

Abstract

Extensive analysis has been carried out to all apoptotic genes through the clustergen overview which categorized the experimental groups to 6 categories into sequence of colors. Levels of gene expressions, as represented by different colors, clearly showed that tested saffron-based bioactive ingredients (SBI) have clear effects on number of genes in the hepatocellular carcinoma-induced groups. SBI showed a significant effect on Caspase-3. Caspase-3 is one of the most important genes involved in apoptotic pathway, and thus a common target in cancer treatments. SBI effects on levels of caspase 3 was consistent with our earlier study using saffron crude extract which indicate that SBI tested here might play a big role in saffron's overall effects protecting against hepatocellular carcinoma.

Introduction

Alternative cancer treatment has grown into a powerful research topic for many scientists; one of these alternative treatments is utilizing plant extracts as anticancer remedies. Saffron is a naturally derived plant product from the dried stigma of the Crocus sativus flower (family Iridaceae) that has significant anticancer effects that have been reported against different cancer types. To study the mechanism of anticancer effect of saffron, we have used gene expression analysis on number of genes in the hepatocellular carcinoma (HCC)-induced groups. HCC is the fifth most common cancer and the third leading cause of cancer mortality in the world. This study sheds light on how saffron regulates rat apoptotic genes, using RT-Profiler PCR system. This array System is the most reliable and accurate tool for analyzing the change in the level of expression of a focused panel of genes using SYBR Green-based real-time PCR.

Materials and Methods

Animals
Male wistar rats (120-200 gm) were used for this study.

Fig. 1. Saffron

Fig. 2. (a) Data sheet of experimental groups, with all the expressions of genes calculated mathematically using RT-Profiler PCR Array system (b) Transformation of the data sheet to a better overview (clustergen) using RT-Profiler PCR array analysis.

Fig. 3. Clustergen overview of selected genes with their experimental groups as follows:
- Control Group 1 (Rats with HCC)
- Group 2 (HCC + SBI-1 alone)
- Group 3 (SBI-2 alone)
- Group 4 (HCC + SBI-1)
- Group 5 (HCC + SBI-2)

Table 1. List of genes information associated with almost all types of control and signal loading. Genes information from SABiosciences database, (Qiagen company): all of those genes were found to be highly associated with apoptosis through literature studies, and 3 of these genes (Bcl2a1d, Tp53, casp2) have been affected.

Table 2. List of differentially expressed genes in the different groups.

Fig. 4. (A) Lysates prepared from HepG2 cells treated with saffron for 6, 24, and 48 hours were analyzed by anti-caspase-3, anti-Bcl2, anti-TP53, anti-HE2AX, anti-GADDH western blotting. GADDH served as an internal control for equal loading. (B) Apoptosis measurement after saffron treatment. Annexin-FITC measurements of untreated cells (control) and HepG2 cells treated with 6 mg/mL saffron for 6, 24, and 48 hours the profile represents annexin V–fluorescin isothiocyanate (FITC) staining in the x axis and PI in the y axis.

Fig. 5. Results and Discussion

Many studies have reported of natural antioxidants for use in food or medical materials to replace synthetic formulations, which are restricted because of their side effects.

Natural antioxidants, found in various plants, can protect cells against oxidative damage and may also provide an exciting preventive and therapeutic prospect for degenerative diseases. Saffron (Fig. 1) is a spice that has been long known for its antioxidant properties and as an anti-cancer agents for different types of cancers. The Antioxidant property of saffron could be credited to its active ingredients (such as safranal, crocin, crocetin, and carotene).

Apoptosis is recognized as an important mechanism in liver diseases and its down regulation is common in cancer development including HCC. Thus, the inhibition of apoptosis holds promise as potential therapeutic strategy against HCC.

The present study provides more details in the role of SBI on regulation of gene expression in an animal model of liver cancer, by using RT-PCR array system (Table 1 and Fig. 2). This system is suitable for different applications including drug toxicology studies, tumor metastasis & cancer biomarker research, as well as cytokine profiling and inflammatory response studies. The array system allowed us through utilizing a real-time PCR to easily examine the changes in gene expression between SBI-treated and control samples and to quickly identify genes with significant up- or down-regulation in response to tested SBI (Fig. 3).

The present results were consistent with our earlier study that reported a potent pro-apoptotic effects of the crude extract of saffron both in vivo and in vitro (Fig. 4; Amín et al., 2011).

References


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