Modulatory Role of Kolaviron on Thromboxane-A2 Activity, Glucose-6-Phosphate Dehydrogenase and Haematological Parameters in Streptozotocin-Induced Diabetes.

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Abstract: The study was aimed at investigating the effect of kolaviron on thromboxane-A2 activity, lipid peroxidation and haematological parameters in Streptozotocin-induced diabetes. Twenty male Wistar rats were selected into four groups of five animals each. Group 1 was control fed on standard rat chow. Group 2 received a single dose of 60mg/kg body weight of streptozotocin intraperitoneally. Group 3, injected with STZ and treated with 100mg/kg body weight of Kolaviron. Group 4 received 100mg/kg body weight of Kolaviron. Treatment lasted for four weeks. Results obtained showed that blood glucose level was significantly (p<0.01) reduced in the diabetic treated. Glucose-6-phosphate dehydrogenase level in the diabetics untreated was significantly (p<0.01) reduced. Lactate dehydrogenase was tremendously (p<0.01) raised in the diabetics but decreased in the kolaviron treated. COX-1 and thromboxane A2 expression was significantly (p<0.05) increased in diabetics untreated, lowered in KV treatment. Malondialdehyde level was significantly (p<0.01) high in Dm, reduced in KV treatment, SOD and catalase were increased in treatment groups but reduced upon treatment. Red blood cell (RBC) count and haemoglobin were not different in all the groups, HCT was higher in the diabetic treated when compared with control. WBC and lymphocytes were significantly (p<0.01) reduced in the diabetic group, but elevated in the KV treated groups, platelets count and neutrophils were significantly (p<0.01) increased in the diabetics when compared to control but decreased upon treatment.

Key Words: Diabetes, Thromboxane, Malondialdehyde, haematological indices, Kolaviron, Glucose-6-phosphate dehydrogenase.

I. Introduction

Diabetes mellitus is a pathological condition where blood glucose level rises consistently and sustained above a level accepted to be normal. Changes in body metabolic processes can result in very significant effect on the tissues there by causing peroxidation and oxidative stress situation where the over production of free radicals and peroxides into circulation cause a break down in the endogenous antioxidant system thereby resulting in tissue damage. For instance, research has shown that ROS can damage RBC¹ decreasing RBC and WBC count ³ and cause changes in the morphology and function of erythrocytes, leucocytes and platelets. Hyperglycemia has been implicated to be pivotal in development of most diabetic complications including insufficient production of insulin or inadequate utilization of glucose in the tissues, cardiovascular challenges, atherosclerosis, inflammatory disease heart attacks, stroke etc. ⁴, renal dysfunction and even retinopathy.⁵,⁶

In inflammatory diseases, cyclooxygenase and its products, prostaglandin E2 gotten from the Metabolism of phospholipids to form arachidonic acid, prostaglandins, thromboxane and leukotrienes have been reported to be present in the pancreatic islets ⁷,⁸ and is implicated in the islets destruction and inhibition of insulin secretion ⁹. Thromboxane on the other hand, is a vasoconstrictor and a potent hypertensive agent produced by activated platelets and is involved majorly in stimulating activation of new platelets and also increasing platelet aggregation.

Despite the use of modern drugs like metformin, insulin etc, in the regulation of blood glucose level, traditionally, the use of herbal products as an alternative means to control sugar level is common¹⁰. This could either be as a result of the expensive nature of the drugs or inclination to the use of herbs due to their low side effects and their diverse physiological actions¹¹. Kolaviron (KV), a biflavonoid complex from the Garcinia kola

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seed is known for its hepatoprotective, hypoglycemic, and hypolipidemic effect in animal model. The aim of this research work therefore, was to find out the effect of the biflavonoid, kolaviron, on Thromboxane-A2 activity, glucose-6-phosphate dehydrogenase and Haematological parameters in Streptozotocin-induced diabetes.

II. Experimental design.

Materials and Methods.

Twenty male Wistar rats weighing between 150 and 200 g were obtained from the animal House, Physiology Department, Cross River University of Technology, Calabar, Nigeria, and were randomly selected into four groups of five animals each. The animals were housed in plastic cages and kept in room temperature of 27°C ± 3°C with 12 h light/dark cycle. Group 1 was control fed on standard rat chow. Group 2 received a single dose of 60mg/kg body weight of streptozotocin intraperitoneally. Group 3 was also injected with STZ (60mg/kg) and treated with 100mg/kg body weight of Kolaviron while Group 4 received 100mg/kg body weight of Kolaviron. The feeding and treatment lasted for four weeks.

Animals were confirmed diabetic after three days by collecting blood sample through the tail vein and blood glucose measured using the glucometer, thereafter blood glucose was determined weekly. At the end of the treatment period, the animals were sacrificed and blood collected via cardiac puncture into EDTA bottles and centrifuged at 3000rpm for 10 min at 4°C and serum collected for biochemical analysis. The liver and pancreas were dissected, rinsed in cold phosphate buffered saline (10 mM pH 7.2) and preserved for histology work. Ethical approval for the study was obtained from the Faculty of Basic Medical Science Animal Research Ethical Committee of Cross River University of Technology, Calabar, Calabar, Nigeria (approval number FBMS/CRUTECH/12/020).

Collection of blood samples and biochemical analysis.

Animals were anesthesized, blood samples were collected by cardiac puncture into EDTA blood sample bottles for determination of G6PDH, LDH, liver enzymes and Haematological indices. Samples were allowed to stand for one hour to clot. The blood was centrifuged at 3000rpm for 10 minutes to obtain serum. The serum was stored at 15°C till further use.

Extraction of Kolaviron.

Bitter kola (Garcinia kola or Gutiferae heckel) seeds were bought from the ancient city of Okuku Kingdom in Yala local Government Area, Cross River State Nigeria. Method of extraction used was that described by Iwu et al. Seeds of bitter kola were peeled, dried and ground to powder and then extracted with light petroleum ether (b.p. 40–60°C) in a soxhlet extractor for 24 hr to get a defatted substance. Defatted substance was again extracted with ethanol, diluted with distilled water and extracted with ethyl acetate to give a yellow solid known as kolaviron. The obtained kolaviron was purified by subjecting it to thin-layer chromatography (TLC) using Silica gel GF 254-coated plates and solvent mixture of methanol and chloroform in a ratio 1:4v/v. UV light view revealed three band at a wavelength of 254nm.

Determination of haematological parameters

Haematological Autoanalyzer (Beckman Coulter, Inc. Fullerton, CA, USA) was used for the determination of haematological parameters: Red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb) concentration, white blood cell (WBC) count, platelet count.

Determination of superoxide dismutase/catalase activity and lipid peroxidation

Superoxide dismutase, catalase activity and lipid peroxidation were determined using the methods described by Misra and Fridovich, Sinha, Buege and Aust respectively.

Statistical analysis

All data obtained in this study are expressed as mean ± standard error of mean (mean ± SEM). Collected data was analyzed using one way simple analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison post hoc tests to compare the level of significance between control and experimental groups. All values at p<0.01 were considered significant. The GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for the analysis.

III. Results

At the end of the four weeks of experimental conditioning, we had a positive increase (p<0.01) in blood glucose level in the Diabetic group (79%) compared to control. Treatment with 100 mg/kg of kolaviron for four weeks significantly (p<0.01) decreased blood glucose level by 56.9%.
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Table no 1. Shows effect of Kolaviron on Weekly blood glucose level in STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>weeks</th>
<th>CONTROL</th>
<th>DM</th>
<th>DM+KV</th>
<th>KV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>76.60±6.9</td>
<td>401.6±56</td>
<td>454.2±53</td>
<td>88.60±5.8</td>
</tr>
<tr>
<td>Start</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>73.75±8.2</td>
<td>452.0±53.1</td>
<td>327.8±100</td>
<td>90.5±0.39</td>
</tr>
<tr>
<td>Week 2</td>
<td>85.60±5.2</td>
<td>294.8±47.7</td>
<td>157.6±4.9</td>
<td>129.2±10.0</td>
</tr>
<tr>
<td>Week 3</td>
<td>90.20±2.4</td>
<td>410.4±17.7</td>
<td>187.4±4.5</td>
<td>90.20±2.4</td>
</tr>
<tr>
<td>Week 4</td>
<td>90.20±2.4</td>
<td>428.6±12.5</td>
<td>184.8±2.5</td>
<td>91.20±2.3</td>
</tr>
</tbody>
</table>

Values are expressed in mean±SEM. n=5, a=p<0.01 Vs Control, b=p<0.01 Vs DM; c=p<0.01 Vs DM+KV

Table no 2: shows the effect of oral administration of 100mg/kg body weight of kolaviron on haematological indices. From our results, there was no statistical difference(p>0.05) between RBC, Hb, and Lymphocytes in the diabetics compared to control. The WBC count in the diabetics was significantly(p<0.01) reduced by 40% compared to control whereas there was an upward improvement by (62.9%) in diabetics treated compared to the untreated. Platelet count was statistically significant in the diabetics. The increase was by 18% than control while treatment brought it down by 20.58%. The hematocrit was significantly(p<0.01) increased in Dm+kv. Neutrophils were increased by 28.9% in the diabetics while upon treatment with 100mg/kg of koloviron reduced the count by 24.78%

Table no 2. Shows effect of Kolaviron(100mg/kg) on haematological parameters in STZ (60mg/kg) - induced diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>control</th>
<th>Dm</th>
<th>Dm+kv</th>
<th>Kv</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (X 10 12 ul)</td>
<td>7.08±0.32</td>
<td>7.37±0.36</td>
<td>7.77±0.13</td>
<td>6.88±0.22</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>WBC (10 9 ul)</td>
<td>7.40±0.78</td>
<td>4.44±0.16*</td>
<td>11.28±0.35**</td>
<td>11.54±0.34***</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.50±0.85</td>
<td>10.98±0.76</td>
<td>13.54±0.79</td>
<td>12.30±0.42</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>27.52±1.5</td>
<td>31.82±1.4</td>
<td>38.56±1.3</td>
<td>38.00±2.5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>PLATELET COUNT (X 10 9 ul)</td>
<td>417.4±1.806</td>
<td>510.0±37.15</td>
<td>405.6±4.67</td>
<td>393.0±7.29</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>LYMPHOCYTE (X 10 5 ul)</td>
<td>46.72±2.3</td>
<td>36.68±3.6</td>
<td>48.32±1.3</td>
<td>57.00±5.3</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>NEUTROPHILS (ul)</td>
<td>36.96±1.3</td>
<td>51.96±3.4</td>
<td>39.08±0.24</td>
<td>40.34±3.7</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Values are expressed in mean±SEM. n=5, a=p<0.01 Vs Control, b=p<0.01 Vs DM; c=p<0.01 Vs DM+KV

Figures 1 and 2: shows effect of kolaviron (100mg/kg) on serum glucose-6-phosphate dehydrogenase G6PD activity and lactate dehydrogenase. G6PD was significantly(p<0.01) decreased in the diabetics untreated (DM) but significantly(p<0.01) increased in activity in the treatment groups when compared to control and the diabetics. LDH was significantly(p<0.01) elevated in the diabetics than all other groups.

Figures 3 and 4: shows the effect of kolaviron (100mg/kg) on thromboxane A2 and cyclooxygenase activity in experimental diabetes. Thromboxane A2 (THX-A2) and cyclooxygenase (Cox) in the diabetics were significantly(p<0.01) elevated compared to all other groups. Treatment with 100mg/kg kolaviron significantly reduced Cox and Thx-A2 expression.
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Figures 5, 6 and 7 shows: the effect of kolaviron on lipid peroxidation product, Malondialdehyde and antioxidant activity in STZ-induced diabetes in rats. Malondialdehyde concentration was significantly (p<0.01) increased in the diabetics but decreased in treatment with 100mg/kg of kolaviron. Superoxide dismutase (SOD) and catalase (CAT) activity were significantly reduced in the diabetics untreated but increased following treatment with 100mg/kg kolaviron.

Figures 8 and 9 are photomicrographs of the pancreas and the liver in control and treatment groups. There were lesions on the cytoarchitecture of the pancreas and hepatic cells. The cytoplasmic basophilia nuclei cells and acinar cells were reduced and the liver cells showed sinusoidal block.
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Figure 8: Showing photomicrograph of the pancreas in (A) control, (b) Diabetic group, Dm, with reduced cytoplasm, less basophilia, condensed nuclei, and altered acinar cells. (c) Dm + Kv showing interlobular ducts (IT), pancreatic islets (IS) and numerous serous Acini (SA). No lesion. (D) Kv with serous acinar of the parenchyma with few pancreatic islets (IS). No lesion. X100. H & E.

Figure 9: Showing photomicrograph of the liver in (A) control, (B) Diabetic group, Dm, with enlarged central vein (ECV) surrounded by numerous hepatic cells (H) and areas showing sinusoidal block. (C) Dm + Kv showing showing engorged/dilated sinusoids and depletion of hepatic cells in the parenchyma. (D) Kv showing marked sinusoidal dilation (SD) and loss of hepatic cells (H) indicating hepatic cirrhosis, portal tract (PT) is seen. X100. H & E.

IV. Discussion

Cytotoxicity of streptozotocin and alloxan are two diabetogenic agents that generate excess reactive oxygen species, and nitrogen species causing derangement in pancreatic islet cells and hepatocytes in experimental animal models thereby precipitating hyperglycemia and other metabolic disorders with associated complications.

In this study, we examined the effect of kolaviron, an active component from Garcinia kola, on the activity of thromboxane A2, glucose-6-phosphate dehydrogenase and haematological parameters in STZ-induced diabetes. Results obtained in this study agreed with an earlier study that reported elevated blood glucose level and cytotoxicity. There was also evidence of suppression of glucose-6-phosphate dehydrogenase activity in this study. Available literature supports the fact that hyperglycemia suppresses G6PD activity via activation of cAMP/PKA signaling pathway and as such decreases the production of nicotinamide adenine dinucleotide phosphate reductase (NADPH). Such G6PD-induced reduction in NADPH results in oxidative stress and a distorted antioxidant system. This cytotoxic actions of experimental diabetes was further demonstrated in this work, by the observed lesions on the cytoarchitecture of the pancreas and hepatic cells where the cytoplasmic basophilia nuclei cells and acinar cells were reduced and the liver cells showing sinusoidal block. Treatment with kolaviron produced an appreciable decrease in blood glucose level and spectacular increase in G6PD concentration. The hypoglycemic activity of kolaviron is reported to result from the available bioactive components such as alkaloids, flavonoids, tannins, and saponins. These components synergistically promote glycemic control via varied mechanisms. For instance, while alkaloids are reported to act by inhibiting glycogen phosphorylase in vivo, flavonoid on the other hand is speculated to promote hepatic free radical scavenging ability of superoxide dismutase, increase glucose transporter 4 (GLUT4) expression in muscles, inhibit lipid peroxidation and glucose-6-phosphatase, which in effect promote the synthesis of glycogen synthesis and upregulation of hepatic glycogen levels. This bioactive substances are also thought to be responsible for the observed increase in G6PD in this study.

Functionally, G6PD promote the production of ribose-5-phosphate and NADPH, a compound that is believed to act as a cofactor for catalase antioxidant status as well as glutathione reductase essential in free radical scavenging. In this research work, we showed that the two endogenous antioxidants, superoxide dismutase and catalase were reduced in hyperglycemic condition as a result of lipid peroxidation evidenced by the high level of malondialdehyde and also a recorded increase in lactate dehydrogenase. Lactate dehydrogenase is usually expressed in blood cells and in heart muscle. During tissue breakdown, LDH becomes released in good amount into the blood and serum and serves as surrogate marker for heart or cellular injury. Apart from diabetic nephropathy, retinopathy and hepatotoxicity etc. often associated with hyperglycemia, research has shown that Diabetes mellitus also cause alteration in haematological indices due to pancreatic beta cell injury. In this study, we noted an unaltered red blood cell (RBC), Haemoglobin(Hb), packed cell volume (PCV) and lymphocyte count in the diabetic rats but with an observed increase in white blood cell (WBC), neutrophils and...
platelet count contrary to an earlier report demonstrating a decrease in RBC, Hb, and packed cell volume in diabetic animals. The increase in WBC count and neutrophils may have been due to the increase in oxidative stress and induced inflammation triggered by hyperglycemia.

In oxidative stress, thromboxane A2 stimulate the activation of new platelets and trigger inflammatory activity leading to various disease conditions such as hepatotoxicity and ischemic reperfusion injury. In our study, we showed that platelet count, cyclooxygenase and thromboxane A2 were tremendously increased in experimental diabetes. Cyclooxygenase-1 is predominantly found in platelets and is a major source of thromboxane while its isoform, COX-2 is constitutively expressed in small amount in circulating platelets in normal conditions. COX catalyze the conversion of arachidonic acid formed from the hydrolysis of membrane phospholipid into prostaglandin H2 which is further converted to THX-A2 and PGE2 by cyclooxygenase-thromboxane synthases. Evidences abound that hyperglycemia in vivo can activate phosphokinase C and cause the release of larger platelets with increased GP Ib and GP IIb/IIIa receptor and increased production of THX-A2. Once THX-A2 is formed, it stimulates the formation of new platelets and also activate available platelets to form more THX-A2 resulting in vasoconstriction and augment activation process. The resultant formation of thromboxane A2 from platelets play very important role both in the aetiology of atherosclerosis and acute thrombotic onslaught. This increases the risk of cardiovascular events. Treatment with 100mg/kg body weight of kolaviron biochemically reversed the hyperglycemia--induced Cox and THX-A2 activity as well as decreasing the platelet turnover. This cytoprotection property may not be unconnected with the physiologic actions of the identified bioactive components of kolaviron and so may be important in reducing cardiovascular risk factors thereby offering cardioprotection. In this study therefore, one thing remains clear and novel, significant reduction in the G6PD, LDH and suppression of Cox and Thx-A2 activity following Kolaviron treatment.

V. Conclusion

Kolaviron (biflavonoid) inhibits Cox and Thx-A2 expression, reduces platelet turnover suggestive of an anti-inflammatory activation and promotes antioxidant activity. Hyperglycemia does not alter erythropoiesis but promotes white blood cell proliferation.

Conflict of interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

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