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Safranal as a novel anti-tubulin binding agent with potential use in cancer therapy: An in vitro study

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ABSTRACT

Safranal, a component of saffron, indicates anti-tumor activities; however, the precise mechanism of this effect has remained elusive. In this study we investigated tubulin assembly and structure in the presence of safranal to open the new horizons about the potential of safranal as an anti-tumor agent via microtubule disfunction. Anti-microtubule activity of safranal was evaluated by turbidimetric method and transmission electron microscopy (TEM). Safranal (0.1–70 μM) was incubated with tubulin (5 μM) and tubulin structural changes was surveyed using fluorometry. Tubulin binding site with safranal was estimated by molecular docking. Microtubule polymerization decreased significantly in the presence of safranal, regardless of its concentration and the IC_{50} value was obtained 72.19 μM. Safranal was situated between α and β tubulin closer to α-tubulin and hydrogen bond with Gly 142 and hydrophobic interactions played critical roles for safranal molecule stabilization in binding site. It seems that decline of tubulin assembly could result from tubulin structural changes through safranal bindings between alpha and beta tubulin with ΔG^0 of −5.63 kcal/mol. Safranal can be taken into account as an anticancer agent; however, in vivo experiments are required to confirm this conclusion.

1. Introduction

Saffron, the most expensive spice with different uses as a food additive or drug, is the dried stigmas of *Crocus sativus* L. flower (Iridaceae family) and is produced mainly in Iran, Spain, India and Greece [1,2]. The yellow-orange color of saffron comes from crocin family which contains glucosyl and gentiobiosyl esters of crocetin, a dicarboxylic 20-carbon water soluble carotenoid [3]. Its odor and aroma is a characteristic derived from safranal, a volatile aldehyde present in saffron [4]. Safranal, $C_{10}H_{14}O$ or 2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde, is made of picrocrocin and 2,6,6-trimethyl-4-hydroxy-l-carboxaldehyde-l-cyclo hexene (HTCC) during the course of saffron drying process (see Fig. 1) [2]. Several studies have been carried out to elucidate the capability of saffron applications in medicine. For instance, it has been reported that safranal and other carotenoids decreased inflammation in mice [5] and could exhibit antihypertensive [6], anticonvulsant [7], antigenotoxic [8], antidepressant [9], learning and behavior improvement [10], and antioxidant [11,12] activities in multiple animal studies and clinical trials.

In the recent years, dried stigmas of *C. sativus* L. has come to be known for its anti-cancer activity and it seems that saffron has no cytotoxic effects on non-malignant cells [8,13]. To date, the precise mechanism of anti-cancer effect of saffron remains elusive; however, all published data from animal and in vitro studies showed that saffron and its fundamental components have anti-cancer and anti-tumor activities. Recently, various hypotheses for anti-tumor and anti-cancer properties of saffron have been drawn from different studies. In more detail, anti-tumor activity of saffron

Abbreviations: ANS, 8-anilino-1-naphthalenesulfonic acid; ATP, adenosine triphosphate; BSA, bovine serum albumin; EGTA, ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid; GTP, guanosine 5′-triphosphate; HTCC, 2,6,6-trimethyl-4-hydroxy-l-carboxaldehyde-l-cyclohexene; MAP, microtubule-associated protein; MT, microtubule; OD, optical density; PIPES, piperazine-1,4-bis(2-ethane sulfonic acid); SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TEM, transmission electron microscopy; Trp, tryptophan.

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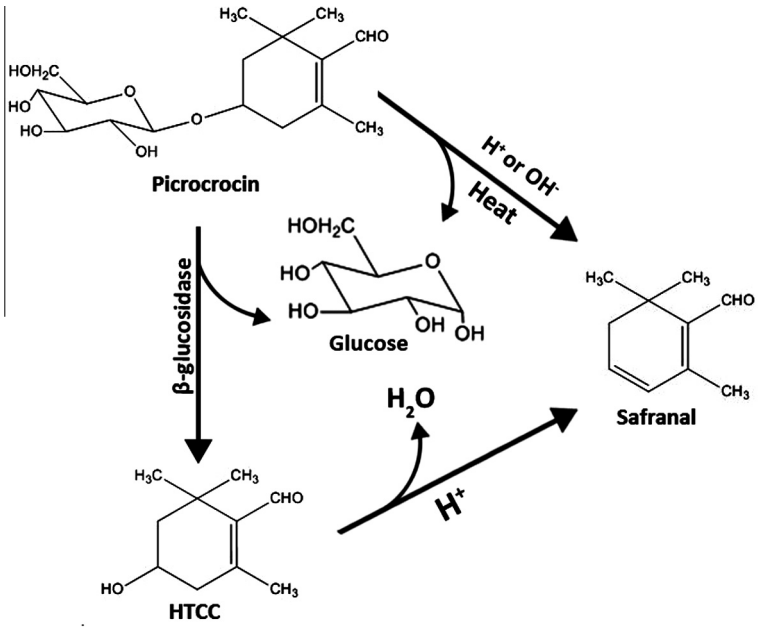

Fig. 1. Scheme of the chemical and enzymatic conversion of picrocrocin to HTCC and safranal.

results from different mechanisms including (1) disruption of the DNA and RNA synthesis, but not protein [14], (2) its ability to remove free radicals [12,15], (3) participation in the metabolic conversion of carotenoids to retinoids [16,17], (4) mediating interactions between carotenoids and topoisomerase II [18], (5) apoptosis caused by cytotoxic influences of carotenoids [19], (6) increase glutathione enzymes GSH and related enzymes due to its antioxidant activity [18] and (7) down-regulation of the expression of the catalytic subunit of the human telomerase, hTERT [20,21].

It has been predicted that saffron constituents require exerting their effects on cytoplasmic component through macromolecular synthesis and cytotoxicity [18]. Hence, the likely cytoplasmic component that is usually attacked by anti-tumor agents is microtubule (MT) proteins. MT proteins are cylindrical, long and hollow polymers which are composed of tubulin heterodimers with α and β subunits [22]. Each tubulin dimer consists of three domains including N-terminal, intermediate and C-terminal domains [23]. MTs along with actin and intermediate filaments organize the fundamental cytoskeletal constituent parts in all eukaryotic cells. They are naturally dynamic polymers that become larger and shorter by the reversible non-covalent association and separation of alpha and beta tubulin heterodimers at their two terminal ends which is associated with the hydrolysis of guanosine 5′-triphosphate (GTP) [23,24]. MT-associated proteins (MAPs), e.g. tau, are essential for accurate function of MT dynamics [25]. The motor proteins, kinesin and dynein, exist in eukaryotic cells to transport cargoes along MTs through ATP hydrolysis and contribute at chromosome segregation [26]. Therefore, MTs play a vital role in cell division, which makes them a very advantageous target for the development of therapeutic agents against the rapidly dividing cancer cells. Inhibition of MT dynamics impairs proper chromosome attachment and movement, and finally obstructs cell cycle at mitosis [27,28]. Based on different mechanisms of MT-targeting agents on inhibition of cell cycle process, such agents can be grouped into two main families; agents that destabilize MTs (e.g. colchicine and vinca alkaloids such as vinblastine, vincristine, etc.) and agents that stabilize MTs such as the taxanes (e.g. paclitaxel). There are three well known binding sites for anti-mitotic agents on tubulin dimer; the vinca domain, the taxane site and the colchicine site [29]. The location of the vinca domain is close to the GTP binding site in β-tubulin [30]; the taxane site is in a deep hydrophobic area within the lumen of MT [31]; and the location of colchicine site is between α and β-tubulin at the intra-dimer interface [32].

Previously, we reported the effect of crocin on MT polymerization and structure in comparison with paclitaxel. Our data showed that crocin is able to increase MT polymerization and nucleation rate and disrupt MT dynamics through acting as stabilizing agent [33]. In the present study, we investigated the in vitro effect of safranal on the MT structure and assembly to figure out the possibility of safranal as a MT-targeting agent with potential application in cancer therapy. Tubulin assembly was studied by turbidity method and transmission electron microscopy (TEM). Structural changes of tubulin dimers were also investigated by monitoring intrinsic and extrinsic fluorescence emissions. Moreover, we succeeded in finding the interaction site between safranal and tubulin dimer and calculated Gibbs free energy of bindings through computational modeling.

2. Material and methods

2.1. Chemicals

Safranal ⩾88% (2,3-dihydro-2,2,6-trimethylbenzaldehyde), ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) and guanosine-5′-triphosphate (GTP) were purchased from Sigma-Aldrich (Deisenhofen, Germany). Phosphocellulose P11 was prepared from Whatman (Florham Park, USA). All other chemicals such as piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES), glycerol, $MgSO_4$, KOH and NaCl were prepared from Merck (Darmstadt, Germany). Solutions were prepared with double-distilled water and were kept at 4 °C before use.

2.2. Animal

This study was carried out on tubulin extracted from sheep brain, to investigate the effects of safranal on MT structure and organization. One adult male sheep (76.85 kg) and of good body