BIOMARKERS OF OXIDATIVE STRESS INCREASE IN DOSE-DEPENDENT MANNER, FOLLOWING PERIODIC ADMINISTRATION OF COFFEE AND CAFFEIN

Abstract:

Scientifically called *Coffea Arabica,* Research interests in Coffee have expanded with the discovery of its antioxidant properties. Coffee is a popular beverage consumed worldwide. Its effect on health has been a global puzzle. In this study, the effect that coffee consumption has on Oxidative stress parameters (Superoxide dismutase, Glutathione peroxidase, Catalase and Malondialdehyde) was examined. A hundred (100) Wistar rats bred in the Animal house of the Faculty of Basic Medical Science of Delta State University were used for the Study. While thirty (30) of them were used for toxicity test, Seventy (70) rats were randomly selected into groups of ten (10) rats with seven (7) groups each. All animals were fed with normal rat chow and water. All the experimental rats were treated for four (4) weeks period. Group 1, control, received food and water only, groups 2, 3 and 4 received 40mg/kg, 60mg/kg and 80mg/kg, doses of Coffee respectively while Groups 5, 6 and 7 received 30mg/kg, 45mg/kg and 60mg/kg doses of Caffeine respectively. After administrations of test substances, animals were sacrificed accordingly and serum samples collected for analysis of oxidative stress parameters. Both Caffeine and Coffee treatments showed a dose-dependent effect on most parameters measured. Coffee was found to greatly increase antioxidant enzymes. All comparisons were done at ($P<0.05$).

Keywords: Coffee, Caffeine, *Coffea Arabica,* oxidative stress

1. INTRODUCTION

Antioxidants, type of molecule that neutralize harmful compounds called *free radicals* that damage living cells, spoil food, and degrade materials such as rubber, gasoline, and lubricating oils. Antioxidants can take the form of enzymes in the body, vitamin supplements, or industrial additives. They are routinely added to metals, oils, foodstuffs, and other materials to prevent free radical damage[1&2].

Antioxidants work to control the levels of free radicals before they do oxidative damage to the body. For example, certain enzymes in the body, such as superoxide dismutase (SOD), work with other chemicals to transform free radicals into harmless molecules. Dietary antioxidants supplement the action of enzymes that occur naturally in the body, and some studies show that a diet high in foods that are rich in antioxidants may decrease the risk of cancer and heart disease[3&4]. Studies are inconclusive, however, and research into the health benefits of antioxidants is ongoing[4].

Vitamins C and E are well known antioxidants that may prevent cataracts and cancers of the stomach, throat, mouth, and pancreas. They may also protect from heart disease and strengthen the immune system. Good sources of vitamin E include wheat germ oil and sunflower seeds[3]. Caffeine in various foods has been variously implicated to have a healthful antioxidant activity against some free radicals inside the body. Caffeine, active ingredient in coffee may increase the effectiveness of gastrointestinal uptake of some pain killers, especially in patients with migraine and headache medications[5, 6&7].

Coffee consumption has been a food culture for centuries, approximately 85% of the world's population today uses substantial amounts of caffeine on a regular basis and 80% of pregnant women consume caffeinated beverages[8]. Caffeine is widely consumed at different levels by most segments of the population. Both the public and the scientific community have expressed concerns about the potential for caffeine to produce adverse effects on human health[9]. Intake of caffeine found in coffee, tea, chocolate, and some soft drinks, particularly cola-containing beverages is high in the industrialized world, and consumption of cola, in particular, has been increasing among children and young adults[8&9].

Caffeine is the most popular pharmacologically active substance consumed[10]. It is a stimulant and is often used to enhance mental alertness. Although there is no high quality evidence that a modest level of caffeine consumption has adverse effects on fertility or pregnancy outcome, putative beliefs about a relationship between caffeine intake and adverse reproductive outcomes are common and caffeine consumption is often perceived to be an unhealthy habit[10].

1.1 Aim of Study

Using wistar rats as experimental model, this study aimed at determining the effect(s) of Coffee and Caffeine on Oxidative stress parameters; Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Catalase and Malondialdehyde (MDA). Study also evaluated the effect of coffee on general body and organ weight.

2. METHODOLOGY

2.1 Research design

One hundred (100) Wistar rats bred in the Animal house of the Faculty of Basic Medical Science of Delta State University were used for this experimental research. Thirty (30) rats

were used for toxicity test, while seventy (70) rats were randomly selected into groups of ten (10) rats for seven (7) groups each. All animals were fed with normal rat chow and water. All experimental rats were treated for four (4) weeks period. Group 1, control, received food and water only, groups 2, 3 and 4 received 40mg/kg, 60mg/kg and 80mg/kg, doses of Coffee respectively while Groups 5, 6 and 7 received 30mg/kg, 45mg/kg and 60mg/kg, doses of Caffeine respectively. After administrations of test solutions, animals were sacrificed by cervical dislocation and serum samples collected for analysis. Following analysis, obtained results were expressed as Mean ± Standard deviation. Evaluation of data for significance was done, using One-way Analysis of Variance (ANOVA). A p-value < 0.05 was considered statistically significant.

2.6 Ethical Considerations

Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State. All animals were treated in line with guidelines, stipulated by the National Institute for Health Guide on the Care and Use of Laboratory Animals (1985).

2.7 Procedure

3.7.1 **Preparation of stock solution of caffeine**

High dose (60mg/kg)

1200mg (1.2g) of Caffeine was weighed with an electronic weighing balance and dissolved in 200ml of distilled water. This gave stock solutions of 1200mg/200ml (6mg/ml).

Medium dose (45mg/kg)

900mg (0.9g) of Caffeine was weighed with an electronic weighing balance and dissolved in 200ml of distilled water. This gave stock solutions of 900mg/200ml (4.5mg/ml).

Low dose (30mg/kg)

600mg (0.6g) of Caffeine was weighed with an electronic weighing balance and dissolved in 200ml of distilled water. This gave stock solutions of 600mg/200ml (3mg/ml).

3.7.2 Preparation of Stock Solutions of Coffee

Low dose (40mg/kg)

800mg (0.8g) of coffee was weighed with electronic weighing balance and constituted in 200ml of distilled water. This gave stock solutions of 800mg/200ml (4mg/ml).

Medium dose (60mg/kg)

1200mg (1.2g) 0f c0ffee was weighed with electronic weighing balance and constituted in 200ml of distilled water. This gave stock solutions of 1200mg/200ml (6mg/ml).

High dose (80mg/kg)

1600mg (1.6g) of coffee was weighed with electronic weighing balance and constituted in 200ml of distilled water. This gave stock solutions of 1600mg/200ml (8mg/ml).

3.7.3 Administration of Coffee Solution

High dose (80mg/kg), Medium dose (60mg/kg) and low dose (40mg/kg) were estimated from the lethal dose of coffee (192mg/kg). For high dose, medium and low dose of coffee, 1.6g, 1.2g and 0.8g were dissolved in 200ml of distilled water making the stock concentration to be (8mg/ml), (6mg/ml) and (4mg/ml) respectively.

The body weight of male Wistar rats was taken and the dose of test drugs in millilitre to be administered was calculated.

3.7.4 Administration of Caffeine Solution

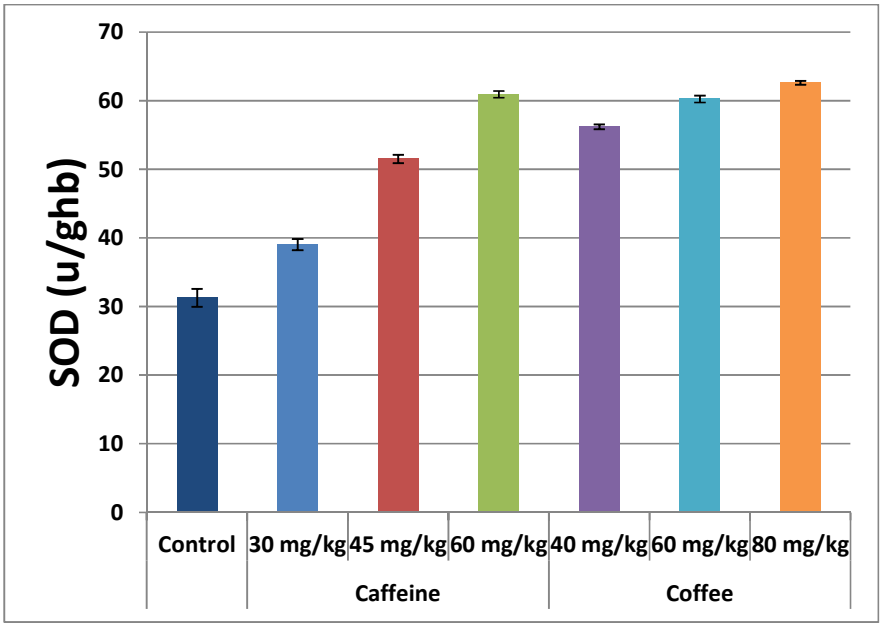
Caffeine was administered to experimental animals according to their body weight, such that animal weighing 200g, 150g, 170g received 2ml, 1.5ml and 1.7ml respectively. Caffeine was administered orally using orogastric canola.

3.7.11 Statistical Analysis

Evaluation of data for statistical significance was done, using one-way Analysis of Variance (ANOVA). Statistical data were analysed using the SPSS version 20, a statistical software. p-value of less than 0.05 was considered statistically significant.

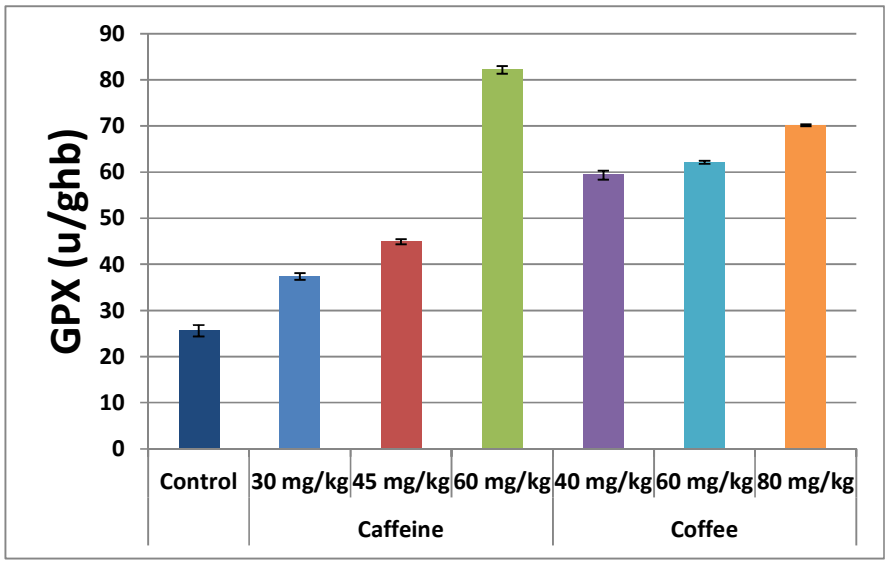
4. RESULTS

Figure 4.1 Showing SOD activities due to administration of Coffee and Caffeine.



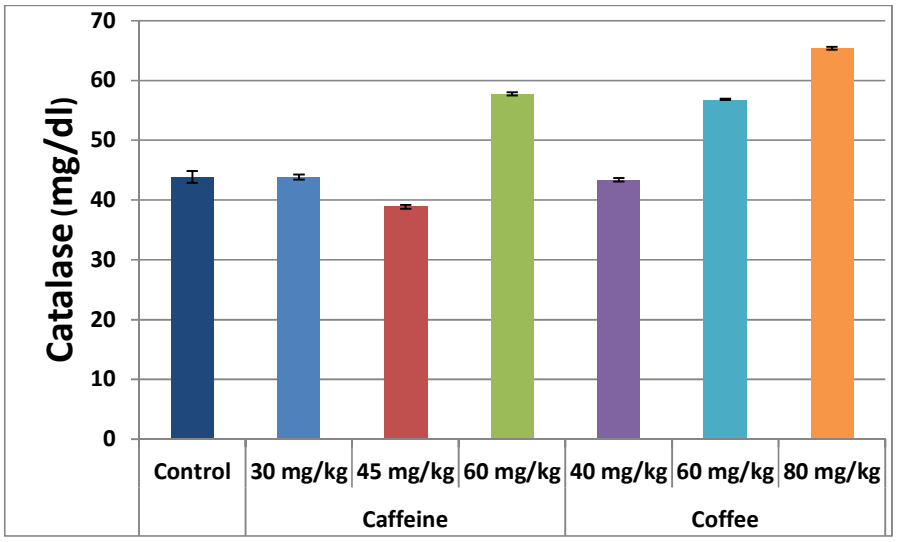
From above Figure, SOD values in all doses significantly (P<0.05) increased when compared to value in control group. Highest values are seen in the highest doses followed by medium and lowest for the low doses for both solutions administered. Both coffee and caffeine showed similar and graded effect on SOD activity.

Figure 4.2 Showing GPx activities due to administration of Coffee and Caffeine.



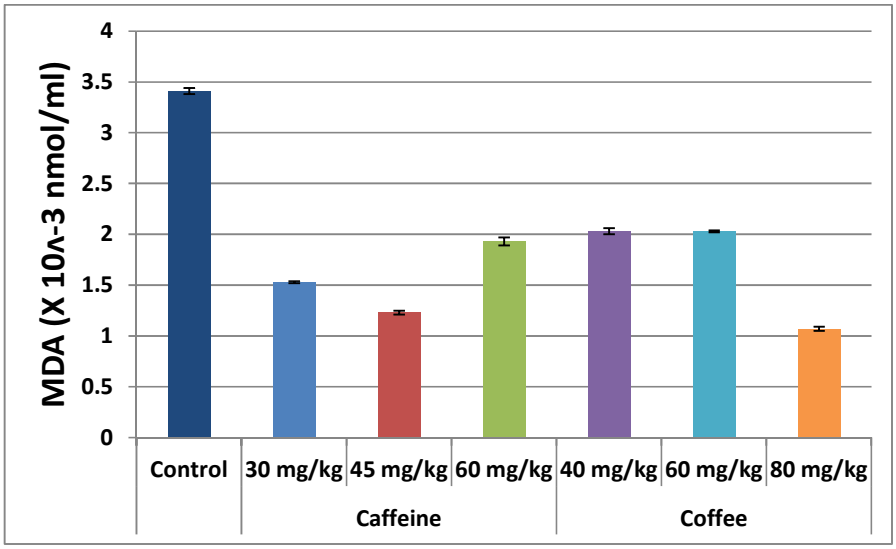
From above figure, GPx value in all doses significantly (P<0.05) increased when compared to GPx value in control group. The highest values are seen in the highest doses followed by medium, while lowest in low doses for both solutions administered. Both coffee and caffeine showed similar and graded effect on GPx activity.

Figure 4.3: Showing Catalase activities due to administration of Coffee and Caffeine.



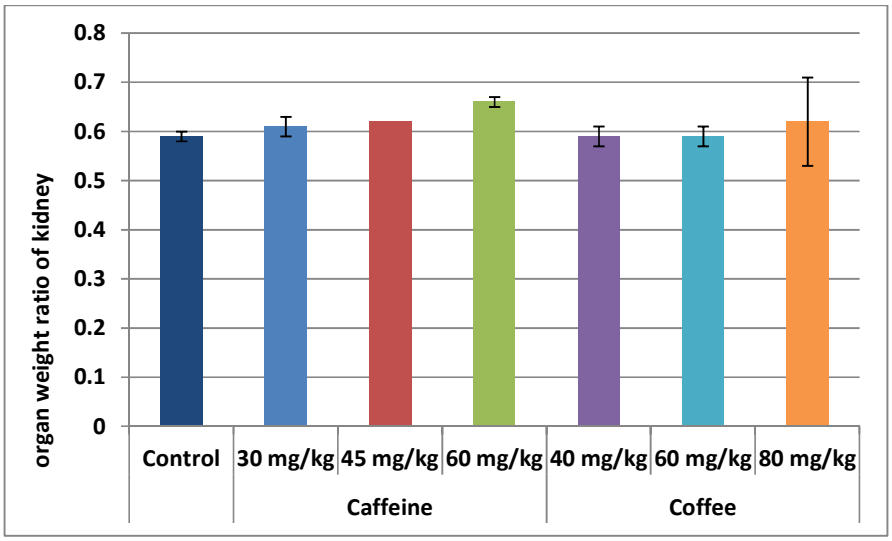
From above Figure, Catalase value in Lowest doses of coffee and caffeine did not show any significant (P<0.05) change when compared to control. Significance (P<0.05) increases were only seen in medium and highest doses of coffee. Also highest doses of caffeine administration showed significant increase when compared with control with exception of medium dose of caffeine which showed significant decrease, all other test groups were higher than control.

Figure 4.4: Showing MDA activities due to administration of Coffee and Caffeine.



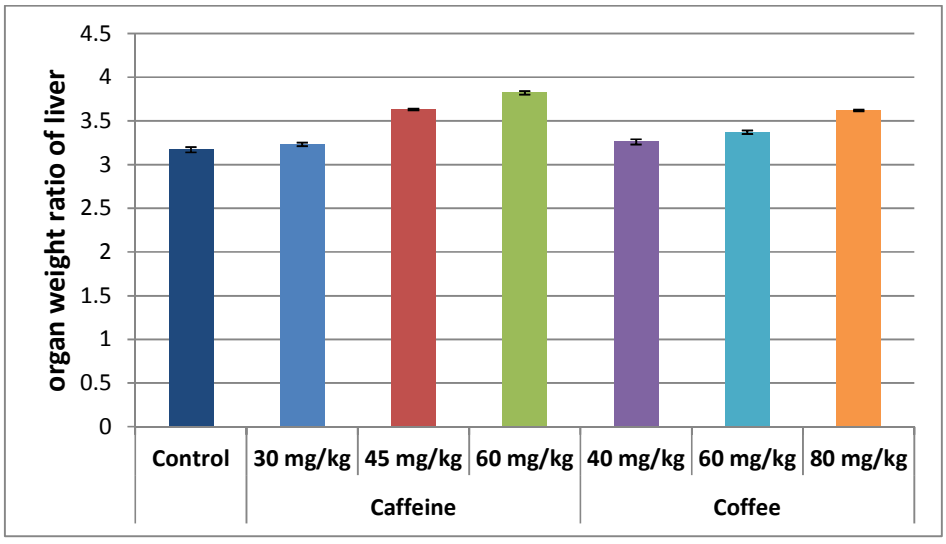
From Fig 4.4 above, there was significant decrease (P>0.05) in serum MDA level among all groups when compared to control. There was no dose dependent pattern effect.

Figure 4.5: Showing organ weight ratio of the Kidney due to treatment with Coffee and Caffeine.



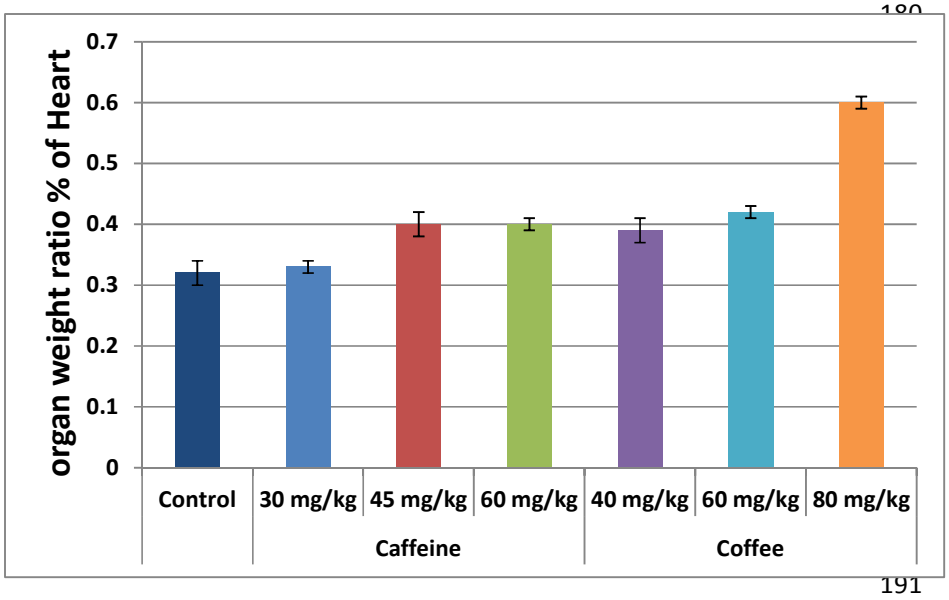
From Fig 4.5 above, no significant difference (P>0.05) was seen in relative kidney weight among groups (control, high dose, medium dose and high dose).

Figure 4.6: Showing organ weight ratio of the Liver due to treatment with Coffee and Caffeine.



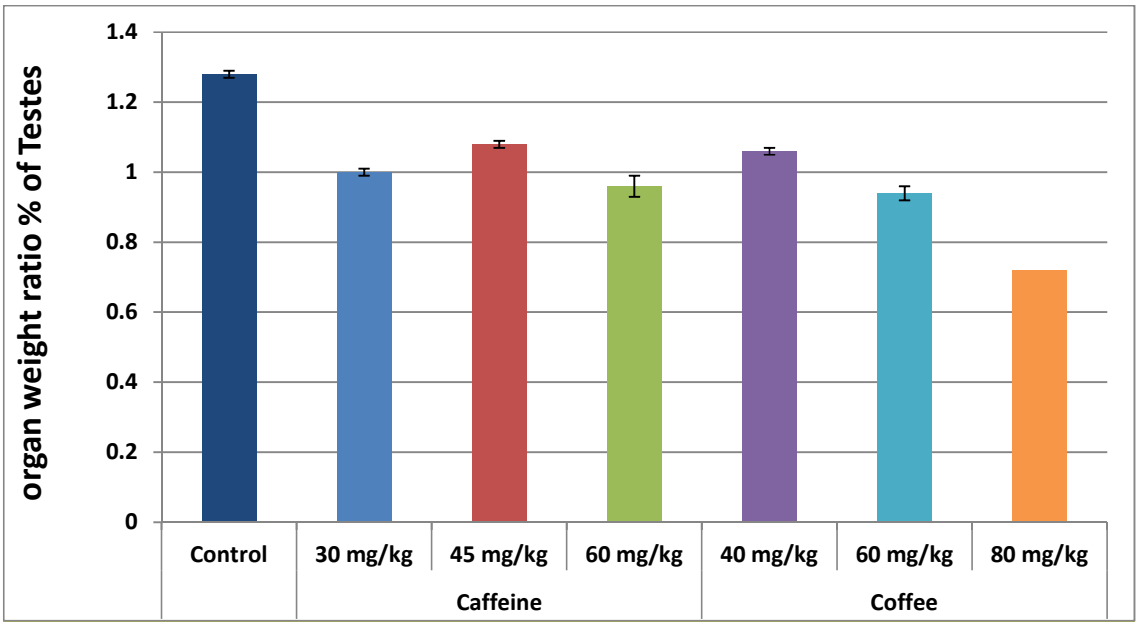
From Fig 4.6 above, no significant (P<0.05) increase was seen in relative liver weight in medium dose and high doses of Caffeine but only in high dose of coffee treatment when compared to control and high dose respectively.

Figure 4.7: Showing organ weight ratio of the Heart due to treatment with Coffee and Caffeine.



From Fig 4.7 above, there was a significant difference (P>0.05) in relative heart weight among groups (control, high dose, medium dose and high dose) except in low dose caffeine.

Figure 4.8: Showing organ weight ratio of the Testes due to treatment with Coffee and Caffeine.



From Fig 4.8 above, there was a significant difference (P>0.05) in relative testes weight among groups (control, high dose, medium dose and high dose) means.

4. DISCUSSION

Controversies on coffee consumption are ranging, because coffee has also been found to produce some negative (undesirable) effects. Regarding the conflicting results of epidemiological studies on caffeine and its effects on reproductive outcomes, caffeine-containing foods and beverages still remain one of the most consumed by most human populations of the world, its health effects have been and are still being studied extensively[11] Coffee is known to have beneficial effects as a result of its antioxidant properties. However, its harmful effects are mainly due to its caffeine content[12].

Caffeine is the World's most widely consumed psycho-active substance, but unlike most other psychoactive substances, it is legal and unregulated in nearly all jurisdictions[11]. An estimated 80% of the world's population consume a caffeine-containing substance daily[13]. Given this widespread use, the potential health effects of coffee are important for public health as well as for helping an individual make an informed choice regarding coffee consumption.

In the present study, the effects of coffee consumption on various oxidative stress markers were studied. Serum levels of Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Catalase and Malondialdehyde (MDA) were evaluated.

Effect on general and organ weight

Findings from this study demonstrated that consumption of coffee may have the potentials of decreasing body weight. The was no significant change in weight ($P< 0.05$), showing that the weight decreased due to treatment must have been counterbalanced by weight gain due to growth and adequate feeding over the duration of experiment. This closely agrees with that reported that coffee reduces body weight and also with that reported that increase in the intakes of coffee were inversely associated with weight gain [14]. More so this agreed with who opined that the significant loss in body weight could be attributed to the diuretic effect of Caffeine and its role in enhancing fat metabolism[15].

Effect on Oxidative Status

Results showed a significant increase ($p<0.05$) in testicular superoxide dismutase (SOD), suggesting that coffee increases superoxide dismutase. This is in agreement with Park, (2010) in his work on the "effect of coffee intake on anti-oxidative activities". He reported that coffee intake increase activities of antioxidant enzymes[16]. It can therefore be said that coffee consumption can increase the activities of SOD and help in the recuperation of antioxidant defence system.

Results also showed a significant increase ($p<0.05$) in the glutathione peroxidase (GPx) level of both medium and high dose when compared to control, just as GPx level of high dose group shows a significant increase when compared to GPx level in control group). This shows that coffee may increase glutathione peroxidase. This is in agreement with Park, (2010) who reported that coffee intake increase the activities of antioxidant enzymes[16]. Results also shows a significant increase ($p<0.05$) in low, medium and high doses when compared with control, suggesting that coffee increases catalase level in a dose dependent manner. This is in agreement with Montavon *et al* (2007) who reported that coffee intake increases catalase and SOD activities[17].

Malondialdehyde (MDA) is the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems. It is an indicator of lipid peroxidation and an indirect indicator of reactive oxygen species (ROS). Superoxide dismutase (SOD), on the

hand, scavenges both extracellular and intracellular superoxide anion and prevents lipid peroxidation of the plasma membrane. Reactive oxygen species (ROS) has potential toxic effects on sperm quality and function[18]. For instance, Agarwal et al. (2009) reported increased formation of ROS is correlated with the reduction of sperm motility[19].

The decrease in MDA indicates a reduction in lipid peroxidation, while the increase in the level of SOD suggests that Coffee has free radical scavenging ability, and therefore antioxidant capacity. This agrees report of earlier studies by Adefegha et al, (2012).

In recent years, evidences have shown that oxidative stress may play a role in the pathogenesis of idiopathic male factor infertility. Oxidative stress results from free radicals, reactive oxygen species and imbalances in antioxidant and oxidants status but can be reduced by consumption of antioxidant supplementation such as honey tea, coffee, vegetables, wine, juice, sprouted grains and other food[20]. Perhaps the greatest benefits of coffee may reside in its antioxidant components. Antioxidants are known to prevent oxidative stress which compromises functions and structures, In a study which underscores the importance of antioxidants containing foods in male reproduction, it was seen that higher antioxidant intake was associated with higher sperm count and motility[19&20]. A study showed that caffeine can protect the antioxidant enzyme superoxide dismutase against high dose of gamma irradiation as compared to other mitochondrial enzymes which are not involved in scavenging of free radical generated during irradiation such as superioxde[20].

5. CONCLUSION

This study shows that coffee induces a favourable turn on the activities of antioxidant enzymes with significant difference on SOD, GPx, Catalase and MDA levels. Administration of medium dose of coffee caused a significant ($P<0.05$) increase in serum Catalase level when compared to coffee high dose. It is said that coffee increases the activities of MDA levels, and help in recuperation of antioxidant defence system in wistar rats. While in serum catalase, there was no significant difference ($P<0.05$) in serum MDA levels among group (control, high dose, medium dose and low dose).

Recommendations

Results from this study necessitates recommendations for further studies on the antioxidant effects of Coffee on other systems like the neuro-endocrine system (eg dopamine,

noradrenaline) in the hypothalamus and other sexual behaviour regulatory centres in the brain.

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