Effects of L- Ascorbic acid and α –Tocopherol and co-administration on Serums Lipid Profile and Liver Enzymes on High Fat Diet – Induced Insulin Resistance in Wistar Rats.

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Abstract: The aim of this experiment was to investigate the effects of L- Ascorbic acid and α –Tocopherol and co-administrations on high fat diet induced diabetes mellitus on serums lipid profile and liver enzymes on Wistar rats. Diabetes was induced by administration of high fat diet 10 % Groundnut oil, 20 % Groundnut mill and 2 % cholesterol eight weeks. After which the animals were randomly assigned into 5 groups of 5 rats each. Group I served negative control were administered 1ml distilled water. Group II were administered 100 mg/kg L- Ascorbic acid. Group III were administered 10 mg/kg α –Tocopherol. Group IV were co- administered 100 mg/kg, L-Ascorbic acid and 10 mg/kg α –Tocopherol respectively. Group V administered Glibenclimide 1 mg/kg served as positive control.. All treatments were given orally for a period of four weeks. The results obtained showed a statistically significant decrease ($p<0.05$) in the serum levels of total cholesterol, triglyceride and low density lipoprotein in in groups administered 100 mg/kg L- Ascorbic acid, 10 mg/kg α –Tocopherol and co-administration when compared with the control group. However, there was a significant increase ($p<0.05$) in the serum level of high density lipoprotein in the groups administered 100 mg/kg L- Ascorbic acid, 10 mg/kg α –Tocopherol and co-administration when compared with the control group. In relation to the liver enzymes, there was a significant decrease ($p<0.05$) in the serum levels of aspatate amino transferase, alanine amino transferase and alkaline phosphatase when compared to control group. As regard to glibenclimide there was a significant decrease ($P<0.05$) when compared with the control. In conclusion the ability of L- Ascorbic acid and α –Tocopherol administrations to high fat diet induced insulin resistance type II diabetic in Wistar Rats, significantly decrease the serum levels of cholesterol, triglyceride, low density lipoprotein, aspatate amino transferase, alanine amino transferase and alkaline phosphatise. Also a significant increase in the serum level of high density lipoprotein.

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1. Introduction

The diet-induced hypercholesterolemia has been recorded to adversely influence the health of humans and animal species. High level of blood cholesterol is usually associated with an increased risk for the development and progression of coronary artery disease and consequently of ischemic heart disease (Hassan *et al*, 2011). It has been reported that high levels of fat increase generation of oxygen free radicals and decrease antioxidant enzyme activity. In contrast, there are several reports indicating the beneficial effects of antioxidant supplementation in detoxification of free radicals. Thus, oxidative damage may result in many chronic health problems like heart attacks and stroke and kidney failure that are ascribed to high fat diet. Vitamin E is a potent lipid-soluble antioxidant protecting against lipid peroxidation and LDL oxidation (Maya *et al*, 2012). It also possesses anti-inflammatory properties, thus exerts beneficial effects in cardiovascular diseases. Hypercholesterolemia, characterized by increased levels of blood cholesterol, is a major risk factor for developing cardiovascular diseases such as myocardial infarction and hypertension, atherosclerosis and its complications. Hypercholesterolemia poses a major problem to many societies as well as health professionals because of the close correlation between cardiovascular diseases and lipid abnormalities (Matos *et al*, 2005).

Total blood cholesterol is the most common measurement of blood cholesterol. Cholesterol-enriched diet has been reported to adversely affect the health of humans and animal species Cholesterol feeding can induce several features of the metabolic

syndrome, such as dyslipidemia and insulin resistance. In animal models, dietary cholesterol appears to be an important risk factor for hepatic steatosis and progression to steatohepatitis. The prevalence of dyslipidemia resulting from excess energy intake and physical inactivity is increasing in Egypt. Dyslipidemia has been known as a risk factor for cardiovascular complications in type 2 diabetes mellitus. Diabetes mellitus is associated with insulin resistance, hyperinsulinemia, hyperglycemia and biochemical alterations in lipid metabolism (Tripathi and Srivastava, 2006). The typical lipid abnormalities in type 2 diabetic adults comprise low levels of HDL cholesterol(good), high levels of triglycerides and a dominance of small, dense LDL(bad) particles. High level of blood cholesterol is a contributory factor of atherosclerosis and many lipid associated ailments like obesity, heart attacks, stroke and kidney failure (Sathivel *et al*,2008).

The liver is the largest organ in the body. It is located below the diaphragm in the right upper quadrant of the abdominal cavity. An adult's liver weighs approximately 3 pounds or 1500g and accounts for approximately 2.5% of adult body weight. It extends approximately from the right 5^{th} rib to the lower border of the rib cage. The working cells of the liver are known as hepatocytes. Liver diseases are a major global concern and this type of disease/disorder still has extremely poor prognosis and high mortality because of the lack of effective preventive/treatment options (Singh, 2008).

There are a number of transaminases present in the body, but two in particular are measured these enzymes are: aspartate transaminase (AST) and alanine transaminase (ALT). Both liver enzymes can be affected by a variety of liver conditions. If the liver has been damaged, each will have leaked into the bloodstream. Elevated levels of these enzymes of hepatocellular origin suggests injury to hepatocytes. Thus, a simple blood test can diagnose liver damage (McClatchey and Kenneth, 2002).

The aim of this experiment was to investigate effects of L- ascorbic acid and α –tocopherol and co-administration on Serums Lipid Profile and Liver Enzymes on High Fat Diet – induced Insulin Resistance in Wistar Rats.

2. Material and Methods

2.1 Drugs and Chemicals

All chemicals were obtained commercially and were of analytical grade: Cholesterol (Mumbai India, M. W 386.67, CAS No. 57-88-5, LoT No. 100413). L-Ascorbic acid and α –Tocopherol were purchased from Zayo Stigma Aldrich Company, Jos, Nigeria

2.2 Animals

Twenty five (25) Wistar rats of either sex (weighting between 150- 200g) were obtained from the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria. The rats were maintained on standard laboratory animal feed (Vital Feeds Company Kaduna Nigeria) and water *ad libitum,* and housed in polypropylene cages at room temperature throughout the study. These studies were carried out in Ahmadu Bello University, Nigeria in accordance with the rules governing the use of laboratory animals as accepted internationally.

2.3 Induction of obesity and diabetes mellitus

The animals were fasted for 16-18 hours before the commencement of the experiment, but were allowed water *ad libitum*. The normal groups were fed with standard animal feeds only, while the high fat-diet groups were fed with standard animal feeds + high fat diet (10 % Groundnut oil, 20 % Groundnut mill and 2 % cholesterol) for the induction of obesity, diabetes and oxidative stress for the experimental period, which lasted for eight weeks. Rats with blood glucose levels between 130 - 150 mg/dl were considered diabetic (Khahn, 2005).

2.4 Experimental Design

In the experiment, a total of 25 rats were used. The rates were randomly divided into 5 groups of 5 rats each as follow:

Group 1: fed with standard animal feeds and high fat diet (10 % Groundnut oil, 20 % Groundnut mill and 2 % cholesterol) administered distilled water as control orally for four weeks.

Group 2: fed with standard animal feeds + high fat diet (10 % Groundnut oil, 20 % Groundnut mill and 2 % cholesterol) administered 100mg/kg L- Ascorbic acid orally administered for four weeks.

Group 3: fed with standard animal feeds + high fat diet (10 % Groundnut oil, 20 % Groundnut mill and 2 % cholesterol) administered 10mg/kg α –Tocopherol orally administered for four weeks.

Group 4: fed with standard animal feeds + high fat diet (10 % Groundnut oil, 20 % Groundnut mill and 2 % cholesterol) administered 100mg/kg L- Ascorbic acid and 10mg/kg α –Tocopherol orally administered for four weeks.

Group 5: fed with standard animal feeds + high fat diet (10 % Groundnut oil, 20 % Groundnut mill and 2 % cholesterol) orally administered Glibenclamide 1mg/kg for four weeks.

After treatment, the animals were subjected to light anaesthesia by exposing them to chloroform soaked in cotton wool placed in anaesthetic box, covered with lid. Blood was collected from the cardiac puncture. Serum was separated by centrifugation using Denley BS 400 centrifuge (England) at 3000 *g* for 10 minutes and the serum were used for the biochemical assays

2.5 Determination of lipid profile

Serum total cholesterol level was estimated by enzymatic method of Allain *et al.*(1974). Serum HDL-C was estimated by the method reported by Burstein *et al*., (1970). Serum triacylglycerol (TAG) was estimated by the method of Trinder (1969). Serum low density Lipoprotein cholesterol (LDL-C) and very low density Lipoprotein cholesterol (VLDL-C) was calculated according to the Friedewald formula: LDL-C = TC- (HDL-C+TAG/5) and VLDL-C= TAG/5 (Friedewald *et al*., 1972).

2.6 Determination of liver enzymes

Serum Alkaline Phosphatase (ALP) activity was assayed by the method of Bassey-lowry-Block. (1946). Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities were assayed by the method of Reitman and Frankel. (1957).

2.7 Statistical analysis

Data obtained from each group were expressed as mean ± SEM. The data were statistically analyzed using (ANOVA) with Tukey's post-hoc test to compare the levels of significant between the control and experimental groups. All statistical analysis was evaluated using SPSS version 17.0 software and Microsoft Excel (2007). The values of $P \leq 0.05$ were considered as significant.

3.0 Results

Table 1: Above showed the effect of L-Ascorbic acid and α –Tocopherol and co-administration on high-fat diet fed rats on Liver enzymes. There was a significant decrease ($p<0.05$) in the serum cholesterol, triglyceride and low density lipoprotein when compared with the control group. As regards to the high density lipoprotein there was a significant increase ($P<0.05$) when compared with the control group. In relation to glibenclimide there was a significant decrease ($P<0.05$) when compared with the control.

Table 1: Effects of L- Ascorbic acid and α –Tocopherol and co-administration on high-fat diet fed rats on Lipid profile.

Treatment Groups (n=5)	Serum Total Cholesterol (mmol/L)	Serum Triglyceride (mmol/L)	Serum High Density Lipoprotein (mmol/L)	Serum Low Density Lipoprotein (mmol/L)
Negative control (1mg/kg)	3.25 ± 1.21	1.92 ± 0.09	1.86 ± 1.21	2.57 ± 0.27
L-ascorbic(100 mg/kg)	2.61 ± 0.75 [b]	0.87 ± 0.03[a]	2.21 ± 0.15[a]	1.46 ± 0.12 [a]
α –tocopherol(10 mg/kg)	2.72 ± 1.00[a]	0.61 ± 0.03[a]	2.26 ± 0.18[a]	1.00 ± 0.18 [a]
L-ascorbic (100mg/kg) and α –tocopherol(10 mg/kg)	2.15 ± 0.04[a]	0.52 ± 0.06[a]	3.22 ± 0.11[a]	0.59 ± 0.22[a]
glibenclimide (1mg/kg)	2.88 ± 1.13 [a]	0.55 ± 0.09[a]	2.21 ± 0.02[a]	1.11 ± 0.23[a]

Values are expressed as mean ± SEM; n = 5. [a,] = $P < 0.05$ significant.

Table 2: Effects of L- Ascorbic acid and α –Tocopherol and Co-administration on high-fat diet fed rats on Liver enzymes.

Treatment	**AST(IU/L)**	**ALT(IU/L)**	**ALP(IU/L)**
control (1mg/kg)	85.00 ± 2.35	93.80 ± 3.65	72.80 ± 0.92
L- ascorbic acid (100 mg/kg)	51.2 ± 0.31[a]	72.6 ± 1.55[a]	44.00 ± 1.30[a]
α –tocopherol (10 mg/kg)	49.0 ± 0.56[a]	48.00 ± 0.64[a]	57.20 ± 2.22[a]
L- ascorbic acid (100mg/kg) and α –tocopherol (10 mg/kg)	39.2 ± 1.00[a]	51.80 ± 0.22 [a]	50.10 ±1.12[a]
Glibenclimide (1mg/kg)	50.0 ± 8.02[a]	61.60 ± 1.09[a]	62.60 ± 2.57[a]

Values are expressed as mean ± SEM; n = 5. [a,] = $P < 0.05$ significant.

Table 2: Above showed the effect of L- Ascorbic acid and α –Tocopherol and co-administration on high-fat diet fed rats on Liver enzymes. There was a significant decrease ($p<0.05$) in the aspatate amino transferase, alanine amino trasferase and alkaline phosphatase when compared to control group. As regard to glibenclimide there was a significant decrease ($P<0.05$) when compared with the control.

4.0 Discussion

Alterations in lipid metabolism and increased mobilization of free fatty acids from muscle and fat deposition occur in tissues such as liver and heart in diabetes mellitus (Bloomgarden, 2003; Shukla *et al.,* 2012). Hyperlipidaemia, a risk factor of cardiovascular diseases is frequently seen among diabetic patients (Mengesha, 2006). Serum lipid levels are commonly increased in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease (Al-Shamaony *et al.,* 1994, Muthulingam, 2010).

The lipid profile obtained in the present study showed a significant ($P < 0.05$) decrease in total cholesterol, total triglyceride, low-density lipoprotein and an increase in high-density lipoprotein levels in groups administered L- Ascorbic acid and α –Tocopherol when compared with control group. This finding is in line with the results obtained by Kolawole *et al*. (2012), who demonstrated the anti-lipidaemic effect of *Perdea americana* on cholesterol diet fed rats for six weeks of the experimental protocol.. This is in agreement with the reports of Fernandes *et al.* (2010); Mironova et *al*. (2000); Odetola *et al*., (2006) and Iweala and Oludare, (2011) who demonstrated increased serum lipids in diabetes in animals. Diabetic-induced hyperlipidemia is attributable to excess mobilization fat from the adipose due to under utilization of glucose (Krishnakumar *et al*., 2000; Nimenibo-uadia, 2003). The lack of insulin and elevations of the counter-regulatory hormones (e.g glucagon) lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissue (Subbiah *et al*., 2006;Rotimi *et al*., 2011; Matsinkou *et al.,* 2012). The fatty acids from adipose tissues are mobilized for energy purpose and excess fatty acids are accumulated in the liver, which are converted to triglyceride (Shih *et al.*, 1997; Suryawanshi *et al*., 2006).

The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of unlimited actions of lipolytic hormones on the fat depots (Ayeleso *et al.,* 2012). Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease in diabetes (Ayeleso *et al.,* 2012). This observation may be as a result of inhibition of deregulation of lipid metabolism cause by the imbalance between the level of intake and expenditure, which hindered mobilisation of excess cholesterol into the body system. The decrease in serum lipid profile may also be as a result of the L-Ascorbic acid and α –Tocopherol inhibiting fat accumulation and fatty acid synthesis by activation of fatty acid oxidation, demonstrated by Sahar and Abdel, (2012). Obesity has been implicated as one of the leading causes of hyperlipidaemia. Hyperlipidaemia itself usually causes no symptoms, but may lead to symptomatic vascular diseases, including coronary artery disease and peripheral arterial disease (Rohilla *et al*., 2011). In addition, the hypocholesterolemic effect of the L- Ascorbic acid and α- Tocopherol, single and co-administered may also be attributable to its antioxidant property, which is responsible for the decrease activity of 3-hydroxyl-3-methyl-glutaryl co-enzyme A (HMG CoA) reductase, which is the key regulatory enzyme in cholesterol biosynthesis (Olooto *et al.,* 2014), reduction in cholesterol absorption by the intestinal wall and/or induction of LDL-receptors within the peripheral tissue (Danesh and Kanwar, 2004; Olooto *et al.,* 2014). This observed improvement in the lipid profile status of diabetic treated rats revealed the cardio-protective properties of the vitamins and may be attributed to antioxidant effects. In addition, the significantly lowered cholesterol level recorded in the current investigation may have contributed to the observed significant high serum high-density lipoprotein cholesterol in the animals. Kwiterovich (2000) and James *et al*. (2010) had reported that about 30% of blood cholesterol is carried in the form of HDL-C. HDL-C function to remove cholesterol atheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease. Therefore, the observed increase in the serum HDL-C level on administration of L- Ascorbic acid l 100 mg/Kg and α –Tocopherol 10 mg/Kg diabetic rats, indicates that the vitamins have HDL-C boosting effect. More so, the stabilization of serum triglyceride and cholesterol levels in rats by Retinol and α –tocopherol may be attributed to glucose utilization and hence depressed mobilization of fat (Momo *et al*., 2006; Iweala and Oludare, 2011). This finding suggests that L- ascorbic acid and α –tocopherol may be useful in reducing the complications of hyperlipidemia and hypercholesterolemia which often coexist in diabetics.

The Serum liver enzyme measurements are valuable tool in clinical diagnosis that provides information on the effect and nature of pathological damage to any tissue (Daisy and Saipriya, 2012). AST, ALT and ALP are biomarkers of damage to the plasma membrane and endoplasmic reticulum and are often used to assess the integrity of the plasma membrane and tissues after being exposed to certain pharmacological agents (Rathod *et al.,* 2009). Result obtained in the present study showed that the activities of serum liver enzymes; aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were significantly increased in the diabetic

untreated animals in comparison with non-diabetic untreated rats. This is consistent with the studies of Daisy and Saipriya (2012), who reported increased transaminase levels in streptozotocin-induced diabetes. Increased gluconeogenesis and ketogenesis might be due to an elevated activity of transaminase (Ghosh and Suryawansi, 2001; Gandhi *et al.,* 2011). Abolfathi *et al.* (2012) reported that the elevation in markers of liver injury such as ALT, AST and ALP indicate hepatocyte damage in experimental diabetes. And this increase in the levels of these enzymes in diabetes may be as a result of leaking out of these enzymes from the tissue into the blood stream (Concepción *et al.,* 1993). AST and ALT are released when injury involves organelles such as the mitochondria (Kumar *et al.,* 2003). Transaminases mediate the catalysis of amino transfer reactions, and are vital markers of liver injury in clinical diagnostics (Li *et al.,* 2007), alkaline phosphatase is a hydrolase enzyme located in the cytoplasm (Han *et al.,* 2006) and is responsible for removing phosphate from nucleotides and proteins released due to hepatic cellular damage. The ability of L- Ascorbic acid and α –Tocopherol administrations to diabetic animals significantly decrease the ALT, AST and ALP serum levels suggest their hepato-cellular protective function and this can be attributed to its antioxidant effects.

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