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(RESEARCH ARTICLE)

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The Biochemical and histological effects of Kola Nitida on the pancreas biomarkers of lipid induced Wistar Rats

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Abstract

This study estimates the biochemical and histological effects of Kola Nitida on the Pancreas biomarkers of Wistar rats subjected to a high lipid diet. High lipid diet is known to induce oxidative stress, inflammation, and pancreatic dysfunction. This study aimed to evaluate whether Kola Nitida, known for its bioactive compounds such flavonoids, caffeine, and theobromine, could protect the pancreas from lipid induced damage. The study was conducted within period of 6 months (April – September 2024). A total of 21 Male Wistar rats were purchased for this study. They were divided into five groups: Control group, Toxicity group, and three Kola Nitida-treated groups (100, 200, 300 mg/kg). After categorization of study animals, blood samples were drawn through cardiac puncture into Lithium heparin bottles using standard antiseptic techniques. Five millitres (5mls) of fasting blood samples collected from each group into Lithium heparin bottles was spun at 3500rpm for 5minutes to obtain plasma. Total cholesterol and Triglycerides were assayed based on enzymatic methods. High density Lipoprotein was assayed using precipitation and enzymatic methods, while Low density Lipoprotein (LDL) was calculated using Friedwald equation. The atherogenic indices; Castelli's 1, Castelli's 2 and Atherogenic Coefficient was calculated with their respective formulas. Additionally, histopathological evaluations were performed to observe structural changes in the pancreas using H&E. The results showed that the rats treated with Kola Nitida exhibited reduced and less fat deposition in the pancreas compared to the toxicity group only. Furthermore, pancreatic B-cell function was better and preserved in the Kola Nitida treated Wistar rats. The ANOVA results from the comparisons of the mean values obtained showed statistically significant difference in the Total cholesterol, Triglycerides, Low Density Lipoprotein and High-Density Lipoprotein of the Toxicity group, 100mg/kg, 200mg/kg and 300mg/kg compared with the control group. In conclusion this study clearly demonstrates that the administration of a high lipid diet and subsequent reversal with Kola Nitida induces statistically significant changes in the biological and histological parameters under investigation.

Keyword: Hyperlipidemia; Kola-Nitida; Pancreas; Histopathological; Castelli 1; Toxicity; Triglycerides; Total Cholesterol**.**

1. Introduction

Hyperlipidemia is a common lipid disorder characterized by elevated levels of lipids in the blood, including cholesterol and triglycerides. (1). It is a major risk factor for cardiovascular diseases, such as atherosclerosis, coronary artery disease, and stroke. (6)

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Metabolic disorders, including obesity and hyperlipidemia, are growing health concerns worldwide (6) Identifying dyslipidemia requires knowledge of the normal distribution of blood lipids in the population. (4). The prevalence of hyperlipidemia varies globally, with higher rates observed in developed countries. (5)

Kola nut, the seed of the kola tree (Cola spp.), is known for its stimulating effects and is a rich source of various bioactive compounds, including caffeine and polyphenols. (2). Caffeine is one of the most well-known components of kola nut and is responsible for its stimulant properties. Kola nut typically contains a higher concentration of caffeine compared to coffee beans, making it a potent stimulant (3).

2. Materials and methods

2.1. Study area

Study was carried out in the Department of Medical Laboratory Science, Joseph Ayo Babalola University, Ikeji- Arakeji, Osun State

2.2. Ethical approval

Ethical considerations were received from the College of Health Science Research and Ethical Committee, Joseph Ayo Babalola University Osun state, Nigeria, and ensured in accordance with the guidelines and regulations approved for the use care of rats.

2.3. Scope of experimental design

This research is a controlled experiment. From the laboratory animal house of Joseph Ayo Babalola University, 20 male Wistar rats were purchased. The animals were fed with a high lipid diet.

2.4. Control group

This group consisted of four (4) Wistar rats that were fed with normal diet.

2.5. Toxicity group

This group consisted of four (4) Wistar rats that were fed with a high lipid diet.

2.6. Experimental group

This group consisted of fifteen (15) Wistar rats that were divided into groups of 100mg/kg, 200mg/kg and 300mg/kg.

2.7. Exclusion criteria

Wistar rats with underlying illness and other conditions were not used for the study.

2.8. Specimen collection and processing

Blood samples were collected from all animals before and halfway to the experiment from the marginal ear vein using a 2ml needle and syringe to determine lipid profile of all animals with a lipid panel diagnostic kit (Randox test kit), which serve as a pointer to whether hypercholesterolemia was induced before and sustained during experimentation. In the end, all animals fasted all-night and was sacrificed by cervical dislocation. The blood samples were put into lithium heparin tubes and centrifuged at 10,000rpm for 5 minutes.

2.9. Statistical analyses

For statistical analysis, data were analyzed by both one-way (for weight analysis) and two-way analysis of variance (ANOVA) (acetaminophen consumption analysis) using Graph Pad Prism (version 9.5.1) software. The results were expressed as mean standard deviation and Statistical significance was considered at a 95% confidence interval ($P<0.05$)

3. Results and discussion

The ANOVA results from the comparisons of the mean values obtained showed statistically significant difference in the Total cholesterol, Triglycerides, Low Density Lipoprotein and High-Density Lipoprotein of the Toxicity group,

100mg/kg, 200mg/kg and 300mg/kg compared with the control group, with a decrease in the lipid parameters in the Kola-Nitida treated groups.

The atherogenic indices as well as the average weight of the animals and the pancreas were decreased in the Kola-Nitida treated group as of when compared to the control.

Table 1 Results (Mean±SD) of Lipid Parameters Treated with Varying Dose of Bitter Kola following treatment with HFD

Parameters	Group 1 (100mg/kg)	Group 2 (200mg/kg)	Group 3 (300mg/kg)	Positive Control (Toxicity)	Negative control	F value	P value	Remark
TC (mmol/L)	2.02±0.08^{a}	1.50±0.02^{b}	2.27±0.07^{c}	2.90±0.01^{d}	1.33±0.04^{e}	478.8	<0.0001	S
TG (mmol/L)	0.96±0.12^{a}	0.75±0.06^{a}	0.87±0.205^{a}	1.21±0.03^{b}	0.34±0.05^{c}	24.36	<0.0001	S
HDL(mmol/L)	0.63±0.07^{a}	0.86±0.06^{b}	0.75±0.057^{c}	0.50±0.02^{a}	1.20±0.10^{d}	49.02	<0.0001	S
LDL (mmol/L)	1.30±0.26^{a}	0.58±0.04^{b}	0.65±0.09^{c}	1.41±0.03^{a}	0.31±0.03^{d}	42.64	<0.0001	S

Keys: TC=Total Cholesterol, TG= Triglyceride, HDL= High Density Lipoprotein, LDL=Low Density Lipoprotein, ; Post Hoc (Tukey's): Values in the row with different superscripts differ significantly at $p<0.05$.

Table 2 Results (Mean±SD) of Atherogenic Indices and weight of Pancreas in Treated with Varying Dose of Bitter Kola following. Treatment with HFD

Parameters	Group 1 (100mg/kg)	Group 2 (200mg/kg)	Group 3 (300mg/kg)	Positive Control (Toxicity)	Negative control	F value	P value	Remark
Castelli 1	3.13±0.31^{a}	1.70±0.10^{b}	2.57±0.25^{c}	5.70±0.17^{d}	1.07±0.15^{e}	217.9	<0.0001	S
Castelli 2	2.07±0.59^{a}	0.68±0.07^{b}	0.85±0.15^{c}	2.77±0.06^{d}	0.26±0.04^{e}	44.22	<0.0001	S
AC	2.13±0.31^{a}	0.74±0.12^{b}	2.02±0.25^{a}	4.70±0.17^{c}	0.17±0.07^{d}	226.3	<0.0001	S
A. Weight (grams)	0.64±0.04^{a}	0.51±0.02^{b}	0.52±0.06^{b}	0.80±0.02^{c}	0.33±0.06^{d}	67.40	<0.0001	S
R. Weight ($x10^{-3}$) (grams)	5.30±0.29^{a}	3.67±0.15^{b}	3.53±0.31^{b}	5.600±0.08^{a}	2.53±0.44^{c}	81.95	<0.0001	S

Keys: AC= Atherogenic Coefficient, A. Weight= Absolute weight of Organ, R. weight =Relative weight of organ.; Post Hoc (Tukey's): Values in the row with different superscripts differ significantly at $p<0.05$.

3.1. Results of Histology Examination

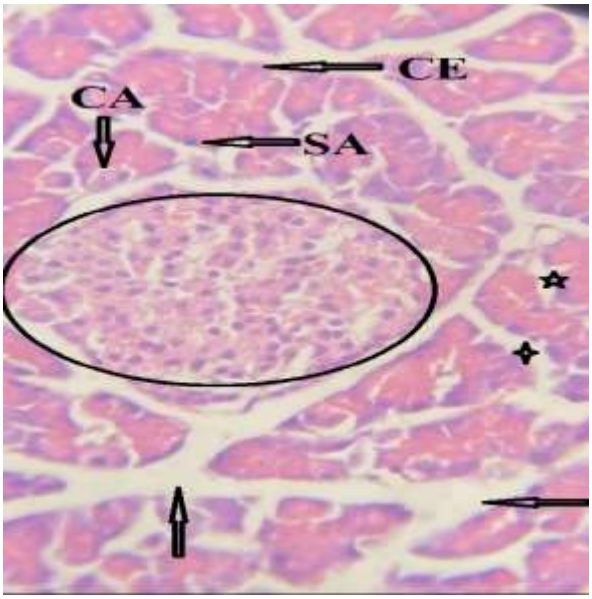

Figure 1 H& E stain of pancreatic tissue (x400). Treatment: 0.00mg/kg. Circle area indicate the islet of Langerhans showing normal blue staining basophilic cells. Arrows (without labels): Indicating the Interlobular ducts while the "stars" indicating the intralobular ducts. The photomicrograph also indicated the presence of pale staining cuboidal epithelial cells (CE) limning the interlobular duct. Also indicated is the serous acini cell (SA) and the Centro acini cells (CA). Inference. A Normal Pancreatic Tissue – Negative control Group.

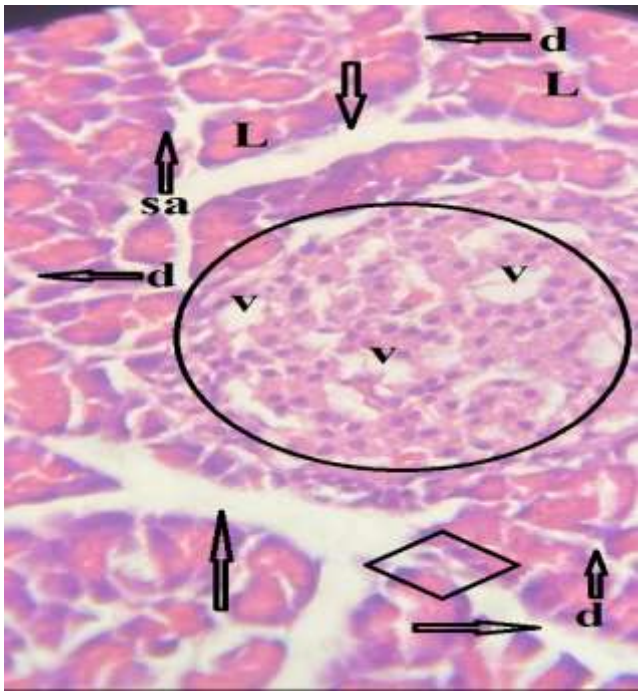

Figure 2 H& E stain of pancreatic tissue (x400). Treatment: 200mg/kg and 300mg/kg Bitter Kola after HFD. Circle area indicates the islet of Langerhans with vacuolation showing normal blue staining basophilic cells. Arrows (without labels): Indicating the Interlobular ducts while "d" indicating the intralobular ducts. The photomicrograph also indicated the presence of poor staining serous acini (SA) cells along the periphery of the lobule (L). Rhombus shaped structure shows infiltration of pancreatic parenchymal materials into the interlobular ducts. Inference: A normal pancreatic tissue recovering from insult–Mild Pancreatitis - Group 3 and Group 2 Rats. HFD= High Fat Diet.

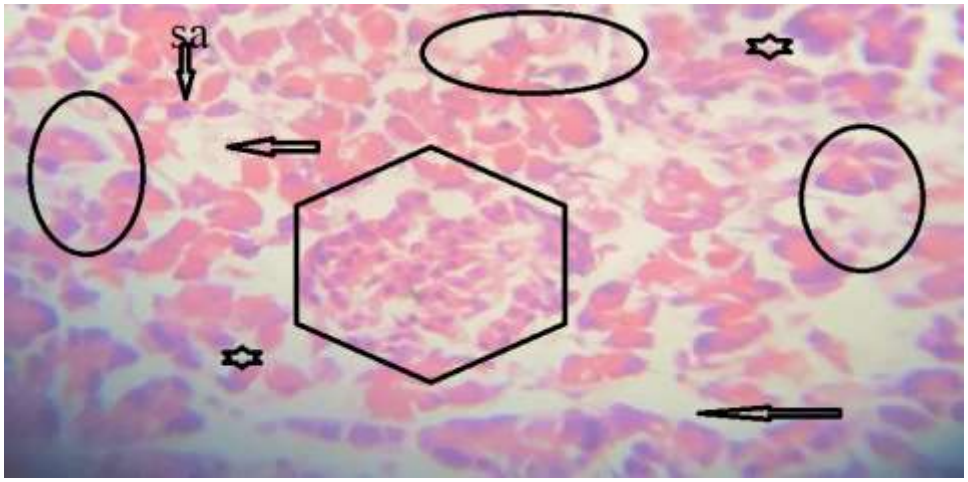

Figure 3 H& E stain of pancreatic tissue (x400). Treatment: 100mg/kg of Bitter Kola after HFD. Circle areas indicate loss of parenchymal materials and grossly distorted lobular structures. Hexagonal region shows grossly distorted islet of the pancreas with severe loss of basophilic endocrine cells and vacuolation. Arrows (without labels) shows degenerated, collapsed/blocked Interlobular ducts and intralobular ducts (star). Hypercellularisation and granulation of the acini and ductular cells (SA) within and without defined lobules. Inference: Severe loss of parenchymal material, distorted acini, ductular and endocrine cells of the pancreas – Severe Pancreatitis – Group 1. HFD= High Fat Diet.

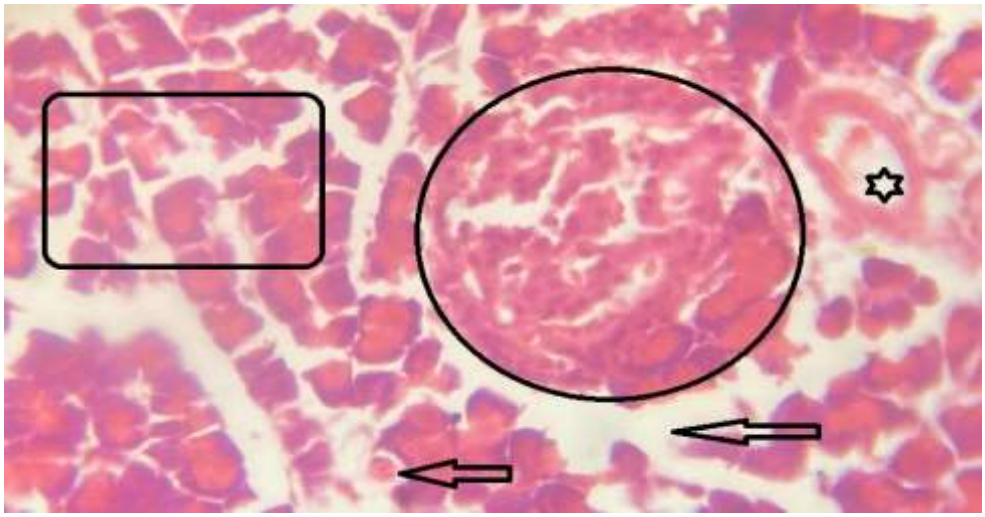

Figure 4 H& E stain of pancreatic tissue (x400). Treatment: 100mg/kg of Bitter Kola after HFD. The circled area shows the islet with fibrous appearance following very severe loss of endocrine cells and materials. Rectangular region shows gross loss of lobular materials, including acini and ductular cells. Arrows shows degenerated, collapsed/blocked Interlobular ducts and intralobular ducts (star). Inference: Presence of fibrous materials + Severe loss of parenchymal material, acini and ductular and endocrine cells of the pancreas – Very severe pancreatitis – Positive Control Group. HFD= High Fat Diet.

4. Conclusion

The administration of a high lipid diet and subsequent reversal with *Kola-Nitida* induces statistically ssignificant changes in the biological and histological parameters when compared with the control group. The strong statistically significant evidence supports the conclusion that *Kola-Nitida* may play a role in mitigating the effects of a high lipid diet in Wistar rats.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical considerations were received from the College of Health Science Research and Ethical Committee, Joseph Ayo Babalola University Osun state, Nigeria, with the approval number **ECR/CHS/036/2024**.

Ethical consideration was received from the Research and Ethical Committee, Joseph Ayo Babalola University Osun state, Nigeria, and ensured in accordance with the guidelines and regulations approved for the use care of rats.

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