The accurate glucose monitoring at cellular level remains a big challenge in the field of clinical diagnosis, control, and therapy of diabetes. Glucose sensors based on fluorescent 2D materials nanocomposites has grabbed the considerable attention owing to their profound optical, electrical, and sensing properties. In our work, the nanocomposite of Graphene decorated zinc oxide nanoflakes (GO/ZnO-NF) were synthesized by one step hydrothermal process which exhibits excellent sensitivity and selectivity due to high surface area, more active sites, enhanced prevalent optical and electrical properties. The surface chemistry and morphology, chemical composition and fluorescent behavior of the prepared samples was confirmed using different characterization tools like SEM, XRD, FTIR, RAMAN spectroscopy, UV-Visible and FLUORESCENCE Spectrophotometry. The designed sensor function is based on the fluorescent quenching of GO/ZnO-NF, treated with different concentrations of glucose in the combination of Rhodamine-B as well as without glucose concentration which effectively enhanced the emission intensity of fluorescent dye Rhodamine-B. The sensor exhibits the sensitivity of 3.34 % mM⁻¹ and lower limit of detection of 0.089 mM (3σ) in the linear range of 0.5mM to 3.5mM of glucose. The selectivity and sensitivity of the sensor has been confirmed through different molar concentrations of glucose and different analytes as well. Hence based on our findings, the proposed sensor has high discerning and responsive behavior of fluorescence quenching efficiency towards glucose.