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Assessing the Protective Effects of *Erythrina Senegalensis* Leafs Extract on Hepatocytes and Diabetes in Albino Wistar Rats after induction of Alloxan-Induced

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Abstract

The protective effect of Erythrina senegalensis leaf extract on albino Wistar rats induced with alloxan was investigated. Methanol was used as an extraction solvent. A single dose of alloxan at 150 mg/kg body weight in 0.1M sodium citrate buffer, and pH 4.5 was used to induce diabetes, after an overnight fasting, and blood glucose concentration was determined after 24 hours. Group 6, normal rats (non-alloxan-induced) were used as controls and other alloxan-induced groups were treated with *E. senegalensi*. They were administered 0.5 ml of distilled water twice daily, for 14 days. The doses administered were 100, 150, and 200 mg/kg body weight (i.e., for groups II, III, and IV, respectively). At the end of the experimental treatments, the animals were sacrificed. Whole blood obtained by cardiac puncture from each animal was allowed to stand for 60 minutes in EDTA tubes to clot before being centrifuged at 300 x g for 10 minutes. The serum was extracted and used for enzyme assays, while their liver tissues were collected and stored in 10 % buffered formalin for histopathological examination. The AST activity of E. senegalensis leaf extract-treated animals did not significantly vary from the control at $p < 0.05$. The data similarly indicate no significant change in the ALT activity of the various doses between the treated rats and the control. The serum AST and ALT (*De Ritis*) ratios were compared. The ratio for all the treated groups did not indicate a significant variation from that of the control at $p < 0.05$. The results provide experimental evidence at the biochemical level showing the E. senegalensis leaf extract is non-toxic.

Keywords: *E. senegalensis, diabetes, hepatic tissues, histopathology, liver-enzymes*

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Introduction

Investigations of the chemical activities of plants have yielded compounds with hypolipidemic, hypoglycaemic, antiplatelet, antitumor, and immune-stimulating properties, for the development of biotherapeutic drugs. Medicinal plants have been used by traditional medicine practitioners for

many years. The background knowledge about their uses was being transferred from generation to generation within human communities (Khan, 2014). These natural products play significant roles in providing the basic active therapeutic compounds currently derivatives of modern pharmacotherapy (Patwardhan *et al.*, 2008). several studies have dealt with the potential role of edible and medicinal plants and different classes of phytochemicals in obesity prevention and management (Shang *et al.*, 2021; Marrelli *et al.*, 2020). Obesity is one of the greatest public health concerns, and different comorbidities related to overweight have been identified, mainly diabetes, resulting in the common metabolic disorder known as metabolic syndrome (Eckel *et al.*, 2005). The potential role of plant extracts or phytochemicals in diabetes management has also been deeply reviewed (Govindappa, 2015; Teoh and Das, 2018). Hepatotoxicity, or liver damage, is caused by hepatotoxins, which may be sourced from chemicals, dietary supplements, pharmaceutical drugs, and medicinal plants. Some medicinal plants are hepatoprotectors against liver damage, while others induce hepatotoxicity. Recent advances in instrumentation and knowledge of active components have allowed research scientists to study the drug metabolic pathways of these phytopharmaceuticals to establish a relationship between medicinal plants and their pharmacological effects on the human liver, as a hepatoprotective or causative agent for hepatotoxicity (Thompson *et al.*, 2017). *Erythrina senegalensis* (referred to as Usiere - Efik, in the Cross River State of Nigeria), leaves have been evaluated for their antidiabetic activity (Eka *et al.*, 2011), and its stem bark evaluated to possess suppressive activity against *Plasmodium berghei* (Saidu *et al.*, 2000), and antibacterial activity (Kone *et al.*, 2004). It is thought that if toxicity occurs, there should be a noticeable change in the level of hepatic enzymes such as aminotransferases. While ALT is a more liver-specific enzyme, AST is also commonly found in non-hepatic tissues such as the heart and skeletal muscle. Since hepatic dysfunction may accompany cardiovascular (CV) diseases, studies have shown that the AST/ALT ratio can predict morbidity and mortality in acute and chronic CV diseases (Liu *et al.*, 2021; Gao *et al.*, 2017). Therefore, aminotransferase activities in serum were assessed and the AST: ALT ratio was computed in *Wistar albino* rats on which graded doses of *E. senegalensis* leaves extract were administered after being administered alloxan to induce diabetes. Therefore, the central idea is to harness the protective effect of *E. senegalensis* for the treatment of diabetes and hepatoprotection.

2. Materials & Methods

I. Preparation of Extract

Fresh leaves of Erythrina senegalensis were obtained from Ikot Ene in Akpabuyo Local Government Area of Cross River State, where they are planted as hedges. The leaves were identified to be E. senegalensis D.C by a taxonomist and voucher specimens (ME 1, 2006) were deposited at the herbarium, University of Calabar, Nigeria botanical garden. The leaves were subsequently sun-dried. The dried *E. sebegalensis* leaves were then blended with an electric blender and extracted using methanol as solvent. The extract

was subsequently concentrated by the air rotor to 10 cm^3.

II. Animal experimentation

Thirty (30) albino rats of Wistar strain weighing between 180 to 230 grams, were obtained from the animal house of the Department of Biochemistry, University of Calabar. The animals were acclimatized for seven days, and their weights were determined before, and during, the last day of experimental treatments. The rats were divided into six groups of six rats per group. they were housed in plastic cages with normal daylight of about 12 hours light (0630 – 1830 hours) and 12 dark. The Animals had free access to standard livestock feeds and tap water ad libitum throughout the experimental period of 14 days. Diabetes was induced via intraperitoneal administration of alloxan at 150 mg/kg body weight in 0.1M sodium citrate buffer pH 4.5, after an overnight fast. 24 hours after administration of alloxan, blood glucose concentration was determined in every group. Groups 6 - normal rats (non–alloxan treated) and alloxan-treated rats without E. senegalensis treatment was used as a control, and administered 0.5 ml distilled water twice daily, for 14 days. The doses administered were 100, 150, and 200 mg/kg body weight. The doses were selected based on previous studies by Saidu *et al,* (2000). The doses were administered in a distilled water volume of 0.5ml twice daily to experimental animals in groups II, III, and IV respectively for 14 days. At the end of the experimental treatment, the animals were sacrificed, and their liver tissues were stored in 10 % buffered formalin for the histopathological examination. Whole blood obtained by cardiac puncture from each animal was allowed to stand for 60 minutes in EDTA tubes to clot before being centrifuged at 300 RMP for 10 minutes. The serum was extracted and used for enzyme assay. Endpoint colorimetric diagnostic kits obtained from Randox Laboratories Ltd. U.K. were used for the estimation of AST and ALT activities in the serum. The AST, ALT ratio was computed for both AST and ALT activities.

III. Histopathological examination of the liver

Reagent: Hematoxylin, Eosin, Xylene, Alcohol, & Chloroform.

Procedure:

a. Section cutting: Liver tissue blocks were sectioned at 5 microns using a microtome.

b. Staining: Sections were stained with hematoxylin and eosin (Hand E) technique. Sections were then brought to xylene for 2 minutes per change. The xylene was cleared with absolute alcohol (95%) for 1 minute per change and then to 70% alcohol for 1 minute. This was then hydrated in running tap water for 15 minutes, stained with hematoxylin for 15 minutes, differentiated in 1 % acid alcohol (3 dips), and washed in running water for 10 minutes. This was then counter stained with 1% alcohol eosin for 1 minute followed by rapid dehydration in ascending grade alcohol, cleared in xylene, and mounted with DPX mutant.

IV. Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS). Data is presented as mean ± SD. One-way analysis of variance was used to determine the degree of significance at $p < 0.05$ confidence interval.

3. Results and Discussion

The assessment of functional status or cellular lesion in cells is normally conducted by monitoring the levels of specific enzymes in serum and urine (Dufour *et al.*, 2000). Aminotransferases

are cytosolic enzymes that have wide tissue distribution with the highest concentration found in the liver tissues (De Ritis and Cacciatore, 1983; Fahad *et al.,* 2019). Thus, they were the first enzymes to be used in diagnostic enzymology when liver damage occurred (Heckmann and Paradisi, 2020). Changes in enzyme concentration in tissue cells should therefore reflect the state of health of the organism involved (Ram *et al*., 2019). Since certain enzymes have correspondingly changed patterns in tissues and serum, they frequently serve as biomarkers for disease states.

The effect of orally administered *Erythrina senegalensis* leaf extract on AST and ALT activities in the serum of experimental animals as well as the AST ALT ratio is summarized in Table 1. Results obtained indicated insignificant variations in both the enzyme activities as well as the AST/ALT ratios of all the experimental groups. The liver-associated enzymes; alanine aminotransferase and aspartate aminotransferase are measures of liver homeostasis. In most acute hepatocellular disorders, the ALT is higher than or equal to the AST. While it is recognized that serum levels of both ALT and AST are elevated several folds in 'acute' viral hepatitis (e.g. hepatitis A and E), De Ritis was the first to describe that the ALT is usually higher than the AST with the AST/ALT ratio well below 1.0, and typically in the range 0.5 to 0.7 (Veropalumbo *et al*., 2012). Nevertheless, when transaminases are elevated severalfold, a low De Ritis ratio still provides an important clue to the aetiology of acute hepatitis.

Table 1 Effect of *E. senegalensis* leaves extract on serum activities of aspartate and alanine aminotransferases of experimental animals

Groups	AST (u/l)	ALT (u/l)	AST:ALT ratio
I	24.20±1.10	57.26±0.53	0.42
II	25.41±0.82	57.80±0.72	0.44
III	25.49±1.79	57.66±0.98	0.44
IV	25.80±1.10	57.60±0.79	0.45
V	26.81±1.02	65.46±0.38	0.41

Plates 1, 2, 3, 4, and 5 show the histological sections of liver cells of experimental animals, of normal control, 100mg/kg body weight, 150mg/kg body weight, 200mg/kg body weight, and alloxan-induced diabetic control respectively. Plate 1 shows a liver section with normal hepatocytes and eosinophilic cytoplasm. There are few inflammatory cells and no cellular atrophy. Plate 2 shows a liver section enlarged central vein. Hepatocytes are enlarged with moderate proliferation of fibroblast around the central vein. The cell cytoplasm is eosinophilic. Moderately foamy macrophages. In plate 3 the liver section shows an enlarged central vein with few fibroblast and inflammatory

cells. There are few foamy macrophages. There are a few areas of cellular degeneration.

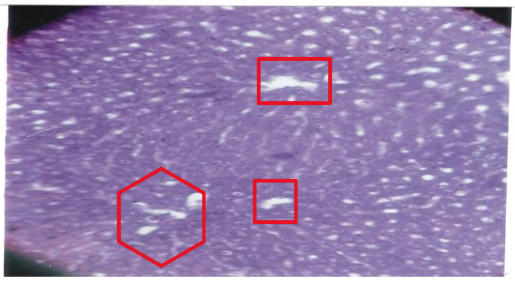


Plate 1: Histological section normal control with normal hepatocytes aneosinophilic cytoplasm (encircled).

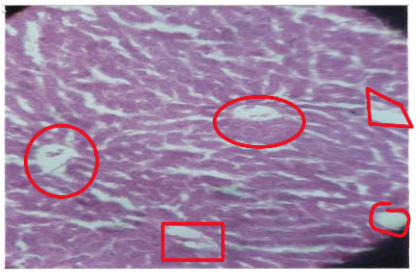


Plate.2: Histological section of the rat's liver, treated with 100mg/kg body weight *Erythrina senegalensis* extract showing enlarged central vein (encircled)

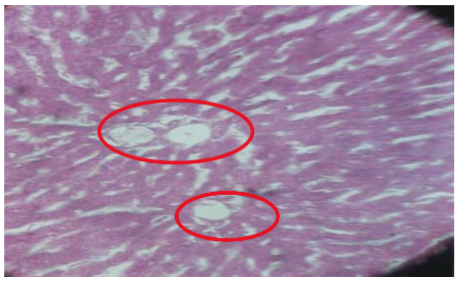
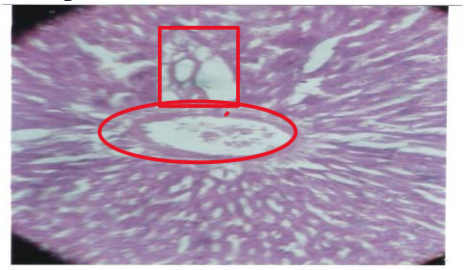


Plate 3: Histological section of liver of rats treated with 150mg/kg body weight *Erythrina senegalensis* extract showing enlarged central veins (encircled)



Effect of *Erythrina senegalensis* leaves extract on the integrity of the liver tissues of experimental animals
Are presented in the slides below. See slide I to 5, respectively.

Plate 4: Histology of the rat liver treated with 200mg/kg body weight *Erythrina senegalensis* extract with an enlarged central vein (encircled).

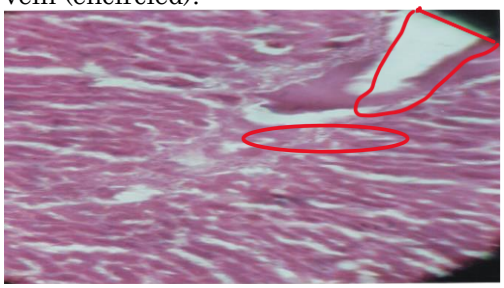


Plate 5: Histological section of the liver of diabetic control showing enlarged hepatocytes and with fibroblast scanty around the blood vessels.

There are a few areas of cellular degeneration. Plate 4 shows a liver section with an enlarged central vein and bile duct with moderate fibroblast. There is mild necrosis around the central vein. There is no bile stasis. Plate 5 shows a liver section with enlarged hepatocytes and fibroblast scanty around the blood vessels. There are moderate inflammatory proliferations.

This study focused on short-term effects of the extract, thereby limiting insights into the long-term consequences of extract administration. By acknowledging these potential limitations, researchers can strengthen the interpretation of their findings and identify areas for future investigation, by exploring the underlying molecular mechanisms by which the extract exerts its protective effects.

Conclusion

Animals treated with 100mg/kg, 150mg/kg, and 200mg/kg *Erythrina senegalensis* leaves displayed tolerance,

and histological studies also confirmed the *Erythrina senegalensis* extract was not hepatotoxic and did not cause increases in the activities of ALT and AST compared to that of the control group at $p < 0.05$. It is thus concluded that the administration of *Erythrina senegalensis* in low and moderate doses would not inflict severe liver tissue damage. The results of this study therefore provide experimental evidence at the biochemical level showing that *Erythrina senegalensis* leaf extract is non-toxic.

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