

## **Metabolic and biochemical alterations in induced-diabetic rats treated with *Annona muricata* and the associated mechanism using NMR-based metabolomics approach**

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### **Abstract**

Patients are increasingly turning to herbs for diabetes treatment, resulting in a rise in demand for alternative therapies derived from plants. In underdeveloped nations, specifically, where most people have limited resources and do not have access to contemporary treatment, plants continue to play a crucial role in the treatment of diabetes. *Annona muricata*, often known as "Soursop" in various regions of the world, is a well-known medicinal plant with multiple applications. It is a common plant used in Africa and South America to manage various types of disease. However, there is insufficient toxicological information or published standard available regarding repeated dose animal toxicity data. As part of the safety assessment, we exposed Sprague Dawley rats to an acute oral toxicity of *A. muricata*. The intent of the current study was to use advanced proton nuclear magnetic resonance (<sup>1</sup>H NMR) in serum and urinary metabolomics evaluation techniques to provide the in vivo acute toxicological profile of *A. muricata* leaf ethanol extract in accordance with the Organization for Economic Co-operation and Development's (OECD) 423 guidelines. A single 2000 mg/kg dose of *A. muricata* leaf ethanol extract was administered to Sprague Dawley rats over an observational period of 14 days. The toxicity evaluation (physical and behavior observation, body weight, renal function test, liver function test and <sup>1</sup>H NMR analysis) showed no abnormal toxicity. Histopathological analysis manifested mild changes, i.e., the treated kidney manifested mild hypercellularity of mesangial cells and mild red blood cell congestion. In addition, there was mild hemorrhage into tissue with scattered inflammatory cells and mild dilated central vein with fibrosis in the liver. However, the changes were very mild and not significant which correlate with other analyses conducted in this study (biochemical test and <sup>1</sup>H NMR metabolomic analysis). On the other hand, urinary <sup>1</sup>H NMR analysis collected on day 15 revealed high similarity on the metabolite variations for both untreated and treated groups. Importantly, the outcomes suggest

that *A. muricata* leaf ethanol extract can be safely consumed at a dose of 2000 mg/kg and the LD<sub>50</sub> must be more than 2000 mg/kg.

Keywords: Metabolomics, *Annona muricata*, biochemical test, toxicity, <sup>1</sup>H NMR metabolomics, histopathology