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Research Article

Anti-hepatocarcinoma Effects of a Food Additive Chrysin Nanosuspension against Human HepG2 Cells

[1]Zhi-ping Wang, [2]Qun Zhou, [3]Hui-zhe Shi, [3]Sheng Xiong and [3]Yi-fei Wang
[1]School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, China
[2]Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, Tongji School of Pharmacy, Huazhong University of Science and Technology, Wuhan 430030, China
[3]Institute of Biological Medicine, Jinan University, Guangzhou 510632, China

Abstract: Hepatocarcinoma, a malignant cancer, threaten human life badly. It is a current issue to seek the effective natural remedy from plant to treat cancer due to the resistance of the advanced hepatocarcinoma to chemotherapy. Chrysin (Chr), a major symbol ingredient in Chinese Propolis, has a wide range of pharmacological properties and is considered to have anti-hepatocarcinoma effects. However its low oral bioavailability restricts its wide application. In this report, Chr-Nanosuspension (Chr-NS) composed of Chr and poloxamer 188 was prepared by high pressure homogenization technique. The *in vitro* anti-hepatocarcinoma effects of Chr-NS relative to efficacy of bulk Chr were evaluated. The particle size and zeta potential of Chr-NS were 291.1 nm and -28.7 mV, respectively. MTT assay showed that Chr-NS effectively inhibited the proliferation of HepG2 cells and the corresponding IC_{50} values of Chr-NS and bulk Chr were 1.55 and 3.76 μg/mL. These results suggest that the delivery of Chr-NS is a promising approach for treating tumors.

Keywords: Antitumor activity, chrysin, cytotoxicity, food additive, HepG2 cells, nanosuspension

INTRODUCTION

Hepatocellular Carcinoma (HCC) is the sixth most common cancer in the world and the third most common cause of cancer death (Parkin, 2001). In last decades, most patients diagnosed with hepatoma have low recovery rates and conventional and modified therapies currently available are rarely beneficial (Thomas and Zhu, 2005). Moreover, the limited responses of hepatoma, mainly hepatocellular carcinoma, to these agents are often due to its multidrug resistance to them. Thus, developing new therapeutic agents for hepatocellular cancer becomes an urgent need to reduce the mortality caused by this disease (Deng *et al*., 2006). At present, the demands for more effective and safer therapeutic agents for cancer have greatly increased. Natural products from medical plants are valued as an important source to find innovative agents for treatment of cancer (Liang *et al*., 2012).

Chrysin (Chr, Fig. 1) is a food additive in a yellow, crystal powder form, a major symbol ingredient in Chinese propolis, which comes from the dried secreta of *Apid mellifera* L. in accordance with Chinese Pharmacopeia (Sun *et al*., 2012). Chr has been shown to exert a variety of pharmacological effects such as antioxidant, antidiarrheal and anti-inflammatory activities. Chr is a natural flavonoid currently under investigation due to its important biological anti-cancer properties including effects on leukemia (Monasterio *et al*., 2004; Lin *et al*., 2012), cervical (Zhang *et al*., 2004), oesophageal (Zhang *et al*., 2008, 2009), prostate (Samarghandian *et al*., 2011), breast (Lirdprapamongkol *et al*., 2013; Yang *et al*., 2014), lung epithelial (Brechbuhl *et al*., 2012; Shao *et al*., 2012), thyroid (Yu *et al*., 2013; Zarebczan *et al*., 2012) and hepatocarcinoma (Sun *et al*., 2011) cancers.

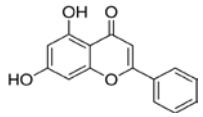

Fig. 1: Chemical structure of Chr

However, Chr is hardly water-soluble and its absorption *in vivo* is very poor after oral administration (Walle *et al*., 2000). A compound as a drug should have favorable Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) characteristics. To circumvent these pitfalls, several strategies like cyclodextrin nanoparticles (Hădărugă *et al*., 2012) and chemically modified prodrugs (Zou *et al*., 2011), have been proposed to deliver Chr in the last few decades.

Nanocrystal suspension, Nanosuspension (NS) for short, is a carrier-free nanoparticle system containing

Corresponding Author: Zhi-ping Wang, School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, China

only pure drug crystal and minimum surfactant and/or polymer for stabilization (Gao *et al*., 2013). Reduction of particle size by nanocrystal technology to the nano-scale usually leads to a significant increase in drug solubility and dissolution rate with an obvious improvement in drug bioavailability (Liversidge and Cundy, 1995). Liversidge and Cundy (1995) reported that, in the same dosage, danazol NS with the average particle size of 169 nm could obtain the C_{max} as high as 3.01 mg/mL and the bioavailability of 82% in beagle dogs, while the commercially available danazol suspension with the average particle size of 10 mm could only obtain the C_{max} of 0.20 mg/mL and the bioavailability of 5%. It could be found that NS significantly enhanced the oral absorption of danazol, a poorly water-soluble drug. A few techniques have been used to prepare drug loaded NS, including nanoprecipitation, pearl-milling, high speed homogenization, sonication and High-Pressure Homogenization (HPH) (Zhang *et al*., 2007). Among these techniques, the HPH method with a high productivity and a lower level contamination which is favorable for implementation of industrial products has shown great superiority over other methods. In this study, we evaluate the human HepG2 cells anti-hepatocarcinoma activity of Chr-NS relative to efficacy of bulk Chr delivery (Mo-Nan *et al*., 2012).

MATERIALS AND METHODS

Materials: Chr form was purchased from Aladdin industrial corporation (Shanghai, China). Chr standard was purchased from the National Institutes for food and drug Control (≥98.0%). Poloxamer 188 (P188, Lutrol® F68) was kindly donated from BASF (Ludwigshafen, Germany). MTT was purchased from Sigma Chemical (St. Louis, MO).

Preparation of the test solution: HPH technique was applied to prepare Chr-NS. Briefly, P188 of 0.75% was dissolved in distilled water. The Chr powder of 0.35% was dispersed in the aqueous surfactant solution using high speed homogenization 5000 rpm for 10 min (IKA T18 basic ULTRA-TURRAX®, Germany). Then the pre-mix was passed through a Lab HPH (APV-2000, Germany), 5 cycles were performed at 500 bar and 15 cycles at 1500 bar.

Characterization of the Chr-NS: The particle size, Polydispersity Index (PDI) and Zeta potential measurements were performed on a Nano-ZS90 (Malvern Instruments Ltd., Malvern, UK) thermostated at 25°C. The sample was diluted 50 times with bidistilled water before the measurements. All values were measured at an analysis angle of 90°C in a 10-mm diameter cell. Each value reported is the average of three measurements.

Cell viability assay: Cells were treated with different concentrations (0, 0.1, 1, 10 and 100 μg/mL,

		Size (d.nm)	Intensity %	S.D. (d.nm)
Z-average (d.nm): 291.1	Peak 1:	466.1	86.6	362.10
Pdl: 0.674	Peak 2:	58.39	7.0	12.81
Intercept: 0.871	Peak 3:	5074	6.4	571.70

Result quality: Good

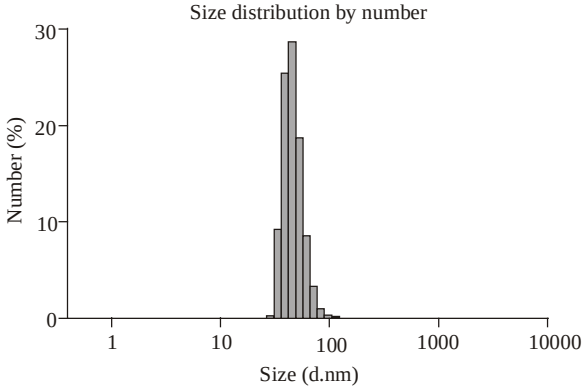

Fig. 2: The particles size of Chr-NS

		Mean (mV)	Area %	S.D. (mV)
Zeta-potential (mV): -28.7	Peak 1:	-28.7	100.0	5.65
Zeta deviation (mV): 5.65	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0210	Peak 3:	0.00	0.0	0.00

Result quality: Good

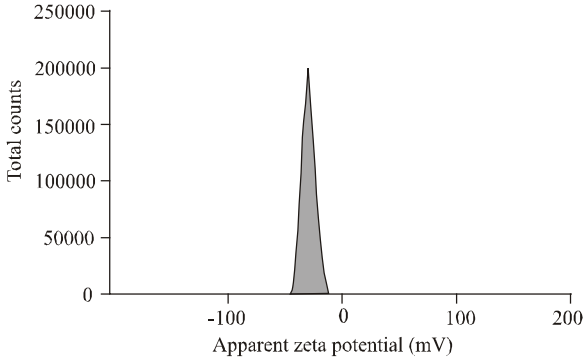

Fig. 3: The zeta potential of Chr-NS

respectively) of Chr solution and Chr-NS respectively. And then, the effect of Chr-NS on the viability of cells was determined by the colorimetric MTT assay. The inhibition rate was expressed as following formula:

Inhibiton rate (%) = [1- (absorbance of experimental group/absorbance of control group)] ×100

Statistical analysis: Results were expressed as mean±Standard Deviation (S.D.). Student's t-test was

used to compare the mean differences between samples using the statistical software SPSS version 16.0 (SPSS, Chicago). In all cases $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Particle size analysis and zeta potential of Chr-NS: The mean particle size and PDI were measured immediately after the preparation of the NS. The mean particle size with PDI 0.674 was 291.1 nm (Fig. 2). The PDI is a measure of particles size distribution. The values less than 0.3 indicate a high degree of homogeneity in particle size and vice versa. The zeta potential of Chr-NS was 28.7 mV (Fig. 3).

Cytotoxicity of Chr-NS: To determine whether Chr-NS has growth-inhibitory effects, HepG2 cells were exposed to different concentrations of Chr-NS for 72 h. Our data showed that the growth of human HepG2 cells were significantly inhibited by Chr-NS, the IC_{50} of Chr-NS and Chr solution were 1.55 and 3.76 μg/mL, respectively.

CONCLUSION

In present study, we demonstrated that Chr-NS effectively inhibited the growth of HepG2 cells *in vitro*. Therefore, Chr-NS may be explored as a novel potential antitumor agent for the functional food and pharmaceutical purpose. This study also provides evidences to support the therapeutic effects of compound for treatment of cancer in China. Despite of the promising results from our current investigation, there are still a plethora of practical issues which may be difficult to reconcile for the ultimate use of Chr-NS for the novel target-therapy in cancer management.

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