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Research Article

Dyslipidemic Molecules and Histology Architecture in Selected Organs of Albino Rat were Altered by Bioactive Compounds of Quail Egg Yolk

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2. Key words: Dyslipidemia; Atherogenic indices; Lipid profile; Quail egg yolk

1. Abstract

The amelioration of dyslipidemia is a pharmacological means of stabilizing the integrity of the cell membrane. The bioactivity of methanolic-ethanolic extract of quail (*Coturnix japonica*) egg yolk on the lipid profile of selected tissues (blood, brain, kidney, pancreas, liver, heart and testes) was investigated in albino rats. Twenty-five (25) albino rats were randomly selected into five groups. Experimental groups 3, 4 and 5 were administered 50 mg/ml, 100mg/ml and 200 mg/ml of extract in olive oil respectively. All treatments were administered orally once a day for twenty-one (21) days. The evaluations were done for lipid profile estimations (High density lipoprotein, total cholesterol and triglyceride, other parameters like very low density lipoprotein, low density lipoprotein and atherogenic indices). The histology of the experimented tissues was carried out. The results confirmed antidyslipidaemic property of the extract; LDL, VLDL, TG and total cholesterol content of the organs in groups 3, 4 and 5 were significantly ($P<0.05$) reduced and dependent on the concentration variation, and competed significantly with the olive oil administered group. The potency to attenuate atherogenicity induced vis-a-vis normal biochemical processes was discovered, to predict the cardiac stimulatory effect of the extract. The result suggested that quail egg yolk consumption has a modulatory effect on lipid profile while 200mg/ml concentration was the most effective for reduction of LDL, total cholesterol (expect for blood), triglyceride (expect for the kidney that showed an increase in TG levels) Quail egg yolk possessed properties that could prevent notable dyslipidaemia related disorders.

3. Introduction

Dyslipidemia can be detected in the body by determining the amount of triglyceride, total cholesterol, high density lipoprotein and the low density lipoprotein or low levels of High-Density Lipoprotein (HDL) cholesterol in the blood and organ tissues. These are referred to as biochemical markers of dyslipidemia [1]. The term 'atherogenic dyslipidemia' denotes a combination of elevated triglycerides and small-dense LDL particles, and low levels of HDL- cholesterol [2]. Dyslipidemia is a risk factor for cardiovascular disease, which is a major contributor to mortality [3]. Heart disease and stroke are usually due to atherosclerosis of large and medium sized arteries. Hypercholesterolemia is the most important factor in the pathogenesis of atherosclerosis. Atherogenic diets have been implicated in atherogenic dyslipidemia and have been identified as modifiable risk factor for heart disease.Eggs contain biologically active compounds which play modulatory roles leading to therapeutic prevention of chronic and infectious diseases. These bioactive compounds possess antimicrobial, immune modulatory, antioxidant, anti-hypertensive and anti-tumor properties. The nutritional value of quail eggs is much higher than those offered by other eggs, as a result of the

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bio-components they are enriched with; antioxidants, minerals and vitamins. The egg has been described as bioactive against physiological intolerance (stress), and the lipid extracts of the yolk has been described to have lipidomics modulation [4,5]. Similar studies also showed the anti-diabetic activity of the quail egg yolk against streptozotocin induced diabetes mellitus [6]. The purpose of this study was to evaluate the dietary and therapeutic effects of the methanolic-ethanolic extracts of quail egg yolk on dyslipidemia in selected organs in albino rat. The study will also justify the contributory role of olive oil used as vehicular agent for the extracts.

4. Materials and Methods

4.1. Materials

4.1.1. Reagents and chemicals: Reagents kits and Chemicals used in this experiment were obtained from different sources such as British Drug House (BDH) and Randox Laboratories Limited and were all of good analytical grades. All the solutions, buffers and reagents were prepared using glass distilled water.

4.1.2. Sample collection and preparation of extract: Extraction of bioactive components involved; approximately 100 g of freeze-dried quail egg yolk was extracted with 500 mL of 80% methanol and 500ml of 80% ethanol (80:20, vol/vol) adjusted to pH 1.5 with 1 mol/L HCl. The samples were then mixed thoroughly using a vortex mixer for 2 min and centrifuged at 6000*g* for 10 min at 4°C. The supernatant was collected, freeze-dried, and stored at 4°C.

4.1.3. Treatment of Experimental Animals: Adult male Wistar albino rats, weighing 210-230 g were received from experimental Animal Care Centre (University of Ilorin, Kwara State. Nigeria). All animals were maintained under controlled conditions of temperature (22 ± 1°C), humidity (50-55%) and light (12 h light/12 h dark cycle). They were acclimatized to the laboratory conditions for 14 days before the start of the experiment. Animals had free access to rat chow and drinking water. All experimental procedure including euthanasia was conducted in accordance with the Ethical Regulation and Guide for the Care and Use of Laboratory Animals. There were 5 groups of 5 rats each. Group 1 (control) was fed normal diet and water (food and water *ad libitum*) only, group 2 (reference) was administered olive oil while groups 3 to 5 were administered varying dosage (50, 100 and 150mg/kg body weight) of ethanolic-methanolic extracts. All administrations were done orally at interval for 21 days and olive oil was used as the vehicle. At the end of the experiment, the animals were sacrificed by cervical dislocation.

Group 1: C- Control group (normal diet and water)

Group2: CREF- Reference group (0.4ml Olive oil, feed and water)

Group3: QEY1- Quail egg yolk extract administered group (50mg/ml of extract in olive oil: 4ml/kg BWT),

Group4: QEY2-Quail egg yolk extract administered group (100mg/ml of extract in olive oil: 4ml/kg BWT), and

Group5: QEY3- Quail egg yolk extract administered group (150mg/ml of extract in olive oil: 4ml/kg BWT).

4.1.4. Preparation of Serum and Tissue Homogenates

Rats were anaesthetized by cervical dislocation. The rats were dissected and blood samples were collected through cardiac punctures into clean plain bottles. Serum was prepared by aspiration of the clear yellowish liquid after clotting and centrifuged at 3000rpm for 15 minutes at 250 C in a bench centrifuge. The liver, kidney, pancreas, brain, testes and heart were quickly excised, washed in ice cold 1.15% potassium chloride solution, blotted with filter paper and weighed. Half of the weights were then chopped into bits separately and homogenized in five volumes of the homogenizing ice cold sodium phosphate buffer (5% w/v, pH 7.4) using a Teflon homogenizer. The resulting homogenates were centrifuge at 3000 for 15 minutes at 4°C. The supernatant was stored at 4°C and then used for biochemical analysis. The remaining half were stained and evaluated for possible tissue lesions.

4.2. Biochemical Estimation

Lipid Profile Test

4.2.1. Determination of total cholesterol concentration: The cholesterol was determined according to the principle described in Randox kit manual. 1ml of the reacting mixture containing 4-aminoantipyrine, phenol, peroxidase, cholesterol esterase, cholesterol oxidase and 80mM Pipes buffer pH 6.8 was mixed with 10µl of plasma and incubated for 5 min at 37°C. The absorbance at 546 nm was then taken against the reagent blank within 60min. The concentration of cholesterol in the sample was subsequently calculated against a standard.

4.2.2. Determination of triglyceride concentration: The triglyceride concentration was determined using colorimetric method as described in Randox kit manual. Briefly, 10 µl of the sample was mixed with 1 ml of Pipes reagent (40 mM phosphatebuffer, 5.5 mM 4-chlorophenol and 17.5 mM mg^{2+}) and enzyme reagent (4-aminophenazone, adenosinetriphosphate, lipase, glycerol kinase, glycerol-3-phosphate oxidase and peroxidase). Thereafter the mixture was incubated for 5 min at 37°C and the absorbance at 546 nm was taken against reagent blank within 60 min. The

triglyceride concentration was subsequently calculated against the standard.

4.2.3. Determination of HDL-cholesterol concentration: The precipitation was carried out according to the method described in the Randox kit manual. Briefly, 200 µl of plasma was mixed with 500 µl of the precipitant (0.55mM phosphotungstic acid and 25mM magnesium chloride) and allowed to sit for 10 min at room temperature. Then, the mixture was centrifuged for 10 min at 800 × *g*. Thereafter, the clear supernatant was separated off and subjected to the same procedure for the determination of cholesterol described above.

4.2.4. Determination of LDL-cholesterol concentration: The LDL-cholesterol concentration of the plasma samples was determined according to the equation in Randox kit manual.

LDL Cholesterol (mg/dl) = Total Cholesterol – Triglycerides/5 – HDL Cholesterol

Determination of VLDL-cholesterol concentration: Very-Low Density Lipoprotein (VLDL) concentration of the plasma was calculated Friedewald's equation [7]. VLDL (mg/dl) = Triglycerides (TG)/5

4.2.5. Determination of Atherogenic Indices

Determination of coronary risk index (CRI)

Coronary Risk Index (CRI) or Cardio Risk Ratio (CRR)is the ratio of Total Cholesterol (TCHL) to High Density Lipoprotein (HDLc). That is CRI or CRR = TCHL/HDLc[8].

Determination of atherogenic coefficient (AC)

Atherogenic coefficient (AC) is the ratio of differences between Total cholesterol and High Density Lipoprotein-cholesterol to High Density Lipoprotein-cholesterol.Atherogenic coefficient (AC) = (TCHL – HDLc)/HDLc[9].

Determination of atherogenic index of plasma (AIP)

AIP is a logarithmically transformed ratio of molar concentrations of triglycerides to HDL-cholesterol.

$\text{Log}_{10}^{(TG/HDLc)}$ [10].All values are expressed as mean ±SD. Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using SPSS 17.0 for windows. The significance level was set at $p<0.05$.

5. Results

(Figure 1) showed the various level of triglyceride in different organs after treatment with quail egg yolk extract of different concentrations. When compared with the negative control group it was evident that the extract significantly ($p<0.05$) reduced the level of triglyceride in all the organs especially at 200mg/ml concentration. The total cholesterol, low density lipoprotein level and all atherogenic indices that were calculated were also reduced significantly ($p<0.05$) in all the organs of the rats administered with quail egg yolk extract the reduction observed here is seen to be proportional to the concentration administered. (Figure 1) revealed the effect of the yolk extract on the triglyceride biosynthesis in the tissues. There was no significant difference ($p<0.05$) in the triglyceride concentrations among the groups when the blood, brain and testes were studied. The Figure revealed significantly highest ($p<0.05$) value for the CREF groups, there was no significant difference ($p<0.05$) among the other groups. CREF had a triglyceride that was least in the blood, brain and kidney compared to the other groups treated with yolk ext ract reconstituted in olive oil. There was no significant difference ($p<0.05$) among the yolk extract administered groups.

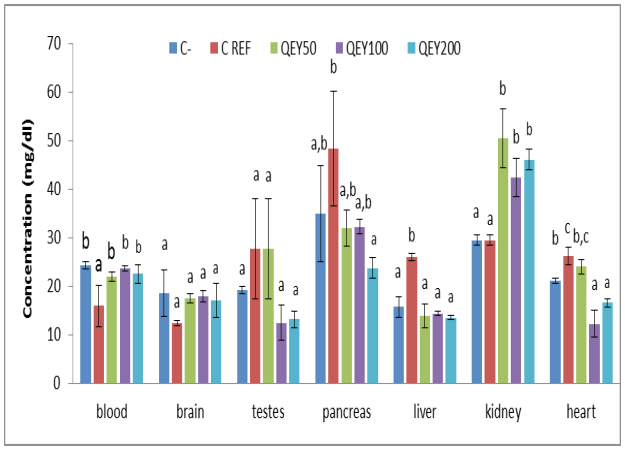


Figure 1: Effect of *Cortunix japonica* extract on triglyceride levels in different organs of albimo rats.

(Figure 2) showed the effect of quail egg yolk extract on the total cholesterol biosynthesis in the tissues. There was a consistent decrease in the cholesterol concentration with increase in the concentration of yolk extract, however, there was no significant difference between QEY100 and QEY200 ($p<0.05$).

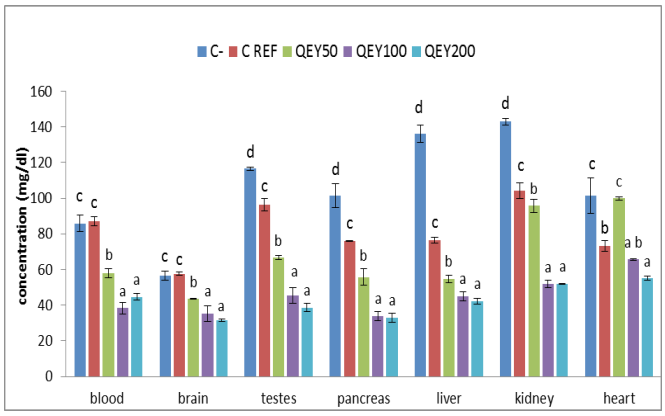


Figure 2: Effect of *Cortunix japonica* extract on total cholesterol levels in different organs of albino rats.

(Figure 3) revealed the LDL concentrations in the different experimental tissues by quail egg yolk extract and olive oil. Among the extract administered groups, there was a significant decrease ($p<0.05$) in the LDL concentration as the extract concentration increases. The olive oil extract exhibited a contradicting effect on the heart, kidney, liver and pancreas compared to its effect in previously discussed results, there was no significant difference ($p<0.05$) between the olive oil group and the QEY50.

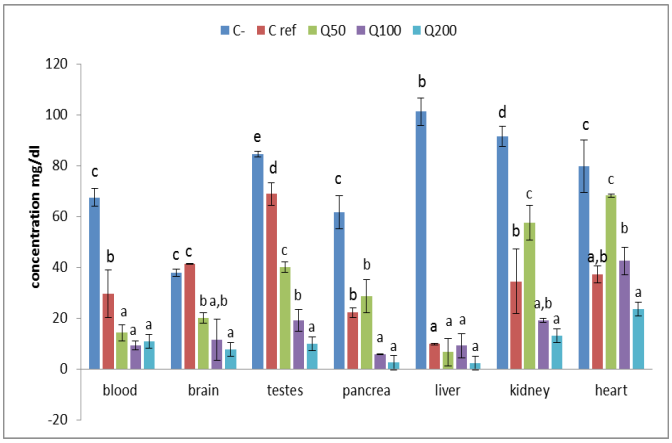


Figure 3: Effect of *Cortunix japonica extract* on low density lipoprotein levels in different organs of albino rats.

(Figure 4) revealed the effect of the extract on HDL biosynthesis and availability in the tissues. The result revealed an increase with increase in extract concentration in testes, brain and pancreas, but the variance was not significant among the extract groups. There was an observable fall in HDL concentration from QEY50 to QEY100 and insignificant ($p<0.05$) rise in QEY200 in the blood, liver, kidney and heart.

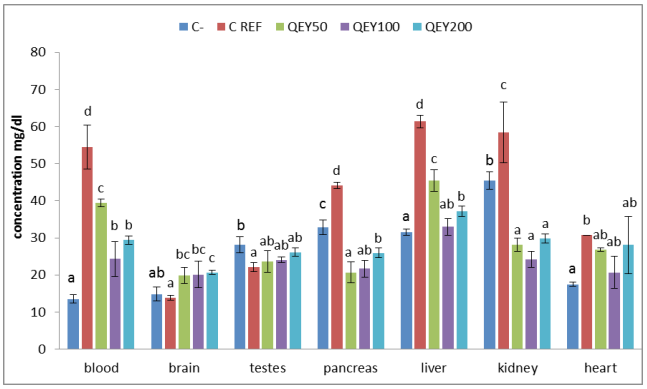


Figure 4: Effect of *Cortunix japonica* extract on high density lipoprotein levels in different organs of albino rats.

(Figure 5) showed the atherogenic indices. There was no significant difference ($p<0.05$) in the CRI and ACI values among the groups. API values for all the treated groups gave negative values, which is more negative with CREF and reduced negativity significantly ($p<0.05$) as the concentration of yolk extract increases.

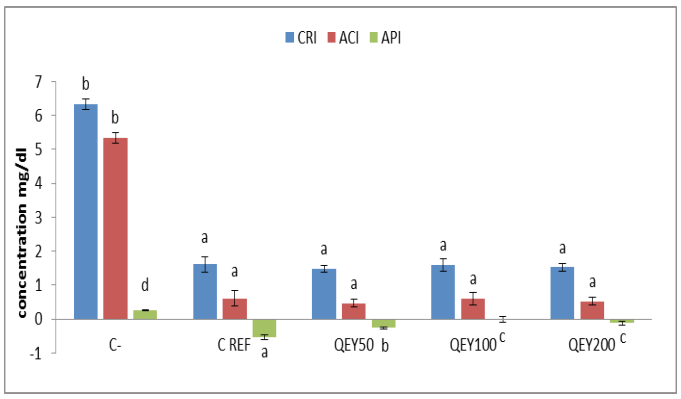
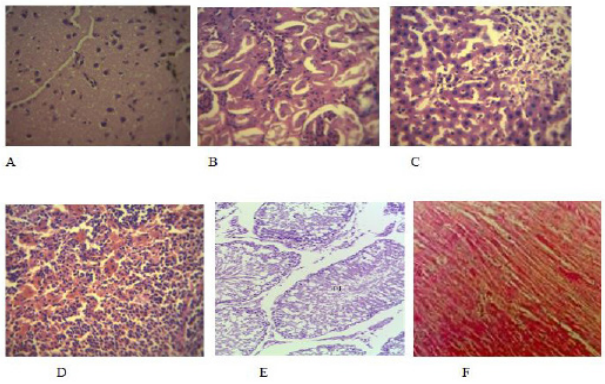


Figure 5: Effect of Cortunix japonica extract on Atherogenic indices of different organs of albino rats.

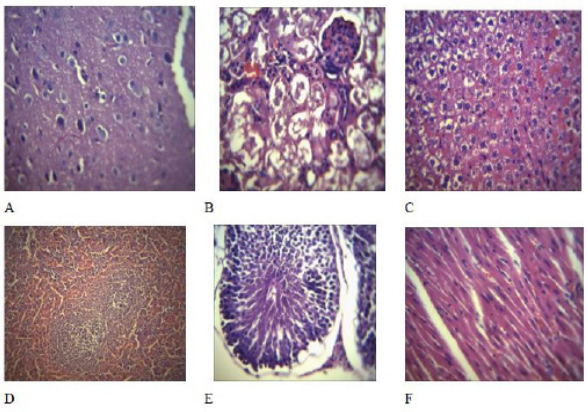
Keys: CRI-coronary risk index
AC- atherogenic coefficient
AIP- atherogenic index

(Figure 6) Histology of tissues A, B, C, D, E and F representing brain (No visible lesions seen), kidney (No visible lesions seen), liver (No visible lesions seen), pancreas (No visible lesions seen), testes (No visible lesions seen) and heart (No visible lesions seen) respectively of Group 1 (C); Control group.



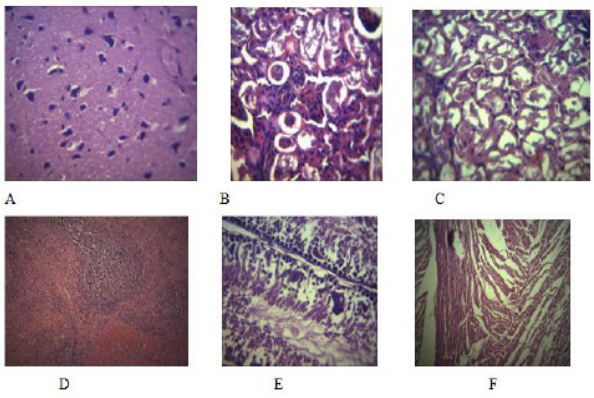
Figures 6: Histology of tissues A, B, C, D, E and F representing brain (No visible lesions seen), kidney (No visible lesions seen), liver (No visible lesions seen), pancreas (No visible lesions seen), testes (No visible lesions seen) and heart (No visible lesions seen) respectively of Group 1 (C); Control group

(Figure 7) Histology of tissues A, B, C, D, E and F representing brain (No visible lesions seen), kidney (There is a severe congestion of the renal interstitium. There are copious amount of proteinaceous material in the lumen of the renal tubules), liver (There is a severe vacuolar degeneration of the hepatocytes. There are few foci of severe congestion of the sinusoids), pancreas (No visible lesions seen), testes (No visible lesions seen) and heart (No visible lesions seen) respectively of Group 2 (CREF); Control Reference group.



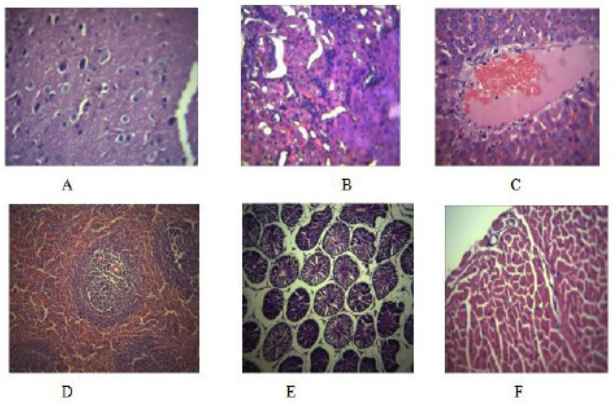
Figures 7: Histology of tissues A, B, C, D, E and F representing brain (No visible lesions seen), kidney (There is a severe congestion of the renal interstitium. There are copious amount of proteinaceous material in the lumen of the renal tubules), liver (There is a severe vacuolar degeneration of the hepatocytes. There are few foci of severe congestion of the sinusoids), pancreas (No visible lesions seen), testes (No visible lesions seen) and heart (No visible lesions seen) respectively of Group 2 (CREF); Control Reference group

(Figure 8) Histology of tissues A, B, C, D, E and F representing brain (no visible lesion seen), kidney (There is a mild to moderate congestion of the renal cortex. Several tubules have protein casts in their lumen), liver (There is a severe diffuse vacuolar degeneration of hepatocytes), pancreas (There is a moderate to severe portal congestion), testes (No visible lesions seen. Sertoli cell outlines are very prominent) and heart (No visible lesions seen) respectively of Group 3 (QEY1).



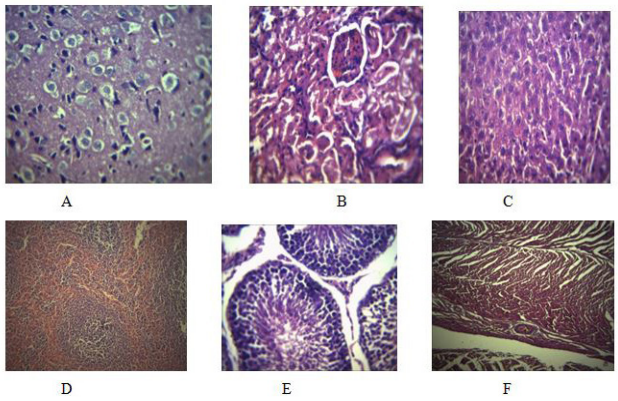
Figures 8: Histology of tissues A, B, C, D, E and F representing brain (no visible lesion seen), kidney (There is a mild to moderate congestion of the renal cortex. Several tubules have protein casts in their lumen), liver (There is a severe diffuse vacuolar degeneration of hepatocytes), pancreas (There is a moderate to severe portal congestion), testes (No visible lesions seen. Sertoli cell outlines are very prominent) and heart (No visible lesions seen) respectively of Group 3 (QEY1)

(Figure 9) Histology of tissues A, B, C, D, E and F representing brain (no visible lesion seen), kidney (There was a mild to moderate congestion of the renal cortex. The glomeruli appear shrunken), liver (There is a mild to moderate portal and central venous congestion), pancreas (The red pulp appears expanded and more prominent), testes (No visible lesions seen) and heart (No visible lesions seen) respectively of Group 4 (QEY2).



Figures 9: Histology of tissues A, B, C, D, E and F representing brain (no visible lesion seen), kidney (There was a mild to moderate congestion of the renal cortex. The glomeruli appear shrunken), liver (There is a mild to moderate portal and central venous congestion), pancreas (The red pulp appears expanded and more prominent), testes (No visible lesions seen) and heart (No visible lesions seen) respectively of Group 4 (QEY2)

(Figure 10) Histology of tissues A, B, C, D, E and F representing brain (There is a mild diffuse spongiosis of the parenchyma. Few neuronal cells are degenerate), kidney (no visible lesion seen), liver (No visible lesions seen), pancreas (No visible lesions seen), testes (No visible lesions seen) and heart (No visible lesions seen) respectively of Group 5 (QEY3).



Figures 10: Histology of tissues A, B, C, D, E and F representing brain (There is a mild diffuse spongiosis of the parenchyma. Few neuronal cells are degenerate), kidney (no visible lesion seen), liver (No visible lesions seen), pancreas (No visible lesions seen), testes (No visible lesions seen) and heart (No visible lesions seen) respectively of Group 5 (QEY3)

6. Discussion

Measurement of major lipids like cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides can give useful information about the functioning of the body system [11]. High concentrations of all lipids except the HDL-C are associated with an increased risk of atherosclerosis, fatty liver and ischemia which could lead to different failure or reduced function. High levels of triglycerides and LDLs are also associated with cardiovascular disease. In this study, *Coturnix japonica* egg was investigated for possible attenuation of dyslipidemia, induced vis-à-vis metabolic processes. A varying concen-

tration of 50-200mg/ml body weight of the methanolic-ethanolic given to the albino rats and its effect monitored in reference to the olive oil used in re-constituting the extract. The aim of monitoring with reference to olive oil is to acknowledge the contribution of olive oil as a drug carrier. Although olive oil is highly potent oil as a result of the considerable amount of omega-3 PUFA, the activity of the extract was revealed in concentration dependent order, revealing the activity which was synergistic with olive oil. The reduction in the total cholesterol levels in all organs following the administration of the extract and olive oil may be attributed to its transportation to the liver metabolic moiety, where it is metabolised to acetyl CoA, since acetyl CoA is a key substrate in the metabolism of cholesterol. The ability of the extract to increase the HDL-c confirmed the anti-hypercholesterolemic activities of the extract at the experimented concentrations. The increase in the concentration of HDL-c in circulatory system and the tissues is a corresponding decrease in the concentration of cholesterol present in those tissues and a marker for reduced dyslipidemia, including the atherogenic indices. The ability to reduce cholesterol vis-à-vis is a positive marker for the extract to attenuate myopathic problems that could occur in these tissues as a result of deregulated cholesterol. Triglycerides are the main storage form of fatty acids. The reduction observed in the triglyceride level of some organs at different concentration can be adduced to inhibition of lipolysis. The significant reduction in total cholesterol and triglyceride levels following the administration of the extracts of *Coturnix japonica* is an indication that the extracts possess anti-dyslipidemic properties and this may help reduce the incidence of cardiovascular diseases like atherosclerosis and hypertension also the incidence of other dyslipidemic related diseases. A similar result was also observed in the study of the effect of Lipid extracts of ginger rhizome on the blood and liver lipid profile [12]. LDL-C is referred to as "bad" cholesterol because it builds up slowly in the walls of arteries feeding the heart, brain and other organs. As a result of this, it forms plague that clots the arteries thereby causing atherosclerosis and increasing the risk of high blood pressure or organ failure (Jackson, 1986). The significant reduction in the LDL-cholesterol is understandable since a reduction in total cholesterol should normally result in reduction of LDL-cholesterol because LDL is needed to transport cholesterol to different extrahepatic tissues. This may be adduced to a possible alteration in the catabolism of VLDL-C (very low density lipoproteins) (Mayes, 1996). Therefore, the reduction in LDL-cholesterol following oral administration of the egg yolk extract of quail had a beneficial effect since it will help to reduce the risk of coronary heart disease. Result showed a significant ($p<0.05$) decrease in LDL-C with increasing concentration of the extract. The reference group also showed a higher concentration of LDL than the experimented groups or administered quail egg groups. VLDL-C concentration in the organs were reduced at different concentrations when compared with the negative control group except for the kidney that showed no reduction in the VLDL-c level at any concentration, the reason for which is unknown. HDL-cholesterol is considered to be anti-atherogenic markers, since there is negative correlation between HDL-cholesterol and risk of cardiovascular disease. HDL-C transports cholesterol from peripheral tissues to the liver where it is converted to bile acids and eventually excreted from the body, thereby reducing the amount stored in the tissue and decreasing the likelihood of getting atherosclerotic [13] or dyslipidemic plagues. The increase in high density lipoprotein cholesterol observed in the blood, brain, heart and liver following the administration of extract of quail egg yolk can be clinically beneficial. It has been demonstrated that an increase in the concentration of HDL-C correlates inversely with coronary heart disease, also it has been estimated that for any 1.0mg/dl (0.026mm0l/ml) increase in HDL-c, there is a 3% decrease in risk of coronary artery disease and a 4.7% decrease in the risk of mortality from cardiovascular disease [14]. A significantly ($p<0.05$) lower value of HDL-c was observed for experimental groups for the following organs; testes, kidney and pancreas when compared to the negative control group meaning that the extract lowered the HDL concentration in this organs. This could be to facilitate effective cholesterol clearance from these organs, thereby modulating dyslipidemia in the organs in-view. These findings could be confirmed by LDL concentration observed after administration, which was obviously low significantly and correlates with concentration dependently.

The ability of the sample to generally reduce the total cholesterol level and triglyceride level brought about a marked decreased in the coronary risk index, atherogenic coefficient and atherogenic index. The Atherogenic index of plasma which is a mathematical relationship between TG and HDL-c has been successfully used as an additional risk index when assessing Cardio Vascular (CV) risk factors [10,15]. Indeed, it has been suggested that AIP values of -0.3 to 0.1 are associated with low, 0.1- 0.24 with medium and above 0.24 with CV high risk [13]. Based on this study it was shown that the negative control group had AIP of 0.25 which is an indication of high CV risks factor, but after the induction of the extract a great decrease was observed in the AIP of all the groups and the values falling between the low risk CV factors. The reference group also had an AIP value of -0.5 which poses no risk at all. Coronary risk index and Atherogenic coefficient are also important assessment in the risk of cardiovascular disease a significant ($p<0.05$) lower value of this factors were observed after the administration of the extract when compared with the control negative.

7. Conclusion

Everyday activities coupled with diet are major determinants of the metabolic stakes of human as well as animal. These diets and activities alter the metabolic processes and could elevate cholesterol, triglyceride, LDL-c and VLDL-c vis-à-vis. The present study revealed that *Cortunix japonica* egg yolk has a beneficial effect on lipid modulation in the organ-system and consequently, attenuates myopathies and pathologies that could evolve from dyslipidemia as a complication.

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