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RESEARCH ARTICLE

Effect of Orange (*Citrus sinensis*) Peel Oil on Lipid Peroxidation, Catalase activity and Hepatic Biomarker levels in Blood Plasma of Normo Rats

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ABSTRACT

Dietary antioxidants are considered beneficial because of their potential protective role against oxidative stress, which is involved in the pathogenesis of multiple diseases such as cancer and coronary heart disease. The effect of feeding orange peel oil on lipid peroxidation, catalase and hepatic biomarkers in blood plasma of normo rats was investigated. Beside mouse chow, four diets were designed to contain 50% of energy as carbohydrate, 35% as fat, and 15% as protein, and one that was lipid-free diet which had distilled water substituted for fat. Groups of five rats were each fed one of these diets, while a fifth group was fed pelletized mouse chow. There was no significant difference in the amount of food consumed, though significant weight lost was observed in all groups except soybean oil. Feeding on orange peel oil led to significant ($p<0.05$) decrease in lipid peroxidation and catalase activities in comparison to soybean oil. Higher AST and lower ALT activities were observed in orange peel oil fed groups. These results suggest the oil from the orange peels possesses antioxidant potentials which could be protective against oxidative stress, thus useful in its treatment and management. However, the elevated levels of hepatic biomarkers pose a threat of hepatotoxicity thus suggesting that it should be consumed or used as a pharmaceutical ingredient at lower concentrations.

KEYWORDS: Orange peel oil; Lipid peroxidation, Catalase; Hepatotoxicity

INTRODUCTION

Sweet orange (*Citrus sinensis* L.) has been described as the most commonly grown tree fruit in the world (Morton, 1987). It belongs to the genus *Citrus* from the family, Rutaceae. Its tree is about 7.5 m or with great age, up to 15 m high. It is globose, subglobose, oblate or to some extent oval (Akpomie, 2010). The outer rind is orange or yellow when ripe and green when unripe; the inner rind is white, spongy and non-aromatic (Akpomie, 2010). The fruits are consumed by sucking the juice or made into orange juice; while the peels and seeds are often discarded (Akpata and Akubor, 2000). However, the peel is edible and mostly consumed particularly when there is scarcity of resources and maximal nutritional value desired. Increased vitamin C and fiber contents have been reported in orange peels but with high concentrations of pesticides (Anon, 2005). Increased dietary vitamin A is however required when consuming orange peel due to the presence of citral, an aldehyde that antagonizes the action of vitamin A (Ensminger, 1983). Flavonoids, consisting mainly of polymethoxylated flavonoids, terpenoids, such as limonene and linalool, and other volatile oils are the major ingredients of orange peel. Orange peel oil is an essential oil produced by cells in the rind of orange fruits. In contrast to most essential oils, it is extracted as a by-product of orange juice production by centrifugation, producing a cold-pressed oil (Wong, 1989). It is composed of mostly d-limonene (Bauer et al., 2001), which is responsible for the characteristic aroma of citrus. Oxidative stress has been associated with increased production of reactive oxygen species (ROS) or a significant decrease in the effectiveness of antioxidant defenses (Schafer and Buettner, 2001). It has been reported to play a major role in the complications of several diseases and aging process. Lipid peroxidation is an indicator of oxidative stress and a cause of cellular injury in animals and tissues (Faix et al., 2005). This paper aims at investigating the effect of feeding orange (*C. sinensis*) peel and/or soybean oil to albino male rats on lipid peroxidation, catalase and hepatic biomarkers in blood plasma of normo albino Wister rats.

MATERIALS AND METHOD:

PLANT MATERIALS:

About 5000 g of fully ripened local sweet oranges were purchased from Ikorodu market, Lagos, Nigeria, and peeled manually. 100 g of the peel was air dried and blended to fine smooth powder before being subjected to soxhlet extraction for 3 h using n-hexane as solvent which was then distilled off and the oily residue concentrated over a rotary vacuum evaporator and was hereafter called orange peel oil.

PREPARATION OF DIETS:

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Four diets were prepared using the formula described by Howell et al. (1998) and designed to contain 50% of energy as carbohydrate, 35% as fat, and 15% as protein. A lipid – free diet had distilled water substituted for the fat. The protein requirement was provided as defatted soybean (15%) as depicted in table 1.

ANIMALS:

Twenty-five male albino rats, each weighing between 90 – 120 g were maintained in accordance with and with the approval of the Animal Ethical Committee, Bells University of Technology, Ota, Nigeria. They were acclimatized for one week on pelletized mouse chow (Ladokee® Feeds Nigeria Ltd, Nigeria) with water provided *ad libitum* at room temperature and a 12-hour light and dark cycle. They were randomly assigned into groups of five animals as shown below:

Group 1: Each group receiving pelletized mouse chow
Group 2: Lipid-free diet (Diet 1)
Group 3: Orange peel oil diet (Diet 2)
Group 4: Soy oil diet (Diet 3)
Group 5: Soy oil + orange peel oil diet, respectively (Diet 4)

The rats were monitored daily for food and water intake, and body weight. At the end of the sixth week, the rats were fasted overnight and sacrificed by cervical dislocation.

PLASMA PREPARATION:

Blood was collected with a 2ml syringe and needle by cardiac puncture and was centrifuged at 3000 rpm for 10 min and the plasma was analyzed to evaluate some biochemical parameters.

PARAMETER ASSAYS:

Enzyme activities were measured by enzymatic colorimetric method using Randox kits which covers for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to the method of Amanvermez et al. (2009). Catalse activity was evaluated according to the method of Chance and Maehly (1955).

The extent of lipid peroxidation fractions was determined by measuring the level of malondialdehyde (MDA) formed according to the method of Chowdhury et al. (2002).

STATISTICAL ANALYSIS:

Statistical significance was established using one-way analysis of variance (ANOVA), and data were reported as mean ± standard error. Significant difference was established at $P < 0.05$. Statistical analyses were carried out using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL).

RESULTS:

No significant difference was observed in food and water intake of rats in all groups throughout the study period as depicted in figure 1. Significant decrease ($p<0.05$) was observed in the weight of the experimental groups except group 4 as shown in figure 2. This was also reflected in the feed efficiency ratio (FER) which was significantly low ($p<0.05$) (figure 2). There was a positive correlation between the feed intake and weight gain as depicted in figure 3. Feeding on orange oil diet led to 58.33% increase in lipid peroixidation as shown in figure 4. However, feeding on soybean oil led to a significant higher increase ($p<0.05$). That of blend of both oils was also observed to be significantly lower ($p<0.05$) than the soybean oil but higher than the orange peel oil. Significant reduced ($p<0.05$) catalase activity was observed in group fed orange peel oil. A significant higher ($p<0.05$) increased activity was observed in the soybean oil fed group. However, blend of both oil had a lower activity compared to the soybean oil diet as shown in figure 5. There were positive correlations between diet intake and the studied parameters respectively (figures 6 and 7). Feeding of orange peel oil led to a significant increase ($p<0.05$) in the AST level, this was observed to be significantly lower ($p<0.05$) in the soybean oil fed group. A higher level was observed in the oil blend (figure 8). Decreased ALT level was observed in the orange peel oil fed group. Feeding on soybean oil led to an increase. Blend of both oils led to a significant reduced level compared to the soybean oil diet (figure 8).

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Corn Starch	50%	50%	50%	50%
Orange peel oil	-	35%	-	17.5%
Soybean powder (defatted)	15%	15%	15%	15%
Soy oil	-	-	35%	17.5%
Distilled water	15%	-	-	-

Table No. 1: Composition of Experimental Diets

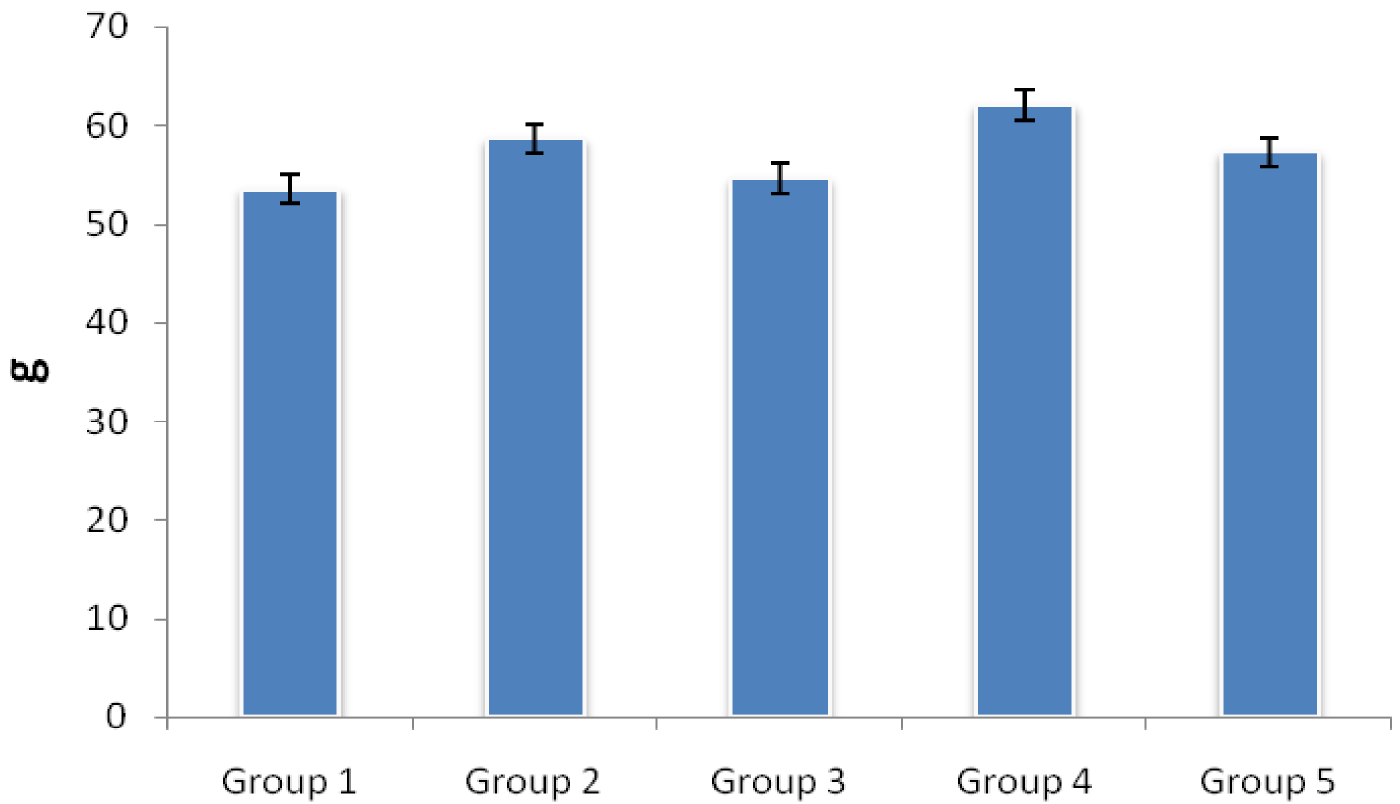

Figure No. 1: weight of feed consumed. Values = mean ± SEM; n = 5.

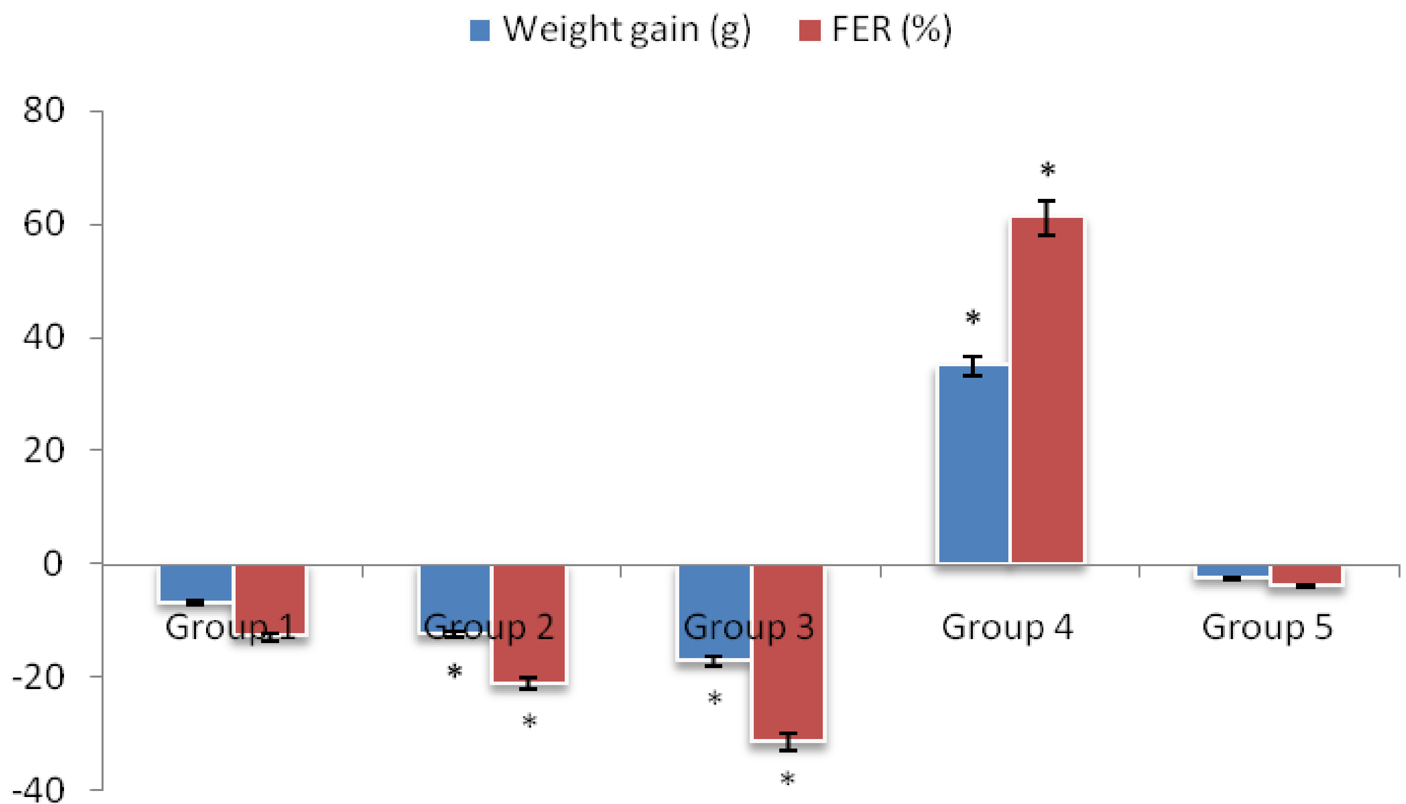

Figure No. 2: Weight gain and Feed effeciency ratio (FER) of experimental groups. Values = mean ± SEM; n = 5. *Significantly different between groups

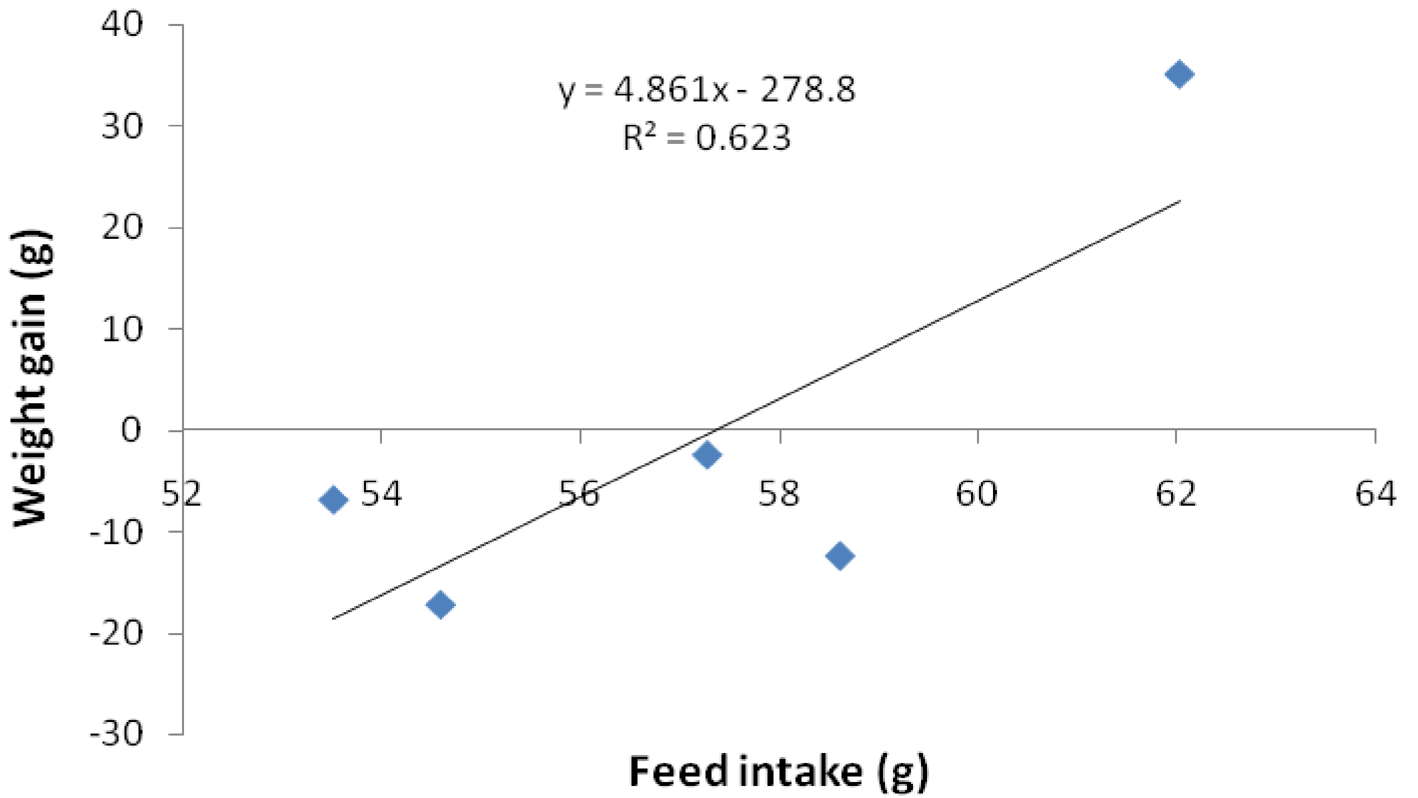

Figure No. 3: Correlation between feed intake and weight gain

μ/mol
3
2.5
2
1.5
1
0.5
0
*
*
*
Group 1
Group 2
Group 3
Group 4
Group 5

Figure No. 4: Lipid peroxidation. Values = mean ± SEM; n = 5. *Significantly different between groups

Figure No. 5: Catalase activities. Values = mean ± SEM; n = 5. *Significantly different between groups

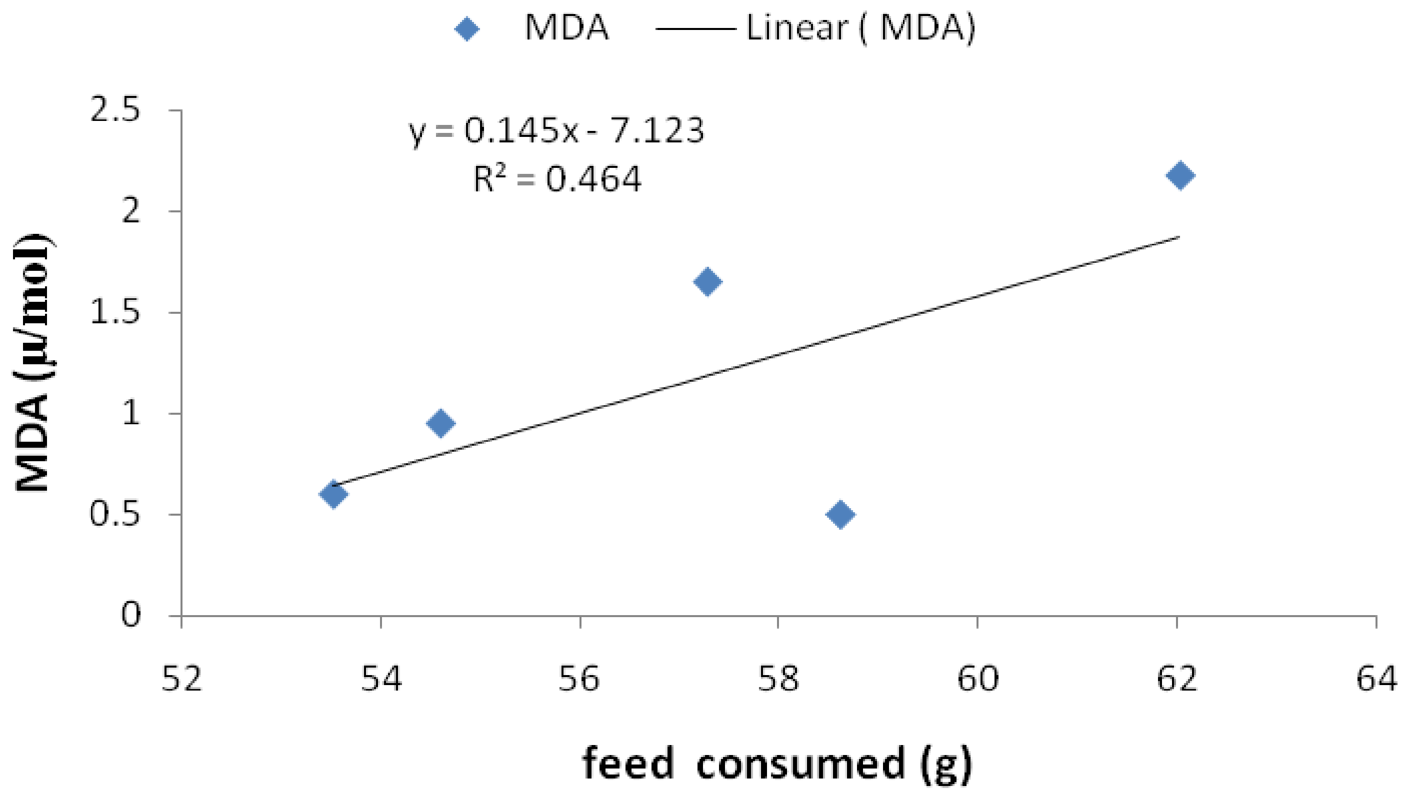

Figure No. 6: Correlation between feed consumed and lipid peroxidation

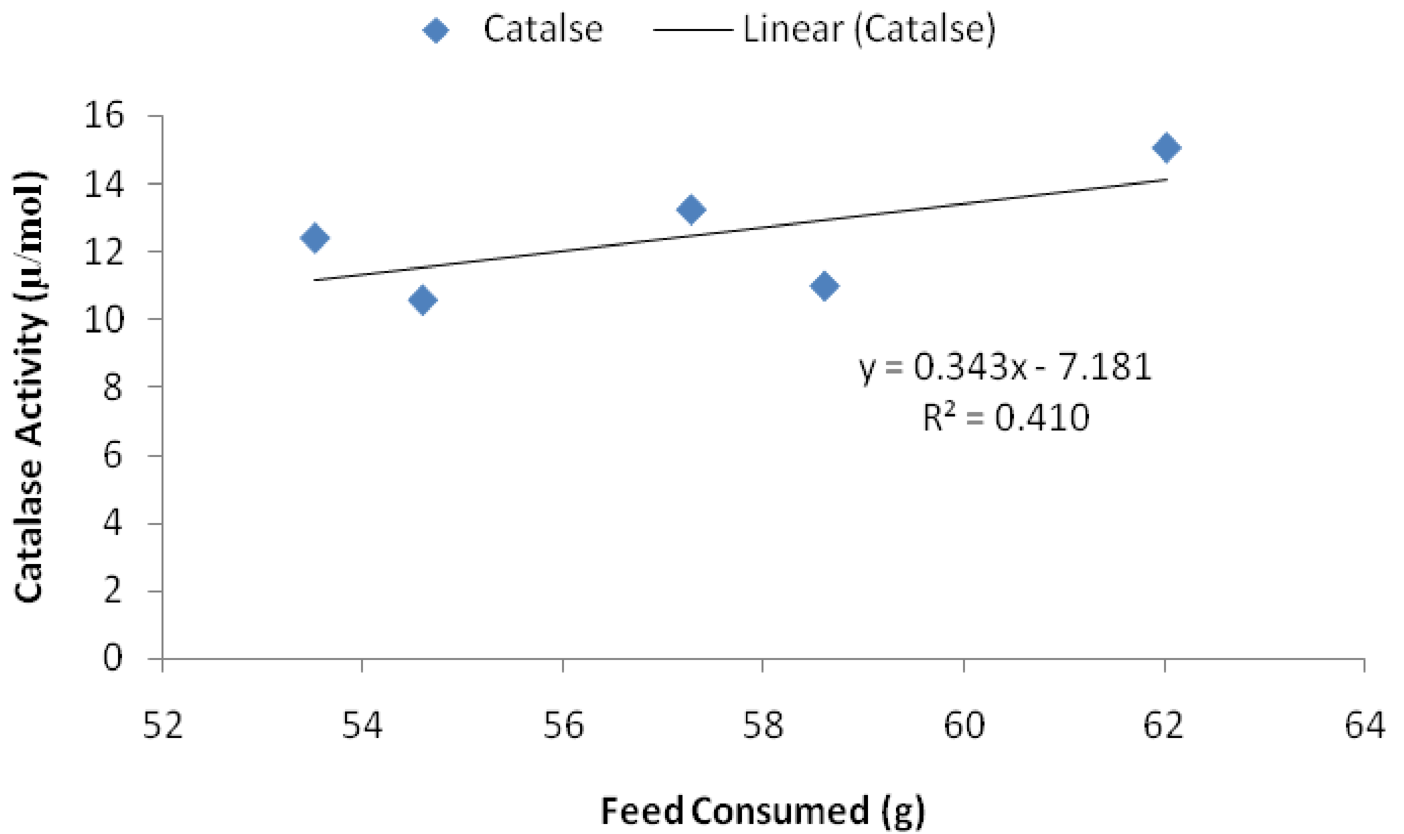

Figure No. 7: Correlation between feed consumed and catalase activity

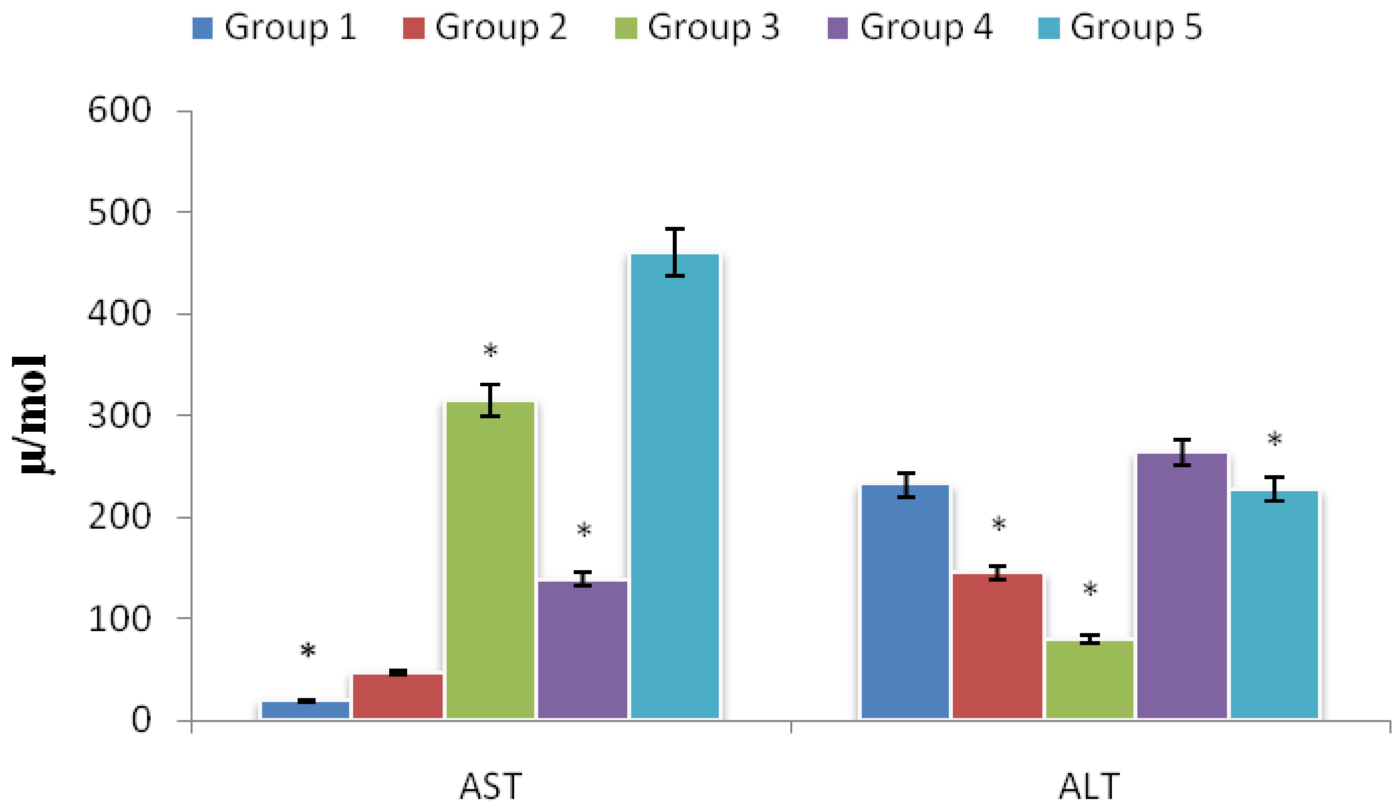

Figure No. 8: Hepatic biomarkers. Values = mean ± SEM; n = 5. *Significantly different between groups

DISCUSSION:

The significant reduction in the body weight of the rats fed orange peel oil diets is of major importance. Obesity plays a role in the leading causes of death, including cardiovascular disease, diabetes, cancer, and asthma. Many developing countries are now faced with a double burden of disease (WHO, 2012). While they continue to deal with infectious disease and under-nutrition, they are experiencing a rapid upsurge in obesity and overweight, particularly in urban settings (WHO, 2012). The observed reduced weight lost indicates that the oil could pose a beneficiary potential in the management of this scourge. Lipid peroxidation (LPO) is has been described as a marker of oxidative stress (Onyema et al., 2005; Abovwe et al., 2010). It induces changes in fluidity and permeability, inhibiting metabolic processes and altering ion transport (Nigam and Schewe, 2000). The reduced MDA content of the orange peel oil diets compared to the soybean oil suggests its potential in the management of oxidative stress. A possible role for orange peel oil as a dietary antioxidant in decreasing lipid peroxidation in blood plasma could be associated with high concentrations of terpenic aldehydes (Lado et al., 2004; Chikhi et al., 2012). These aldehydes are major constituents of essential oils and their antioxidant activities have been reported (Lado et al., 2004). The major constituent of orange peel oil is limonene and its antioxidant activities have been reported (Roberto et al., 2009). The low lipid peroxidation observed in the rats fed lipid free diet can be attributed to the absence of fatty acid. Catalase has been reported to be induced in response to oxidative stress (Checa et al. 1997). The observed reduced activities on feeding orange peel oil compared to the soybean oil further illustrates the antioxidant potentials of the oil. The activities of ALT and AST have been shown to be elevated following hepatocellular injury (Kaneko et al., 1997). They are released into the circulation after cellular damage (Naik and Panda, 2007). In this study, orange peel oil diets had significant higher level of AST activity compared to soybean oil suggesting it may induce hepatic injury at a higher rate than the soybean oil. In most types of liver disease, the ALT level is higher than AST suggesting a low AST/ALT ratio. However, in few exceptions the AST level is higher as observed in the rats fed orange peel oil. This may be as a result of injury from bile duct obstruction.

CONCLUSION:

These results suggest the oil from the orange peels possesses antioxidant potentials which could be protective against oxidative stress, thus useful in its treatment and management. However, the elevated levels of hepatic biomarkers pose a threat of hepatotoxicity thus suggesting that it should be consumed or used as a pharmaceutical ingredient at lower concentrations.

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