooth muscles [19]. Research has shown that caffeine increases endurance in well-trained athletes, thereby increasing the athlete's ability to increase the intensity or duration of exercise [20]. However, evidence has been accumulating that frequent consumption of coffee may reduce the risk of type II diabetes and liver cancer [18]. Increased coffee consumption was associated with higher insulin sensitivity [19] and a lower risk for type II diabetes [20, 21, 22, 23, and 24] in many populations.
In contrast, some metabolic studies have shown that caffeine intake can acutely lower insulin sensitivity [25, 26] and increase glucose concentrations [27]. Tunnicliffe and Shearer, 2008 found that coffee consumption may also mediate levels of gut peptides (glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1), hormones intimately involved in the regulation of insulin secretion [28]. Finally, coffee may have prebiotic-like properties, altering gut flora, and digestion[29]. Reports have shown that Coffee intake might have an antiatherogenic property by increasing ATP-binding cassette transporter (ABC) G1 and scavenger receptor class B type I (SR-BI) expression and enhancing HDL-mediated cholesterol efflux from macrophages via its plasma phenolic acids [29].

Experimental studies and clinical models have implicated cellular injury due to autophagy and apoptosis as a result of the release of reactive oxygen species and oxidative stress to be the primary mediator of multisystem failure during diabetes mellitus [10, 11]. With reports projecting diabetes mellitus to be the leading cause of death worldwide in 2030, new therapeutic opportunities are essential in combating the threat of this condition [11]. The absence of data on the effect of caffeine on oxidative stress in diabetes state and the conflicting reports on the effect of caffeine on lipid peroxidation made this study essential. In this experiment, we assessed the two weeks effect of caffeinated drinks on the antioxidant status in diabetic rat models. We hypothesized that caffeinated drinks would favorably turn on the total antioxidant status, glutathione peroxidase, catalase, superoxide dismutase, but reduce the malondialdehyde level.

II. Materials And Methods

EXPERIMENTAL ANIMALS

We used fifty adult albino male rats. We avoided the interference of reproductive hormones on biochemical parameters in the study by excluding female rat while the experiment lasted. We obtained eight weeks old healthy male albino rats of weight between 140g –159g from the animal house of Ladoke Akintola University of Technology, and we kept the animals in a proper cage and made them acclimatize for seven days. We kept the animals in a well-ventilated cage made with wood and wire mesh under the controlled environmental conditions of temperature between 21 -31^{o} Celsius, 12 hours light/day circle and relative humidity between 45%-55%. Each animal was made to receive humane care correspondingly with the norm of laboratory animals care of the National Society of Medical Research (National Institutes of Health Publications no. 80-23, revised 1978) and approved by Ladoke Akintola University of Technology College of health sciences ethics committee.

EXPERIMENTAL DRINK

Caffeinated drink- 32 fl. oz (946ml) containing 302.6mg caffeine Using distilled water as diluents, 25%, 50%, 100% of Caffeinated drink- 32 fl. oz (946ml) was prepared daily according to table 1.

Table 1: Preparation of different concentration of caffeinated drinks

% dilution of caffeinated drink	Volume of diluent	Volume of caffeinated drink
25%	75%	25% of Caffeinated drink- 32 fl. oz (946ml)
50%	50%	50% of Caffeinated drink- 32 fl. oz (946ml)
100%	Nil	100%of Caffeinated drink- 32 fl. oz (946ml)

Experimental Design

We divided the entire fifty animals into five groups, as described in Table 2 below. The experiments lasted for two weeks.

Induction Of Diabetes In Experimental Animals

Induction of diabetes mellitus was done via the usage of single intraperitoneal injection which was prepared fresh by a solution of alloxan monohydrate (160 mg/kg). The assessment of established diabetes in the rats was done by the determination of the concentration of glucose after 48 hours of the injection. Tail veins of the rats were used for the collection of blood, and the blood glucose level was recorded in the unit of mg/dl by using a digital glucometer. Blood glucose level was measured after the 16 hours of fasting. The rats having a blood glucose level exceeding ≥170mg/dl were chosen for the positive control and experimental groups, respectively. The animals in group 1- negative control were injected with normal saline.

Experimental Procedure

Animals were grouped, as shown in Table 2 below. All the animal groups have access to rat diets, water, and the relative percentage of caffeinated drink, respectively.

Sample Collection And Storage

Animals fasted overnight after two weeks of treatment. However, water was made available to them during the fasting period. 2-3mls of Anesthetic- chloroform was given to the animals. The blood was collected from the heart puncture into the plain bottles and allowed to clot. After clotting occurred, we centrifuged the blood at 3000 g for 10 minutes, and the supernatant was separated into a sample bottle for the biochemical analysis.

TABLE 2:Animal groups withrespective treatments.

Groups	Species	Gender	Treatment	Total number of Animals
1	RATS	MALE	Healthy rats without DM+ Rat diet + water	10
2	RATS	MALE	Induced diabetic rats + Rat diet+water	10
3	RATS	MALE	Induced diabetic rats + Rat diet + water +25% Caffeinated drink	10
4	RATS	MALE	Induced diabetic rats + Rat diet + water +50% Caffeinated drink	10
5	RATS	MALE	Induced diabetic rats + Rat diet + water +100% Caffeinated drink	10

Biochemical Analysis

The total antioxidant status of the serum was measured using the Calbiochem total antioxidant status assay kits. Following the method of the manufacturer, total antioxidant status was measured spectrophotometrically @600nm absorbance under controlled temperature. The malondialdehyde (MDA) level was assayed using Genway Biotech Inc commercial kit method, which measures malondialdehyde level at 532nm absorbance based on the colorimetric method. OxiSelect™ Catalase Activity Assay Kit, which is based on colorimetric method, was used to measure serum catalase activity. We followed the manufacturer procedure of OxiSelect™ Superoxide Dismutase Activity Assay kit to determine the serum activity of superoxide dismutase. Glutathione peroxidase activity was measured using the commercial assay kit method (Sigma-Aldrich®).

ANIMAL WEIGHT MEASUREMENTS

Digital balance (VWR® P-Series Portable Balances, Model: VWR-500P) was used to weigh the body of the Rat at the start of the experiment, and after two weeks.

DATA AND STATISTICAL ANALYSIS:

Statistical Package for Social Sciences (SPSS) was used in this study, and all the collected data were analyzed statistically. All experimental data are indicated as the mean ± standard error of the mean (SEM). The means of the groups were compared using one-way ANOVA. The significant difference between control groups and test groups was examined using a t-test, and the level of momentousness was taken at P<0.05.

III. Results

Total Bodyweight

Table 3 and Figure 1 below present the initial and final mean weight results of the animals in each group. The data are illustrated as the mean ± SEM of 10 animal weights in each group. One-way ANOVA shows a significant decrease in the final weight of animals in group 2, 4, and 5 compared to their initial weight after 2 weeks of the experiment. However, there is an increase in the final mean weight of animals in group 1 compared to their initial mean weight, which is not significant at P<0.05.

Table 3: The effect of caffeinated drink- 32 fl. oz (946ml) containing 302.6mg caffeine on body weight gain of animals

GROUP	INITIAL MEAN WEIGHT±SEM (g)	FINAL WEIGHT ±SEM (g)
1	183.2±4.87	193.2±2.08
2	188.4±2.16	171.8±1.39*
3	180±8.63	166.2±5.82
4	172±5.32	149.4±10.12*
5	179±4.04	144.2±3.51*

* represents the level of significance taken at P<0.05 when final mean weight is compared with initial mean weight.

There are 10 animals in each group.

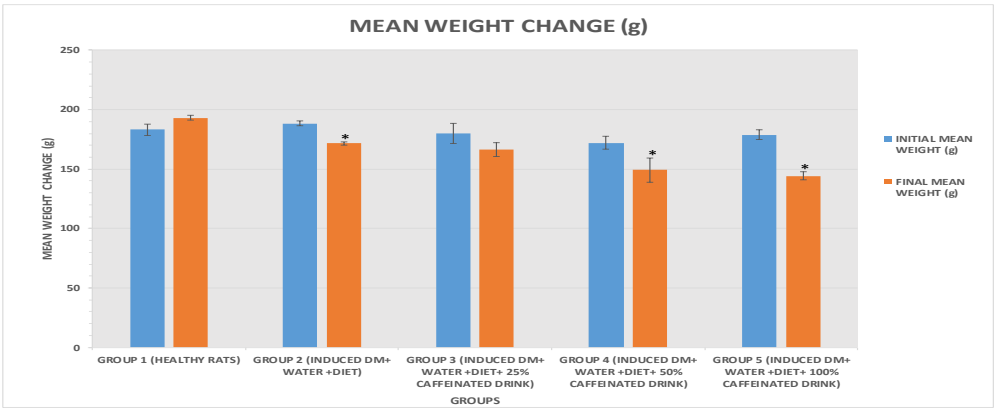

Figure 1:The effect of caffeinated drinkon body weight of animals.

The data are shown as the mean ± SEM of 10 animal weights in each group. * Denote the level of significance at P<0.05 when final mean weight is compared with initial mean weight. N represents the number of white male albino rats in each group (N=10).

BIOCHEMICAL ANALYSIS

The result of our study in table 4, figure 2, & figure 3 below, shows a significant increase in Superoxide dismutase level in the group 3, group 4 and group 5 when compared to group 1 (*P< 0.05). Group 4 and group 5 shows a significant increase in Glutathione peroxidase, catalase, and total antioxidant level when compared to group 1 but was not significant when group 2 and 3 were compared to group 1 in Table 4.

Malondialdehyde estimation in group 2, group 3, and group 4 were significantly increased when compared to group 1 in Table 4. However, there is no significant difference in malondialdehyde level of group 5 compared to group 1 in Table 4 and figure 4 below. Serum glucose level increased remarkably in Group 2, 3, 4, and 5 animals injected with a solution of alloxan monohydrate (160 mg/kg) compared to group 1 (negative control) which was injected with normal saline.

Also, in Table 5, our data analysis showed a significant increase in glutathione peroxidase, total antioxidant, and catalase in group 4 and 5 when compared to group 2, but Group 3 shows no significance when compared with group 2. There is a noteworthy increase in the activity of superoxide dismutase of group 3, 4, and 5 compared to group 2 in Table 5. Malondialdehyde, catalase, and glucose levels are not significant in group 3, group 4, and group 5 compared to group 2 in Table 5.

GROUP	**GLUTATHIONE PEROXIDASE MEAN ± SEM U/ml**	**TOTAL ANTIOXIDANT STATUS MEAN ± SEM mmol/l**	**MALONDIALDEHYDE MEAN ± SEM µmol/l**	**CATALASE MEAN ± SEM U/ml**	**SUPEROXIDE DISMUTASE MEAN ± SEM U/ml**	**GLUCOSE MEAN± SEM (mmol/L)**
1	22.860±6.060	1.966±0.283	1.922 ± 0.372	11.25 ±1.521	1.1790±0.168	5.58±0.13
2	24.600±8.449	1.658±0.169	5.406 ± 0.479*	12.660±2.685	1.1540±0.292	16.86±0.19*
3	30.800±4.733	1.660±0.180	6.723± 0.835*	13.060±1.331	2.498±0.150*	17.55±0.04*
4	41.320±5.638*	2.748±0.274*	7.029 ± 1.652*	24.670±2.320*	5.656±0.120*	15.30±0.46*
5	47.400±4.600*	3.876±0.149*	3.674 ± 1.539	33.780 ±3.885*	6.760±0.189*	16.40±0.17*

Table 4:TheEffect of caffeinated drink on Malondialdehyde level, Glutathione peroxidase, Total antioxidant status, catalase, superoxide dismutase activities, and glucose level. Data in the table above showsthe comparison of the mean ± SEM of total antioxidant status, glutathione peroxidase, catalase, superoxide dismutase activities, and malondialdehyde and glucose level of group 1 with test groups (group 2, group 3, group 4 and group 5). * represent the level of significance at P<0.05 when juxtaposed with group 1- negative control group. There are 10 animals in each group.

GROUP	**GLUTATHIONE PEROXIDASE MEAN ± SEM U/ml**	**TOTAL ANTIOXIDANT STATUS MEAN ± SEM mmol/l**	**MALONDIALDEHYDE MEAN ± SEM µmol/l**	**CATALASE MEAN ± SEM U/ml**	**SUPEROXIDE DISMUTASE MEAN ± SEM U/ml**	**GLUCOSE MEAN± SEM (mmol/L)**
2	24.600±8.449	1.658±0.169	5.406± 0.479	12.660±2.685	1.154±0.292	16.86±0.19
3	30.800±4.733	1.660±0.180	6.723± 0.835	13.060±1.331	2.498±0.150*	17.55±0.04
4	41.320±5.638*	2.748±0.274*	7.029± 1.652	24.670±2.320*	5.656±0.120*	15.30±0.46
5	47.400±4.600*	3.876±0.149*	3.674± 1.539	33.780 ±3.885*	6.760±0.189*	16.40±0.17

Table 5: TheEffect of caffeinated drink on Malondialdehyde level, Glutathione peroxidase, Total antioxidant status, catalase, superoxide dismutase activities, and glucose level. Data in the table above shows comparison of the mean ± SEM of total antioxidant status, glutathione peroxidase, catalase, superoxide dismutase activities, and malondialdehyde and glucose level of group 2 with group 3, group 4 and group 5. * represent the level of significance at P<0.05 when compared with group 2- Positive control group. There are 10 animals in each group.

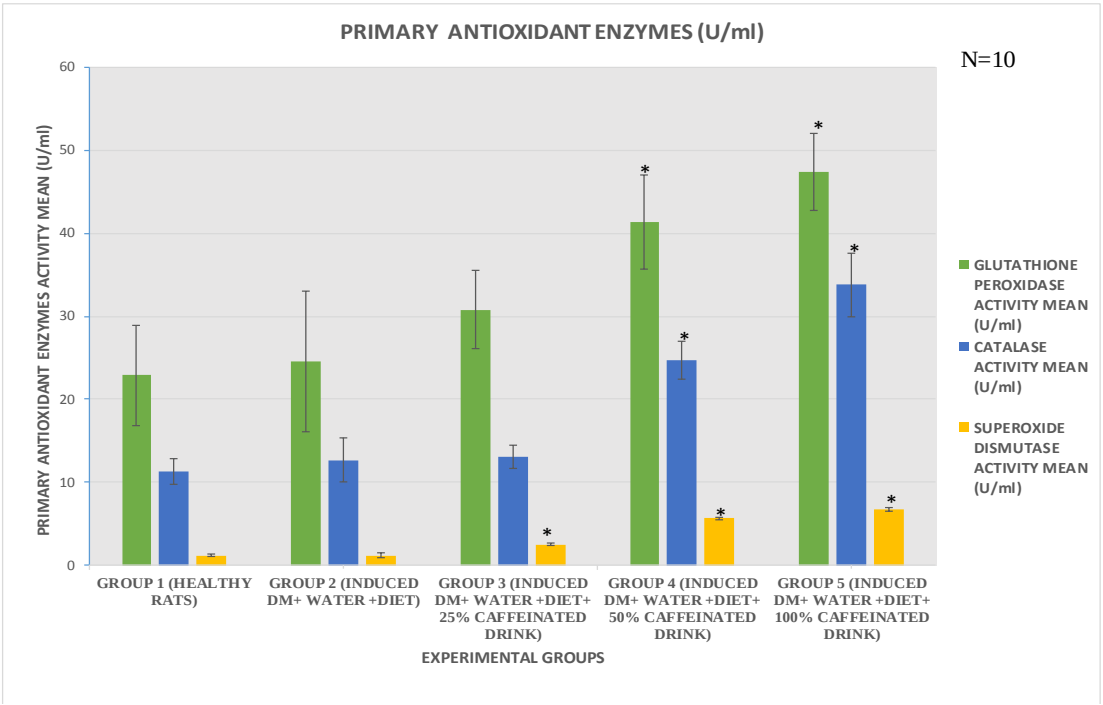

Figure 2:The Effect of caffeinated drink on glutathione, catalase, and superoxide dismutase activities. Data are shown as mean ± SEM of Glutathione peroxidase, catalase, and superoxide dismutase activities. * represent the level of significance at P<0.05 when juxtaposed with group 1- negative control group. N denotes the total number of animals in each group.

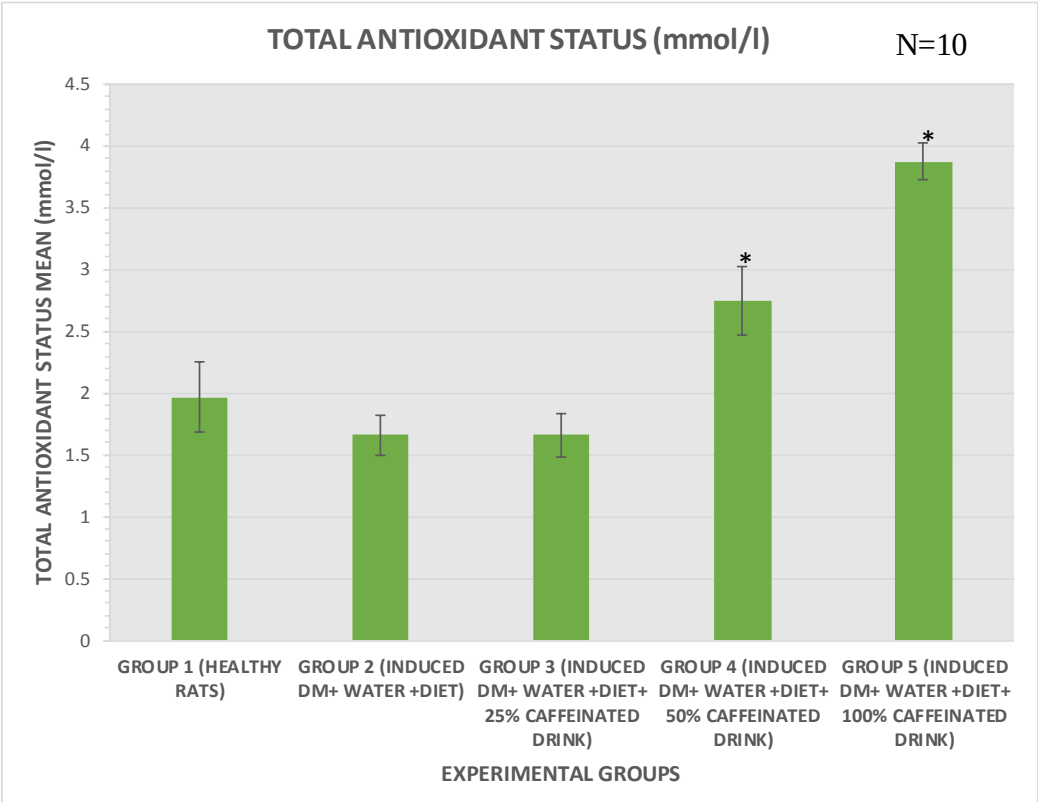

Figure 3:The Effect of caffeinated drink on Total antioxidants status. Data are shown as mean ± SEM of Glutathione peroxidase, catalase, and superoxide dismutase activities. * represent the level of significance at P<0.05 when juxtaposed with group 1- negative control group. N denotes the total number of animals in each group.

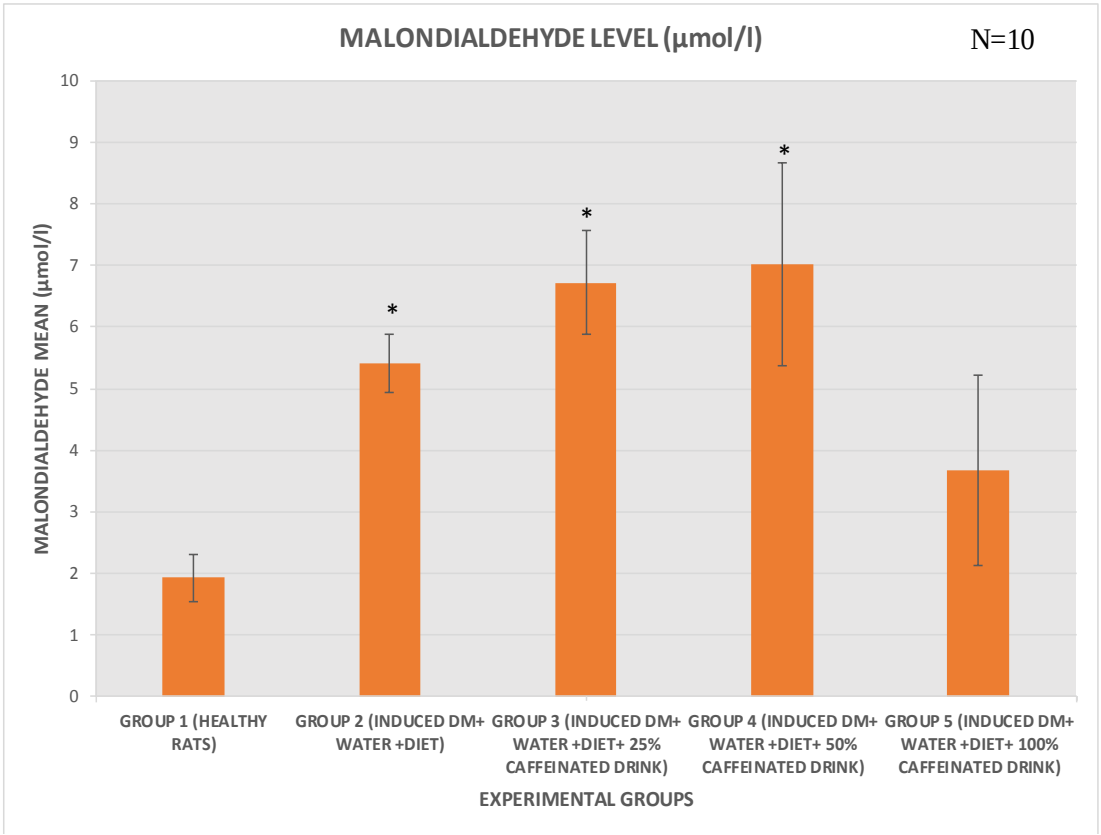

Figure 4:The Effect of caffeinated drink on Malondialdehyde level. Data are shown as mean ± SEM of Glutathione peroxidase, catalase, and superoxide dismutase activities. * represent the level of significance at P<0.05 when juxtaposed with group 1- negative control group.N denotes the total number of animals in each group.

IV. Discussion

Our present study revealed that two weeks administration of caffeinated drink induced a significant increase in antioxidant status but elevated MDA level at diluted concentration and reduced level of MDA at higher concentration (undiluted caffeinated drink) in alloxan-induced diabetic male rats (table 4 &5 and figure 2-5). We hypothesized that the administration of caffeinated drink would favorably turn on the antioxidant enzymes activities and in turn react adequately with diabetic-induced oxidative stress. Impairment of antioxidant defense mechanism with concomitant hyperglycemia has been suggested to accompany diabetic episode in humans as demonstrated in this experiment [(9)].

We examined three primary antioxidant enzymes; Catalase, Glutathione peroxidase, superoxide dismutase, and the total antioxidant capacity to evaluate the antioxidant status and MDA level to determine diabetic-induced oxidative stress damage. In our experimental study, primary antioxidant enzymes, specifically, glutathione peroxidase, catalase, and SOD activities were remarkably augmented in diabetic groups given a caffeinated drink with most significant elevation observed in the group given undiluted caffeinated drink (figure 2). The trend of increase in antioxidant enzymes activities observed in experimental groups compared to negative and positive control was presumably due to the relative concentration of caffeinated drink administered to experimental groups, respectively. The significantly increased glutathione peroxidase and superoxide dismutase level observed in this experiment when group 4 and group 5 were compared with group 1 in Table 4 were supported by the induction of compensatory response against hyperglycemic-oxidative stress [(12)]. Glutathione peroxidase and superoxide dismutase were shown to minimize reactive oxygen species-mediated damage and eliminate exercise-related reactive oxygen species as an adaptation to exercise-induced oxidative stress [(12)]. The rise in the activities of all the three antioxidant enzymes (GPx, catalase, and SOD) of all diabetic groups compared to the negative control in table 4 and figure 2 could be in part due to response to generated free radical, induced by the diabetic condition. Similarly, in table 4 and figure 3 above, caffeinated drink significantly enhanced total antioxidant status in experimental group 4 and 5 given 1:1 diluted and undiluted caffeinated drink respectively compared to the negative control. A recent study suggested that the caffeine content of coffee has in vitro antioxidant effect [(30)]**.** It can be suggested that caffeinated beverages can promote antioxidant status in diabetic condition and thereby ameliorate diabetic-induced oxidative stress.

In addition, there was a remarkablerise in malondialdehyde (MDA) level in group 2, group 3, and group 4 when compared to group 1 in Table 4 and figure 4 above. However, there was no significant difference in the level of malondialdehyde (MDA) when group 3, group 4, and group 5 were compared with group 2 in Table 5. Several studies have revealed that diabetes mellitus induced generation of free radical [(31)] which could be responsible for the rise in MDA level of all diabetic groups except group 5, which has reduced MDA level. Also, researchers have demonstrated caffeine to function in vitro as a pro-oxidant[(30)]. Reduced MDA level observed in group 5 compared to negative and positive control groups is suggestive of caffeine antioxidant action [(30)]. Therefore, despite the different mode of action caffeine relative to concentration, our results reveal a significant increase in antioxidant enzymes activities after administration of caffeinated drink in diabetic rats, but it also increases MDA level in the diabetic state.

Studies have demonstrated the correlation between the diabetic condition and weight loss of experimental animal [(26)]. The difference in weight is a basic predictive of the abnormal and healthy condition of experimental animals. Our study shows a noteworthy decrease in the weight of all diabetic animal groups. This indicates an impairment of glucose metabolism pathways during the experiment, which culminates into wasting away of the muscle. However, the net weight gain in the negative control group can be attributed to the active control of glucose metabolism [(32)].

V. Conclusion

The caffeinated drink can, therefore, be said to possess antioxidant properties and promote primary antioxidant enzymes activities, which can effectively ameliorate hyperglycemia-induced oxidative stress if administered for a considerable period as demonstrated in our experiment. The caffeinated drink can also be said to possess pro-oxidant properties at a lower concentration in diabetic conditions. We recommend studies of this nature to be conducted for an extended period longer than two weeks to establish therapeutic action of caffeinated beverages in induced-diabetic rats.

CONFLICT OF INTEREST: None declared

References

[1]. Khursheed M., Bikha R., Syed Z., Tarachand D., Thanwar D., Samar R. and Naseem. Lipid profile of patients with diabetes mellitus. WASJ 2011; 12(9): 1382-1384.
[2]. Vats, V., Grover, K. and Rathi, S. Evaluation of antihyperglycaemic and hypoglycaemic effect of Trigonellafoenum-graecum Linn, ocimum sanctum Linn and Pterocarpus marsupium Linn in normal and alloxanised diabetic rats. J. Ethnopharmacol 2002; 79: 95-100.
[3]. Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. Global prevalence of diabetes; estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-1053.
[4]. Kumar, V., Abass, A., Fausto, N. and Aster, J. Pathologic basis of disease. 8th edition. Saunders Elsevier 2010; 1131-1146.
[5]. Pinhas-Hamiel, O. and Zeitler, P. Acute and chronic complications of type II diabetes mellitus in children and adolscents. Drug Alcohol Depend2007; 99(1-3): 1-10.
[6]. Chattopadhy, R. and Bandyopadhyay, M. Effects of Azadirachtaindica leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. African Journal of Biomedical Research2005; 8: 101-104.
[7]. Kahn, R. Banting Lecture: Insulin action, diabetogenes and cause of type II diabetes. Diabetes1994; 43: 1046-1084.
[8]. Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. Global prevalence of diabetes; estimates for the year 2000 and projections for 2030. Diabetes Care2004; 27: 1047-1053.
[9]. Bajaj, S. and A. Khan. Antioxidants and diabetes. Indian Journal of Endocrinology and Metabolism2012; 16(2): S267-S271.
[10]. Kashiwagi, A. Complications of diabetes mellitus and oxidative stress. Journal of the Japan Medical Association2001; 44(12): 521-528
[11]. Maiese, K. New insights for oxidative stress and diabetes mellitus. Oxidative Medicine and Cellular Longevity2014-2015.
[12]. Barcelos, R., Souza, G., Amaral, S., Stefanello, G., Bresciani, M., Fighera, F., Soares, A. and Barbosa, N. Caffeine supplementation modulates oxidative stress markers in the liver of trained rats. Life Sciences 2014; 96: 40-45.
[13]. Metro, D., Cernaro, D. Santoro, M. Papa, M. Buemi, S. Benvenga, and L. Manasseri. Beneficial effects of oral pure caffeine on oxidative stress. Journal of Clinical and Translational Endocrinology2017; 10: 22-27.
[14]. Dews, P.B. Caffeine. Ann Rev Nutr1982; 2: 323-341.
[15]. Yukawa, G., Mune, M., Otani, H., Tone, Y., Liang, X. and Iwahashi, H. Effects of coffee consumption on oxidative susceptibility of Low-Density Lipoproteins and serum lipid levels in humans. Biochem (Moscow)2004; 69(1): 70-74.
[16]. Sinclair, C. and Geiger, L. Caffeine use in sports: A pharmacological review. J Sport Med Phys Fitness2000; 40(1):71-79.
[17]. Amos-Tautua, W., Martin, B. and Diepreye, E. R. E. Ultra-violet spectrophotometric determination of caffeine in soft and energy drinks available in Yenagoa, Nigeria. Advance Journal of Food Science and Technology 2014; 6(2): 155-158.
[18]. Lynn, L. and Kissinger, J. Coronary precautions: Should caffeine be restricted in patients with myocardial infarction? Heart Lung1992; 21(4): 365-371.
[19]. Massey, L. Caffeine and the elderly. Drugs Aging1998; 13(1): 43-50.
[20]. Eteng, M., Eyoung, E., Akpanyoung, Agiang, M. and Aremu, C. Recent advances in caffeine and theobromine toxicities: a review. Plant Food Hum Nutr1997; 51(3): 231-243.
[21]. Van Dam, R. Coffee consumption and risk of type 2 diabetes, cardiovascular diseses and cancer. Appl.Physiol.Nut.Met2008; 33(6): 1269-1283.
[22]. Arnlov, J., Vessby, B. and Riserus, U. Coffee consumption and insulin sensitivity. JAMA2004; 291: 1199–1201.
[23]. Van Dam, R. and Feskens, E. Coffee consumption and risk of type 2 diabetes mellitus. Lancet2002; 360:1477–1478.

[24]. Rosengren, A., Dotevall, A., Wilhelmsen, L., Thelle, D. and Johansson, S. Coffee and incidence of diabetes in Swedish women: a prospective 18-year follow-up study. J Intern Med2004; 255: 89–95.
[25]. Salazar-Martinez, E., Willett, W.C. and Ascherio, A. Coffee consumption and risk for type 2 diabetes mellitus. Ann Intern Med2004; 140: 1-8.
[26]. Tuomilehto, J., Hu, G., Bidel, S., Lindstrom, J. and Jousilahti, P. Coffee consumption and risk of type 2 diabetes mellitus among middle-aged Finnish men and women. JAMA2004; 291: 1213–1219.
[27]. Carlsson, S., Hammar, N., Grill, V. and Kaprio, J. Coffee consumption and risk of type II diabetes in Finnish twins (Letter). Int J Epidemiol2004; 33: 616–617.
[28]. Tunnicliffe, J. and Shearer, J. Coffee, glucose homeostasis, and insulin resistance: physiological mechanisms and mediators. Appl PhysiolNutrMetab2008; 33(6): 1290-1300.
[29]. Kempf, K., Herder, C., Erlund, I., Kolb, H., Martin, S., Carstensen, M., Koenig, W., Sundvall, J., Bidel, S., Kuha, S. and Jaakko, T. Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial. Am J Clin Nutr2010; 91(4):950-7.
[30]. Lee, C. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. Clin Chim Acta 2000; 295:141–154.
[31]. Bashan, N., Kovsan, J., Kachko, I., Ovadia, H. and Rudich, A. Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. Physiol Rev2009; 89: 27-71.
[32]. Nagarajan, N., Murugesh, N., ThirupathyKumaresan, P., Radha, N. and Murali, A. Antidiabetic and Antihyperlipemic Effects of Clemeo feline. Fitoterapia2005; 76; 310-315.

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