EFFECTS OF FOLIC ACID SUPPLEMENTATION ON SERUM LIPID PROFILE IN MALE ALBINO RATS

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ABSTRACT

Cardiovascular disease (CVD) are caused by disorder of the heart and blood vessels. Risk factors may include elevated serum levels of total cholesterol, triglyceride, low density lipoprotein and reduced serum concentration of high density lipoprotein among other things. Different reports have revealed interfaces between supplementation with vitamin B complex and decrease in oxidative stress and inflammation, which are related with cardiovascular disorders. This study is therefore designed to assess the effects of folic acid in the regulation of serum lipids such as total cholesterol, phospholipids, triglycerides, and high density lipoprotein cholesterol (HDL-C). Serum lipid profile were assayed for using standard procedures. Results showed that serum total cholesterol and triglyceride were significantly reduced ($p \leq 0.05$) in rats placed on folic acid supplements compared with control group. Serum phospholipids and high density lipoprotein cholesterol were significantly elevated ($p \leq 0.05$) in rats placed on vitamin B6 supplements when compared with control group. The data showed anti-hyperlipidaemic effects of folic acid supplementation in rats.

Keywords: cardiovascular disease, lipoprotein, folic acid, serum lipids.

INTRODUCTION

Cardiovascular disease is the most common cause of death in industrialized countries such as the US, and is on the rise in developing countries too. The National Heart, Lung, and Blood Institute of the National Institutes of Health has identified many risk factors for cardiovascular disease, including an elevated LDL-cholesterol level, high blood pressure, a low HDL-cholesterol level, obesity and diabetes (NIH, 2002). In recent years, researchers have identified another risk factor for cardiovascular disease, an elevated homocysteine level. Homocysteine is an amino acid normally found in blood, but elevated levels have been linked with higher incidence of cardiovascular diseases (Moustapha and Robinson, 1999; Assanelli

et al., 2004) and in patients with hyperhomocysteinemia and myocardial infarction, folic acid supplementation has been found to significantly decrease the homocysteine level and improved arterial endothelial function (Lawrence, 2000; Nelson and Cox, 2000; Assanelli *et al*., 2004). Studies have shown that a folate-rich diet is as effective as folic acid from supplements in decreasing plasma homocysteine concentrations in hyperhomocysteinemia and coronary artery disease patients (Pinto *et al.,* 2005).

Recent studies have already described an inverse relationship between folate and high-density lipoprotein cholesterol (HDL-C) levels and low folate levels have therefore been suggested to be a cardiovascular risk factor and that the subjects with lower folate levels should be recommended for dietary folic acid supplementation to HDL-C levels (Imamura et al., 2010; Villa et al., 2005).

Several studies have also proposed a direct biochemical link between the lipoprotein and homocysteine metabolism. In rats with experimentally induced hyperhomocysteinemia, plasma cholesterol levels increase, probably because of increased expression and activity of hepatic HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis (Sharma et al., 2007; Hirche et al., 2006; Woo et al., 2005). Homocysteine supplementation leads to an inhibition of phospholipid methylation and triacylglycerol accumulation in yeast (Malanovic et al., 2008), and diet-induced hyperhomocysteinemia leads to cholesterol and triacylglycerol accumulation in mouse liver (Werstuck et al., 2001). The nutritional intake of folate and, to a lower extent, of vitamin B12 are major determinants of plasma homocysteine levels (Finkelstein, 2000). Therefore, this study was also carried out in order to assess the effect of folic acid supplementation on the lipid profiles of normal male albino rats.

MATERIALS AND METHODS

Animal Grouping and Folic acid Administration

Twenty albino rats with an average weight of 136g were purchased from Animal house of the College of Medicine, University of Lagos. They were kept in a well-ventilated cage in the animal house of the department of Biochemistry, University of Lagos with conducive atmospheric pressure and temperature range between 25^0-30^0C. The animals do not suffer any observable disorder and have unrestricted access to clean water.

Rats were randomized into four groups consisting of five rats as follows: (i) an untreated control group; (ii) treated group administered 0.2mg/Kg body weight of Folic acid (iii) treated group administered 0.4mg/Kg body weight of Folic acid (iv) treated group administered 0.6mg/Kg body weight of Folic acid for 14 days.

Sample Collection

After 14 days, rats were killed by mild chloroform anaesthesia and tissues were collected for the subsequent biochemical analysis of specific parameters in the pancreas and other vital organs. After autopsy under mild ether anaesthesia, 5 mL of blood was collected directly from the heart into appropriately labelled sample bottles, serum was separated.

All samples were kept in a refrigerator alongside the reagent kit at a temperature range of 2-6^0C, for analysis of biochemical parameters.

Determination of Total Cholesterol

Total cholesterol was determined using enzymatic method described by Allain *et al*. (1974). Cholesterol esterase hydrolyses cholesterol esters to free cholesterol. The free cholesterol produced is oxidized by cholesterol oxidase to cholesten-4-ene-3-one with simultaneous production of hydrogen peroxide which couples with 4-aminoantipyrine and phenol in the

presence of peroxidase to yield chromogen with maximum absorption at wavelength 510 nm, The colour intensity is proportional to the cholesterol concentration.

Determination of triglycerides

Triglyceride was determined using enzymatic method described by Buccolo and David (1973). Triglycerides are hydrolyzed by lipases to yield glycerol and fatty acids. The glycerol produced is oxidized to dihydroxylacetone phosphate with the production of hydrogen peroxide which couples with 4-aminophenazone and 4- chlorophenol to produce a chromogen referred to as quinoneimine. The reaction is catalyzed by peroxidase. The degree of absorbance of the chromogen is directly proportional to the concentration of triglyceride measured at 505 nm.

Determination of Phospholipid

Phospholipids are hydrolysed by phospholipase D and the liberated choline is subsequently oxidized by choline oxidase (CHO) to betaine with the simultaneous production of hydrogen peroxide. In the presence of peroxidase (POD) the hydrogen peroxide couples oxidatively the 4 –aminophenazone (4-AP) and dichlorophenol to form a quinoimine dye.

Determination of High Density Lipoprotein-Cholesterol (HDL-cholesterol)

The precipitation method by Assmann *et al*. (1983) was used to determine HDL-cholesterol. The addition of phosphotungistic acid in the presence of magnesium ions precipitates quantitatively low density lipoprotein, very low density lipoprotein and chylomicron fractions from whole plasma, leaving the HDL fraction in the supernate. The cholesterol in the HDL which remains in the supernatant after centrifugation is estimated using the enzymatic method of Allain *et al*. (1974).

Haematology

Samples of blood were gathered in heparinized anticoagulant bottles and subjected to hematological examination. The hematological examination was done utilizing a

hematological analyser (HMX complete blood count analyser, Japan). Red blood cell count (RBC), Packed cell volume (PCV), Hemoglobin concentration (Hb), Mean Corposcular Volume (MCV), White platelet count (WBC) were resolved.

Statistical Analysis

Results were expressed as mean ± standard deviation (Mean ± SEM) and subjected to statistical analysis utilizing one-way analysis of variance (ANOVA) and Tukey Post-Hoc Test (Graph Pad Prism version 6). Statistical significance was considered at $p< 0.05$.

RESULTS AND DISCUSSION

The effects of administration of folic acid on principal serum lipids in male rats were evaluated in this study because of the possible role of folic acid on tissue metabolism. Such tissue metabolism is likely to involve biochemical reactions or pathways that often determine serum lipid profile in health or disease (Robert *et al*., 2002). Therefore, the hypocholesterolaemia observed in this study (Figure 2) may be important in this respect.
The hypocholesterolaemia observed in this study (Figure 2) following the acute consumption of folic acids (0.4 mg/Kg and 0.6 mg/Kg) may be attributed to reduction in the concentration of acetyl CoA resulting from decreased ß-oxidation of fatty acids, since acetyl CoA is a key substrate in the biosynthesis of cholesterol (Rang *et al*., 1995).

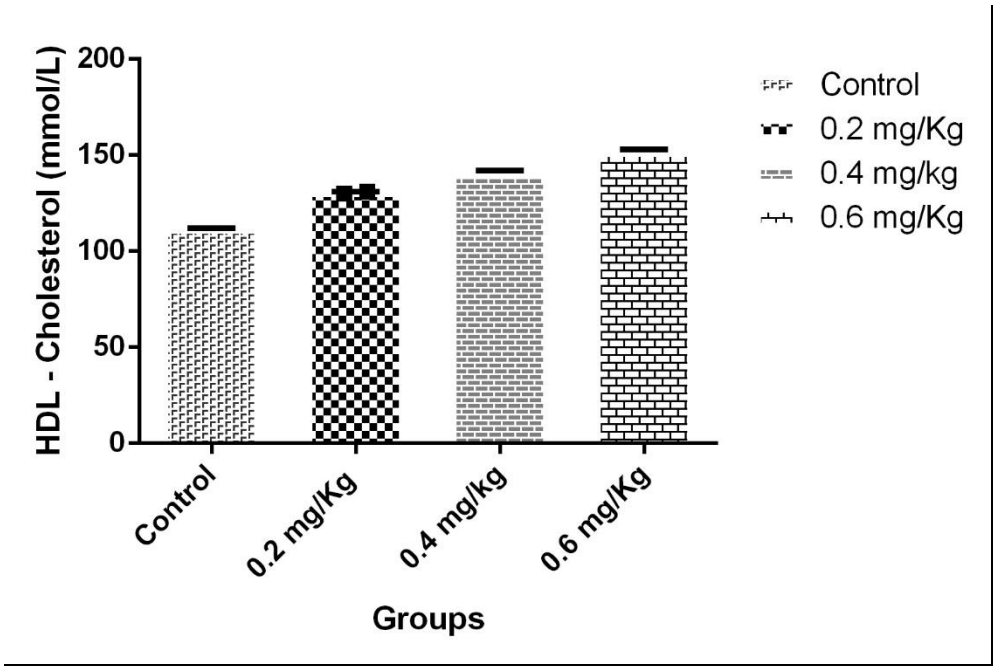

Figure 1: HDL-Cholesterol concentration of rats treated with doses of Folic acid

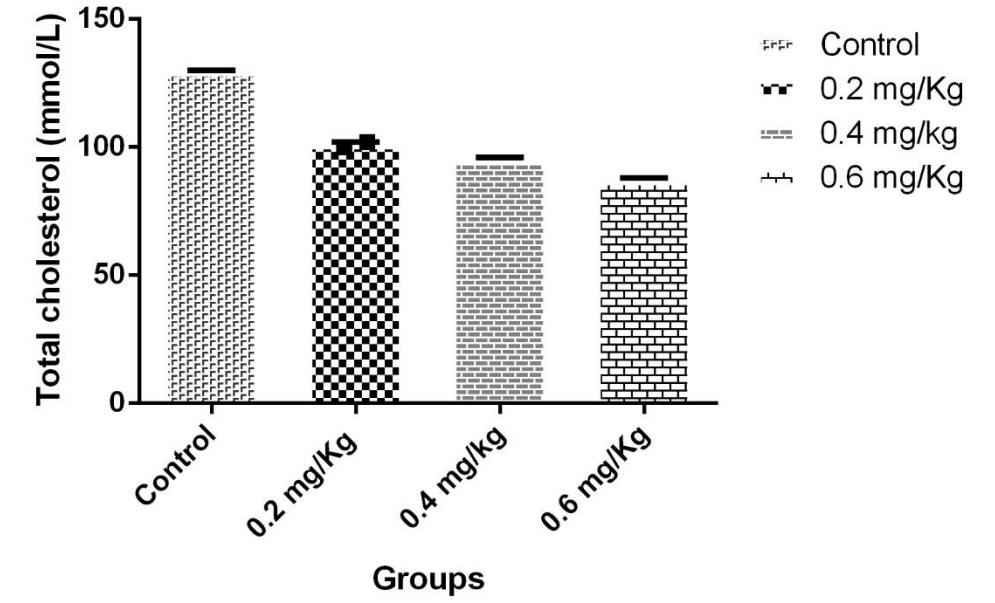

Figure 2: Total Cholesterol concentration of rats treated with doses of Folic acid

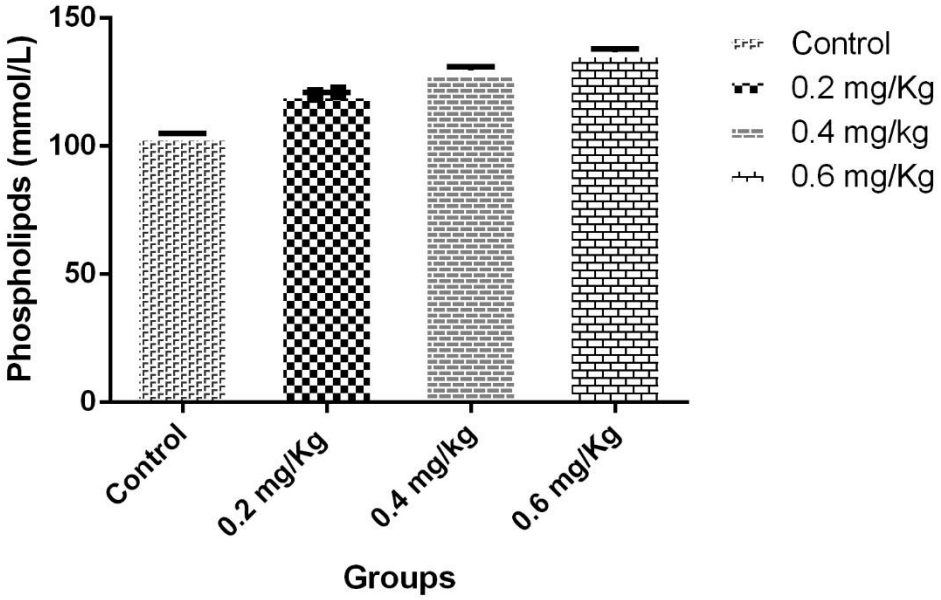

Figure 3: Phospholipids concentration of rats treated with doses of Folic acid

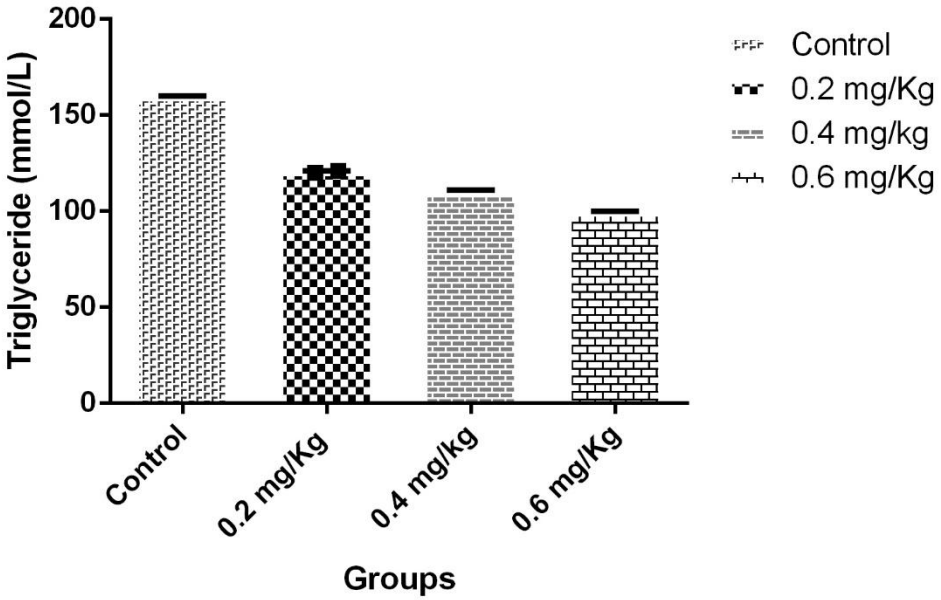

Figure 4: Triglyceride concentration of rats treated with doses of Folic acid

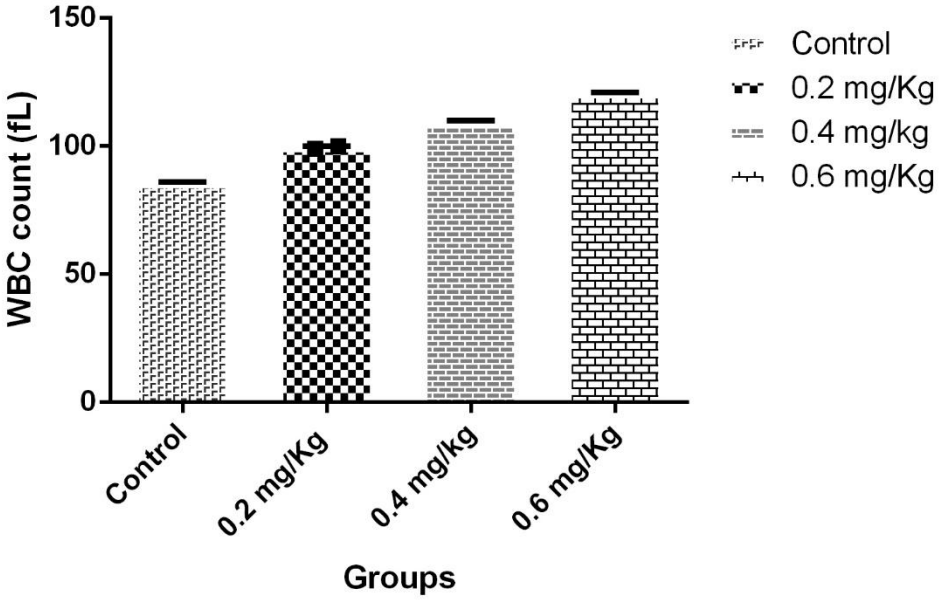

Figure 5: WBC count of rats treated with doses of Folic acid

However, acetyl CoA level was not measured; nevertheless, the hypocholesterolaemia is beneficial as it may help reduce the incidence of arteriosclerosis and hypertension since the two diseased states are associated with high LDLCholesterol (Enas, 1999).

The significant reduction in the serum content of LDL-Cholesterol is understandable since a reduction in Total Cholesterol should normally result in reduction in LDL-Cholesterol. This may be adduced to a possible alteration in the catabolism of VLDL since LDL represents the final stage in the catabolism of LDL (Mayes, 1996). The reduction in LDL following the intake of folic acid is of beneficial effect since numerous epidemiological studies have shown that elevated levels of low-density lipoprotein cholesterol are associated with an increased risk of coronary heart disease (Nelson and Cox , 2000; Woo *et al*., 2002).

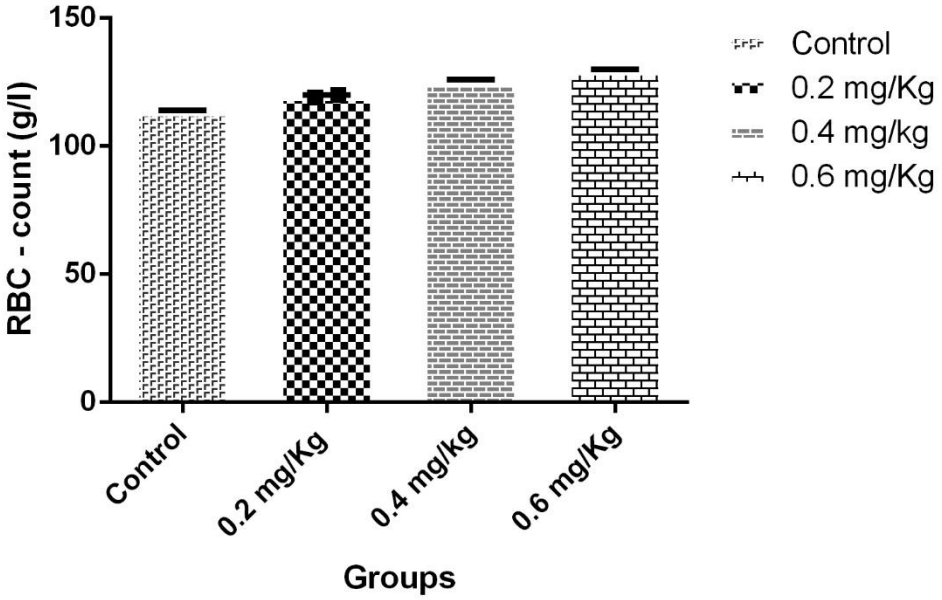

Figure 6: RBC count of rats treated with doses of Folic acid

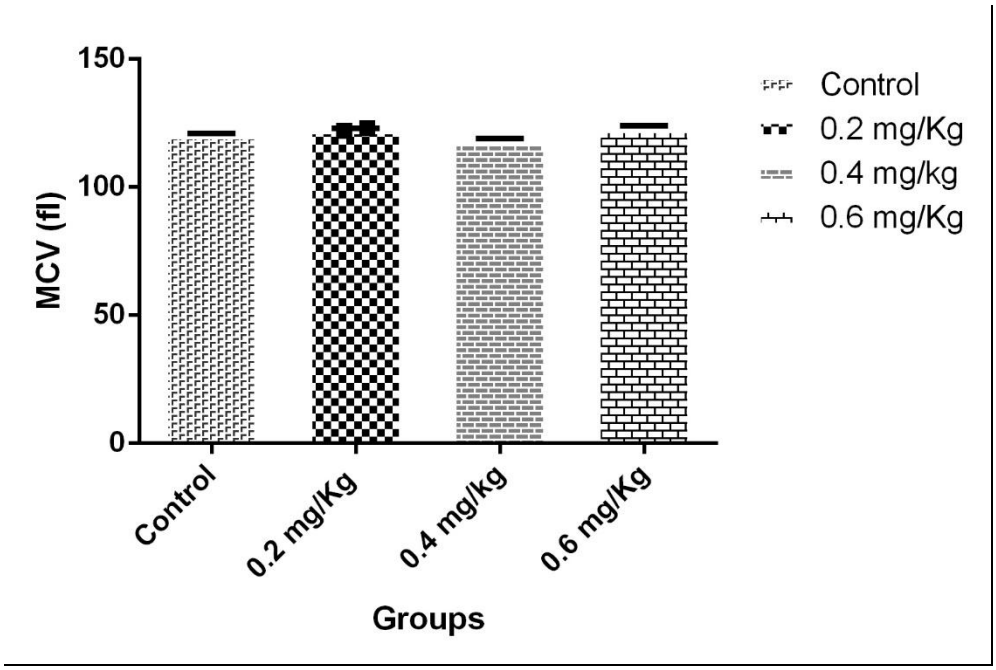

Figure 7: MCV concentration of rats treated with doses of Folic acid

The increase in high density lipoprotein cholesterol (HDL-C), also known as 'good cholesterol' following the intake of folic acid (0.2 mg and 0.4 mg) may also be clinically

beneficial. This view is in consonant with the reported finding where an increase in the concentration of HDL-C correlates inversely with coronary heart disease (Philip, 1995). The biochemical importance of HDL-C is in the fact that it removes cellular cholesterol, transferring it to the liver for excretion (Mayes, 1996).

The significant decrease in triglycerides, (the main storage fatty acids) following the intake of 0.2 mg/Kg, 0.4 mg/Kg, 0.6 mg/Kg folic acid may be adduced to accelerated lipolysis. However, the intake of 0.6 mg folic acid produced a contrasting effect (Figure 4). This might be due to the inhibitory effect on lipolysis.

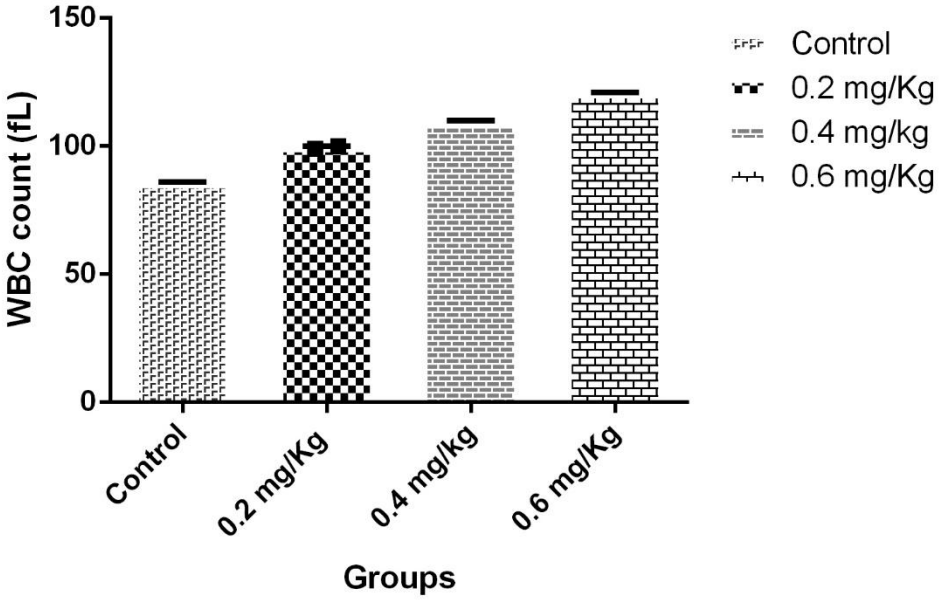

Figure 8: WBC count of rats treated with doses of Folic acid

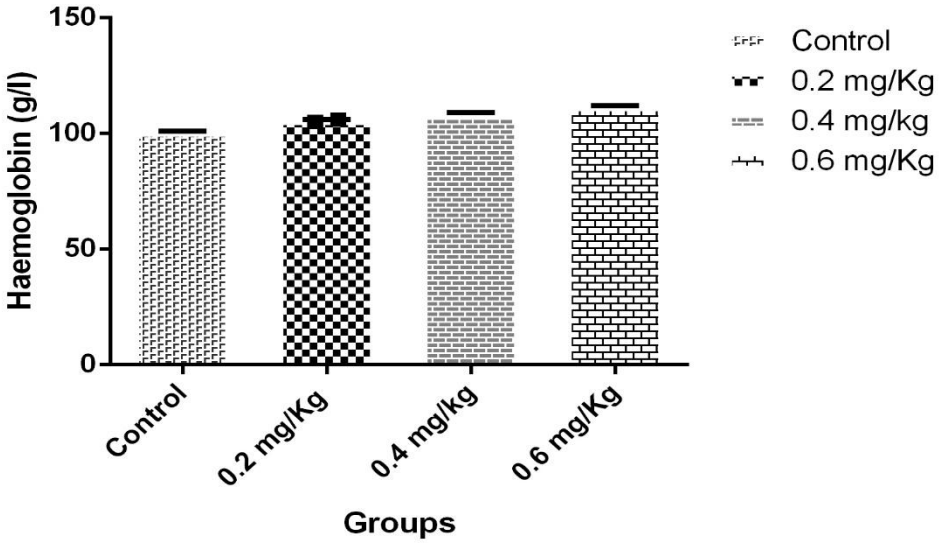

Figure 9: Haemoglobin count of rats treated with doses of Folic acid

Hematological profiles were standardized in the groups of rats treated with compelling dosages of folic acid from the MCV concentration which did not change essentially in the different groups of experimental mice, a typical component of normocytic-normochromic iron deficiency (Menezes et al., 2004).

Although, folic acid supplementation has been found to lower plasma homocysteine level and ultimately reduce the incidence of cardiovascular diseases. The present study shows that folic acid can also reduce one of the risk factors (LDL-C) of cardiovascular diseases through its action on serum lipids. Nevertheless, the clinical application of these findings must await further studies.

CONCLUSION

From this study, it was observed that the administration of Folic acid resulted in an increased serum concentration of HDL-cholesterol and phospholipids and a reduction in the serum level of total cholesterol and triglyceride. High levels of HDL-cholesterol and low levels of total

cholesterol and triglyceride are indications of a good cardiovascular health. It may be suggested that administration of Folic acid should be encouraged as this may help in reducing the rate of accumulation of total cholesterol and triglyceride, which is the two major factors of atherogenicity.

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