Ananas comosus loaded nanoemulsion a promising therapeutic approach for cancer

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Abstract
Cancer has a major impact on society across the world. There are different strategies such as chemotherapy, radiotherapy, surgical intervention, and immunotherapy for treating carcinoma. Nowadays, phytochemicals are gaining a lot of interest for their chemopreventive action in the treatment of carcinoma. Recent investigations demonstrated that extract/bioactive compounds from Ananas comosus (A. comosus) resulting in the reduction of inflammatory cytokines release and proliferation of T-cell was also inhibited. Therefore, A. comosus nanoemulsion have been proposed in the treatment of cancer. Based on previous literature, we have postulated that nanoemulsion of A. comosus may be beneficial because of its safety and efficacy profile.

Keywords: pineapple; nanoemulsion; cancer; targeted drug delivery; herbal; bioactive compounds
Introduction

Worldwide, cancer is second after cardiovascular disorder as the front runner cause of death [1, 2]. Developed and developing countries have an identical scenario of reported cases of carcinoma. Cancer is an aggressive disease and is hard to treat. This dynamic disease grows over a period with accumulation of new mutations [3, 4]. A number of new cancer cases rise over 10 million every year, with annual deaths over 6 million as per report of World Health Organization. Recent data indicates that the number of fresh cancer cases will be beyond 15 million by 2020 [5]. The treatment of carcinoma is powered with different strategies such as chemotherapy, radiotherapy, surgical intervention, and immunotherapy. The chemotherapy comprises of diverse antineoplastic drugs those have higher rate of anti-cancer cell activity [6]. Several side effects are associated with many anti-cancer drugs owing to nonspecific uptake such as the requirement of high doses, secondary malignancies, and molecular resistance phenotype [7]. Thereby, new methodologies are required, as tumors have reoccurrence in a short period of time afterwards maiden chemotherapy treatment [8]. These chemotherapeutic drugs have multiple stern effects on body. Natural plants have been employed in counteracting and treating various diseases for many countries. The natural agents are presently investigated as potential targets for exploration to be a potential target for the invention alongside the advancement of a lead compound in cancer prevention. Epidemiological, clinical, and pre-clinical studies are being conducted for cancer treatment with bioactive agents of medical importance [9–12].

Most anti-cancer approaches have adverse effects whereby natural occurring substances have the edge over usage being less toxic and more effective in the long terms. This has resulted in an abundance of natural anti-cancer agents for the treatment of various cancers as anti-cancer natural products [13, 14]. A. comosus (L.) Merr. is prominent viable fruit crop around the world that is available in various countries. This has been used as a medicine in various native cultures [15]. Pineapple aqueous and organic extract has many therapeutic qualities, and pineapple, as such is a fruit that has biological agents like ascorbic acid, minerals, proteins, lipoids, phenolic, carotenoids, and flavonoids which have treating capabilities [16, 17]. It has been reported to express assorted biological impacts in humans such as anti-oxidant, anti-diarrhoal, antidysslipidemic, anti-diabetic and cardioprotective actions. This also has been found a valuable chemotherapeutic power for prostate, colon and breast carcinoma [18–20]. Pineapple contains the enzyme bromelain (BL), which is a cysteine proteinase utilized for many therapeutic applications. The examples are inhibition of platelet aggregation and the anti-tumor action [21]. Nowadays pineapple extract/BL acceptance as significant bioactive among researchers is rising due to its advanced efficacy having no toxicity. Very few reports indicate that there exists anti-cancer activity of BL upon oral delivery [22]. Thus, activity against cancer of pineapple cannot be explored with traditional folk. Hence, there is a need for inventing a novel methodology for the delivery of BL by oral route. It is necessary to increase the concentration of BL at the site of the tumor for targeted drug delivery systems. To achieve the goal, an alternate drug delivery system was developed, which shall safeguard BL from acidic pH conditions and deliver it at the therapeutic dose more effectively. Hydrophilic and hydrophobic substances are very effectively used in newer drug delivery formulation having nanoparticulate bases [23, 24]. Chang and his coworker’s formulated anti-cancer nanoemulsion and found out the same effective in the treatment of lung cancer [25]. Phase 1 clinical trials are being conducted (ID: NCT03865992) for nanoemulsion have curcumin as anti-cancer drug [26]. Any possible drug interactions of multiple drug therapy can be easily avoided using a nanoparticulate based drug delivery system. This also prevents degradation in the gastrointestinal tract. Absorption of drug is enhanced. Thereby oral bioavailability is increased whereby toxic effects are reduced in target and non-target tissues [27, 28].

Role of BL in cancer

Cancer being an aggressive disease, which evolves and accumulates new mutations on progression. The treatment of cancer involves different approaches, which include chemotherapy, radiation therapy, surgery, and immunotherapy radiation therapy. Chemotherapy uses anti-neoplastic agents, which have higher rate of cancer cell killing but have a lot of adverse effects due to the lack of targeting [29–31]. Therefore, different anti-cancer therapeutic modalities are required, which possess more potent anti-cancer activity with fewer side effects. Improvements have been seen in the pharmacokinetics and dynamics of the drugs owing to the advancements in drug delivery overcoming drawbacks such as high systemic toxicity, poor bio-availability and stability [32–35]. However, drug delivery still faces difficulties in selectivity and specificity of the drug at the target site and immune clearance, which could be overcome by using a carrier system [36–38]. Several nanoemulsion based therapeutic agents have been developed since the last few years which offer improved efficacy and bioavailability, reduced toxicity, enhanced permeability, and retention (EPR) [39–42].

In recent years, many compounds derived naturally from plants have been explored for possessing significant anti-cancer properties [43]. One of compounds among these is the extract of pineapple which has displayed significant therapeutic activities. Pineapple extract or BL has been used for various beneficial effects such as anti-cancer, anti-inflammatory, and in absorption enhancement of antibiotics [44, 45]. BL or pineapple extract has become a potential candidate for anti-cancer activity due to being non-toxic and highly efficacious [46]. Few reports propose that BL anti-cancer activity upon oral administration is due to its protease components [46]. BL proteinases inhibit the in vitro metastases by altering the expression of CD44 on human SK-Me1 28 melanoma cells and Molt 4/8 leukemia cells [47].

Many literature studies have been conducted on A. comosus extracts, revealing that it affects a wide variety of biological systems [48]. Among these, anti-cancer activity requires further investigation [49–52]. Debnath, et al. showed improvement in the survival rate of induced lymphoma animals using BL peroxidase [53]. Recently, Raeisi et al. investigated a BL-based combination with cisplatin on human cancer cell lines and found promising effects on inhibition of cell growth and apoptotic induction on MCF7. Furthermore, similar results were observed with a BL-5-FU combination to AGS cells [54]. Bhuil et al. reported that depending on time, nick formation of DNA is decreasing and increase in the percentage of preventing tumour by using BL in tumor model namely 2-stage mouse skin. Therefore, BL can be used in a synergistic approach for achieving chemoprevention through modulating inappropriate cell signaling cascades [55]. Higashi et al. evaluated the capability of delivery of drug in pancreatic cancer. They have used the technology named Self-assembly PEGylation Retaining Activity technology for preparing reversible BL which was PEGylated having potent effects, extended retention in the blood and significant tumour accumulation [56]. Hayat et. al found that extracted BL from fruit stalk was most effective for inhibiting the proliferation in MCF7 and MDA-MB-231 breast cancer cell lines. The extracted BL prevented BC cells growth by upregulating the level of Bax, p53, expression of Cox-2, Bcl-2 get reduced and decreased in nuclear factor-kappa B (NF-kB) was also reported [57]. Raesi et al. demonstrated that BL produced dose-dependent inhibition and hampered colony formation on AGS, PC3, and MCF7 human cancer cells [58]. Park et al. findings compared cytotoxic effects exerted by BL on Kras mutant and Kras wild-type cells of colorectal cancer and found that ferroptotic cell death was due to the differential expression of ASCl-4 [59]. Romano et. al displayed the anti-proliferative and proapoptotic effects exerted by BL in cells of colorectal carcinoma and anti-cancer activity in colon cancer. It promoted apoptosis as well as decreased cell growth in cell line of colorectal carcinoma e.g. in Caco-2. The mechanism of action of BL was related to reduction of
reactive oxygen species production and downregulating the expression of ERK, pERK1/2-total, pAkt/Akt [60]. Amini et al. observed that pineapple extract exert anti-cancer effect in cell line of colon and human gastric carcinoma. Here pineapple induced apoptosis and disrupts survival of cancer cell by blockage of the Akt and attenuation of MUC1 oncoproteins and Bcl2 [61]. Another study by Bhui et al. showed BL facilitates apoptotic response by inducing autophagy in mammary carcinoma cells. BL induced positive regulation of p38 and JNK but negative regulation of ERK1/2 [62].

Hypothesis
This study hypothesizes: (1) To formulate nanoemulsion of extract or marker compound of A. comosa for the treatment of cancer. (2) The extract-marker compound with maximum activity will be subjected to pineapple nanoemulsion by sonoication/high-pressure homogenization using pharmaceutical experimental design. (3) Characterization followed by comparison of formulated nanoemulsion with marketed formulation. (4) Surfactants (ween 80, poloxamer 188 and others) used in the formulation of nanoemulsion of A. comosa.

Evaluation of hypothesis
Recently many studies emphasize that commonly used phytochemicals have shown potency in cancer protection against different types of human cancer [63]. Further 25% of drugs have been directly derived from plants since the last two decades whereas other of 25% are natural products which are chemically altered [64]. At therapeutic doses, these compounds pose no or very fewer toxic effects in the treatment of cancer. Among these natural phytochemicals, BL derived from pineapple is proteolytic enzymes with many activities like anti-metastatic and anti-proliferative in vitro and in vivo in various models of tumor.

Increased pieces of evidence affirm that pineapple extract is quite effective in treating cancer in a natural way instead of harmful treatments [64]. The fermented methanolic extract of pineapple demonstrated anti-cancer activity closed to the value of the doxorubicin drug against MCF-7, A549, and HCT116 cell lines [64]. BL from pineapple is a phyto-therapeutical drug among researchers due to its nontoxic nature [65]. Recent studies demonstrated that pineapple stem extract/BL delayed in and on set process of tumorigenesis. It also caused reduction in no of tumors, cumulatively. BL treatment resulted in induction of proteins related to apoptosis and expression of NF-kB-driven Cox-2 also inhibited by blockade of signaling of Akt/protein kinase B and MAPK in mouse skin tumors. These properties are responsible for its anti-cancer effect [66]. Eckert et al. performed clinical studies involving oral administration of BL in healthy volunteers and in breast cancer patients. In that study, they observed that there was stimulation of immunocytotoxicity of immune cells of cancer patients [67].

Sekor et al. demonstrated the cleavage CD25 effect of BL. Consequently, it would result in the potentiation of IL-2 regulator and benefit in treatment [68]. It is evident from the studies that BL modulate TGF-β which is an important regulator of cancer-induced immunosuppression and inflammation [69]. Furthermore, it has been reported that BL also stimulates the inherent immune system, producing reactive oxygen species and killing properties of tumour cells [70]. Pineapple also showed anti-thrombotic and fibrinolytic action. The amount of soluble fibrin in circulation was decreased due to fibrinolytic activity of BL. BL caused uncoating of the cells of tumour thus making it visible to immune system beneficial in treating in cancer [71].

From the other studies, it was also observed that BL possessed anti-platelet property which interfere with the progression of cancer and inhibit the formation of platelet aggregates of tumour [72]. From the various studies, we concluded that though mode of action of pineapple as anti-cancer is known, but therapeutic activity is on lower side because high concentration is required at tumour target site. Hence for increasing the potency of pineapple extract/BL for different tumour, concept of nanoemulsion has been made.

There are many studies based on in vitro or in vivo models or clinical trials, too, which have shown the activity of pineapple extract against cancer [22, 73–75]. In different ways, pineapple could be used to control or treat cancer. Extract of pineapple can be utilized against cancer by directly acting on cells of cancer or the environment of cancer, or by acting indirectly via modulating the immune system as immunomodulator or by acting on hematopoietic system. Amini et al. demonstrated the cytotoxic potential of pineapple in combination with other medicament on cell line of human gastrointestinal carcinoma. They reported that pineapple stem extract/BL in various cancers inhibited cell growth and proliferation. BL not only activated the caspase-dependent apoptotic pathways but also concomitantly inhibited cell survival [76, 77]. Raoet, et al evaluates the activity of pineapple extract on mouse breast cancer (4T1) cells under in vitro conditions. Tausig, et al observed that extract of pineapple plant showed anticancer activity in a dose dependent manner in cancer cell line e.g. MCA-1 acetinic tumour, Lewis lung carcinoma YC-8 lymphoma cells [78]. Hence foods containing BL or pineapple extract may be potential candidate for chemo-prevention of colorectal cancer [79]. Hence pineapple extract can be used in respect of other anti-cancer agents that have more side effects.

Pineapple extract loaded nanoemulsion can be prepared for increasing the uptake of nanomedicines in macrophages, endothelial-cells and cells of various cancers. It also has the ability to degrade the components of tumour extracellular matrix. Hence for better diffusivity, pineapple can be loaded in nanoemulsion. Nanoemulsion has gained much interest as a drug-delivery system because it allows the encapsulation of drug effectively that are poorly water-soluble and have biocompatibility and stability [80]. Nanoemulsion delivers the drug to cancer site because of their safety profile, offering a controlled release pattern, enhancing safety and stability, providing targeted drug delivery, and decrease toxic effects. Lipophilic anti-cancer drugs can easily be delivered by using nanoemulsion. Properties of nanoemulsion which makes it ideal candidate for efficiently delivering the drug in cancer include non-immunogenic, nanometric size, a larger surface area controlled/sustained release, and ease of method of preparation. After extensive literature survey, it was found that nanoemulsion overcomes the issue of bioavailability and non-compliance with conventional chemotherapeutical agents [81]. Nanomaterial based drug delivery induce cytotoxicity, kill various cancer cells and eliminate tumour [81]. Thus proposed A. comosa nanoemulsions can be a promising therapy for treatment of cancer.

Conclusion
Conventional chemotherapeutics are associated with no side effects and are also far away for the better management of cancer. By considering the abovementioned studies, we can say that extract or marker compounds derived from A. comosa play a vital role in cancer treatment. Moreover, nanoemulsion has good potential for delivering the active compound at the target site. Thus, proposed nanoemulsion of A. comosa might be a potential role in the treatment of cancer.

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