Chemoprevention Effect of mixed Medicinal Herb *Hibiscus sabdariffa L.* (contains Anthocyanins) and Nanoselenium on DEN-induced Hepatocellular Carcinoma in Rats

Thesis submitted to Faculty of Science Ain Shams University
In Fulfillment of Ph.D. degree of Science in Biochemistry

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Dedication

The present work is especially dedicated to:

Soul of my father

Patience of my mother

Encouragement and support of my wife

Patience and hope of our kids

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Originality declaration

I hereby declare that this thesis is my own work and contains no materials previously published or written by another person, or material which has been submitted for award of any other degree or diploma by me at Ain Shams University or any Educational Academy

Adam Osman Abaker

Abstract

The present study aimed at evaluating the efficacy of *Hibiscus sabdariffa* L. (Karkadeh) water extract, containing anthocyanins and nanoselenium as chemopreventive substances against hepatocellular carcinoma (HCC) induced by *N*-diethylnitrosamine (DEN) in experimental rats. Assessment of chemopreventive efficacy of *H. sabdariffa* extract (HEx) and nanoselenium (nSe) that conducted in this study included *in vitro* and *in vivo* tests. *In vitro* tests of the preventers under investigation revealed high toxicity towards human cell line HepG2. The IC_{50} that recorded for HEx was 9.1 µg/ml. IC_{50} of nSe against HepG2 was 11.6 µg/ml, whereas for the combination of HEx and nSe was 13.6 µg/ml. ensure their high toxicity against this human cell line. *In vivo* tests comprise assessment of liver and kidney function biomarkers oxidative stress, antioxidant, apoptosis, inflammation and tumor markers. Moreover, lipid profile was measured and in addition to the concentration of selenium accumulation in different organs using inductively coupled plasma optical emission spectrometry (ICP-OES) beside histopathological examination. DEN-induction led to liver intoxication which appears as rise in the diagnostic enzymes of liver function, aspartate aminotransferase (AST) alanine aminotransferase (ALT), and Gamma Glutamate transferase (γ-GT) in plasma. Kidney function was affected also by the carcinogen induction which appears as increase in plasma urea and creatinine concentration. Elevation of the level of lipid peroxidation which expressed as malondialdehyde (MDA) due to oxidative stress was recorded. The level of antioxidant parameters, reduced glutathione (GSH) as well as superoxide dismutase (SOD), and catalase (CAT) activities was decreased. On the other hand liver intoxication revealed as reduction in the level of caspase-3 and increase in tumor necrosis factor alpha **(**TNF-α**),** and tumor marker, alpha fetoprotein (AFP). These findings which were significant to the normal control of each parameter, were changed significantly compared with corresponding DEN group of each parameter after drugs administration, particularly post-treatment of HEx (250 mg/Kg body weight thrice a week for ~ 6 months) nSe (2 mg/Kg body weight twice per week for ~ 6 months), indicating the recovery of natural antioxidant defense system and function of affected tissues. The results emphasize that *H. sabdariffa* aqueous extracted anthocyanins contains potent attenuation effect against DEN-hepatotoxicity. ICP-OES findings recorded that nSe is accumulated in high concentrations in spleen, very low accumulation was found in kidney. Synergetic test by combination of roselle extract and nanoselenium showed no better results than individually administration of each. The present study showed that the aqueous extract of *H. sabdariffa* containing anthocyanins and nanoselenium have antioxidant capacity and chemopreventive effect against HCC induced by DEN. However, further study should be conducted to establish the synergetic mechanisms of roselle extract and nanoselenium.

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