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Phenolic compounds based conjugates from dextran aldehyde and BSA: Preparation, characterization and evaluation of their anti-cancer efficacy for therapeutic applications

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ABSTRACT

Here, we have synthesized phenolic compounds (pc) based on conjugates from dextran aldehyde (dex-ald) and bovine serum albumin (BSA) and screening their potential activity as therapeutic agents in cancer disease. The synthesized conjugates were analyzed by UV-vis, FT-IR, XRD and SEM analysis. UV–vis spectra of conjugates showed the shifting of spectral peak at UV to visible region revealed the enhanced conjugation due to formation of linkage. The XRD peaks of conjugates found broader and indicating the amorphous phase of conjugating materials in compared to free components. The SEM images showed that the conjugated materials having numerous pores on its surface, which proved its porous nature. The amount of phenolic compounds conjugated with (dex-ald-pc) and (BSA-pc) were found to be 65.4 and 73.91 mg/g of conjugates, respectively. Cells viability was significantly decreased approximately 80–85% at concentration of 100 μg conjugates whereas the free polymers or phenolics did not affect the viability of cancer cells. Generation of high quantity of reactive oxygen species (ROS) in cells treated with conjugate materials, which may caused cell apoptosis in cancer cell line. The results clearly showed that conjugation of phenolic compounds were an effective method to improve the functional properties for therapeutic applications.

1. Introduction

It is well-known about the value of phenolic compounds for their various health profits in areas of antioxidant and antimicrobial activity [1,2]. Dietary phenols have been received more attention to their many others biological functions such as anticarcinogens, anti-ageing, antimutagens, antidibetic and immunostimulatory action [3,4], which have led to their recognition as a potential nutraceuticals [5]. Phenolic compounds are simple and naturally occurring compounds containing one or several fused phenolic rings [6]. Presently, more than 8000 phenolic compounds have been identified and classified into four main groups – flavonoids, stilbenes, lignans, and phenolic acids [1]. On the basis of structure, it is divided into two major groups, simple phenolic compounds (containing single phenol ring) and complex phenolic compounds (containing more than one phenol ring) [7,1]. The basic structural features of simple phenolic compounds are bearing an aromatic ring with one or more hydroxyl groups and with or without short aliphatic side chain [3]. Various types of natural phenolic compounds such as hydroxycinnamic acid, hydroxybenzoic acid, ferulic acid, caffeic, *p*-coumaric acid, gallic acid, syringic acid, vanillin, syringic acid, benzoic acid, catechol etc. have occurrence in fruits, vegetables, cereals, rice bran etc. [8,9]. A number of studies have been appearing in recent literatures and reviews which had also highlighted their potential effectiveness as anti-cancerous and antitumor activity due to their lipophilic nature [2,7,10–14].

Agro-wastes are cheap and huge source of phenolic compounds [15,16]. Earlier, some studies have been reported on extraction and isolation of phenolic compounds from various types of agro-wastes residues [17–19]. In agro-wastes, most of the phenolic compounds can be generated by the lignin or lignans transformations by using various chemical methods [20,22]. Acid catalysis at high temperature & pressure has been proved to be most effective method, which generating various types of phenolic compounds from lignin degradation [21,22]. Therefore, chemical transformation of lignin in to the potential and useful phenolic compounds, it enhances

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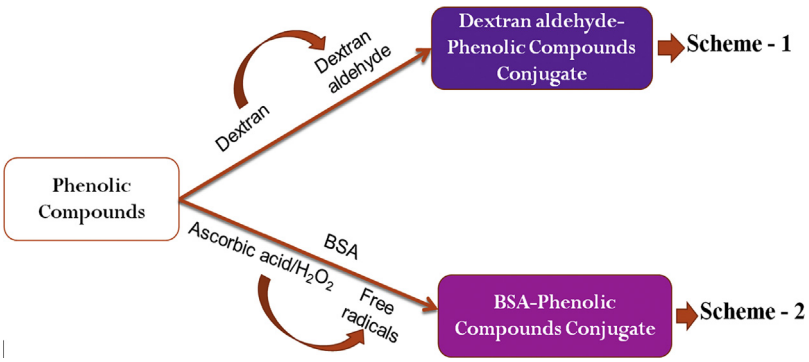

Fig. 1. Scheme for synthesis of phenolic compounds based conjugates with dextran and BSA.

the commercial and economic value of agrowastes. In spite of above medicinal value of phenolic compounds, it suffers from many drawbacks such as high cost, extraction process, solubility, direct consumption and instability when exposed with air, light, heat, in basic or acidic conditions [15,16].

To overcome above problems associated with utilization of phenolic compounds. Recently, some researchers have been successfully conjugated phenolic compounds (flavonoid) with many types of biopolymers such as chitin/chitosan [23,24], protein [25], dextran aldehyde [1,26], polylactic acid [27], starch [28], inulin [29], and alginate [30] which improved stability and effectiveness of phenolic compounds. Conjugation of phenolic compounds with bio-origin polymers also enhances value of conjugates. Very limited reports have been available on conjugation of phenolic compounds with two novel biopolymers such as dextran aldehyde [1,26] and bovine serum albumin [31,32]. Dextran is a natural and complex bacterial homopolysaccharides of glucose and composed of chains of varying lengths from 3 to 2000 kDa [32,33]. The straight chain of dextran is consisting of α-1,6 glycosidic linkages between glucose molecules, while branches to begin from α-1,3 linkages. Dextran has been widely used in various biomedical applications due to its biocompatibility, low toxicity and simple modification [1]. Dextran derivative like dextran aldehyde has been more preferably used in preparation of therapeutic functional materials [1,32]. Dextran is converted into dextran aldehyde via oxidation reaction in presence of sodium periodate [33–35]. Bovine serum albumin (BSA) also known as serum albumin protein produced from cows. Serum albumins are globular in nature and most abundant plasma proteins (about 52%) in the circulatory system [36]. The full-length BSA precursor is 583 amino acids (Mw 66–67 kDa) and its amino acid sequence contains 17 disulfide groups, one thiol group, and one tryptophan residue which have mutual binding potential towards many types of compounds [37]. BSA has various physiological functions such as contributing to the osmotic blood pressure, drug and others molecules carrier. Many drugs and other molecules bind reversibly to albumin [37].

In above described phenolic groups, phenolic acids such as ferulic, vanillic, syringic, cinnamic, benzoic acids etc. have anticancerous and antitumor attributes [2,3,6]. Phenolic acids with more number of hydroxyl group (-OH), degree of saturation of the carbon chain within the molecule and short aliphatic chain exhibits better anticancer activity compared to the ones with no hydroxyl groups [10–12]. The main cytotoxicity mechanism of phenols is due to their lipophilicity and pro-oxidant properties. In fact, this kind of phenolic acid becomes highly reactive within the cell [38]. Phenolic acids kill the cancer cell by disturbing the function of DNA binding protein, inhibiting the ATP formation in mitochondria, apoptosis and ROS generation inside the cell [39]. Conjugation of phenolic acids with biopolymers may enhance the desired therapeutic effect by administrating it in high concentration inside the cell which might create a new chemotherapy approach against cancer. Herein, we have first time described the conjugation of mixture of phenolic acids isolated from agro-waste, to dextran aldehyde and BSA via condensation and free radicals grafting reaction respectively. Prepared conjugate materials have been evaluated for their anti-cancer activity against cancer and normal cell line. The conjugates were tested *in vitro* against malignant (cancerous) cell lines (SW480, colon cancer) as well as in non-malignant (normal) cell line (CCD-18Co is a human female colon normal cell line) to assess both their cytotoxicity in cancer cells and their protection in normal cells.

2. Experimental section

2.1. Materials

Dextran (average molecular weight 10,000) and dialysis membrane with pore size of 2.4 nm were purchased from Himedia, India. Sodium periodate, folin-ciocalteu reagent, sodium carbonate, glacial acetic acid and organic solvents (dimethyl sulfoxide, DMSO and acetone) were purchased from Merck, India. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and bovine serum albumin (pH 6–7) fraction V crystalline powder were purchased from SRL, India. Hydrophobic resin (XAD-16), DMEM medium and alfa-MEM medium were purchased from Sigma Aldrich, India. Milli-Q grade water was used throughout the experiment.

2.2. Extraction and isolation of phenolic compounds

Agrowastes, sugarcane baggasse has collected from local areas of Allahabad, India. Collected biomass were dried in oven (42 °C) and fractioned in gridding machine, broken into a particle dimension between average lengths of ~3–2 mm (with sieve). Agro-waste (AW) was first dewaxed (to remove extractives) with toluene-ethanol in ratio of (2:1) by soxhlet apparatus. Acid catalysis has been applied for the degradation of lignin into phenolic compounds. Dried AW residues (1 gm) were dissolved in 10 ml of H_2SO_4 solution (0.3%, v/v). Then the mixture was transferred into a 20 ml Teflon-lined autoclave reactor and heated at 200 °C for a period of 3 h. After cooling, the solid and liquid fractions were separated by muslin cloth, centrifuged and pH was adjusted to 4.0 by 1N NaOH. Phenolic compounds were separated from liquid fraction by a hydrophobic resin (XAD 16). Before the utilization, resin was activated and washed with isopropanol and water (milli-Q) for 2 h. Resin was mixed (5%, w/v) with liquid samples (100 ml) in 250 ml flask at 30 °C, 150 rpm for 6 h in batch mode. Phenolic adsorbed resin was separated and filtered from liquid fraction. Resin was then washed with milli Q water twice and treated with acetone and water (50:50) for desorption of phenolic compounds. Phenolic

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