INTRODUCTION

Unmitigated increase in the incidence of cancer and the incomplete success with the chemotherapy has presented the lookout for an effective but safer anticancer drug. The past decades have assessed the dietary role for modern diseases and indicated a strong relationship between the diet and the ailment via both population studies and experimental reports. Specific dietary designs have been proposed for various ailments among different human communities. Hence, different sources for the known dietary principles have been hunted for in the recent past which provides an economic and efficient supply of the same [1,2].

Although plenty of drugs were explored for cancer treatment, the clinical relevance of drug development for a routine practice remains distant due to the high costs and side effects. Inflammation dictates the initiation and progression. The n-3 fatty acids demonstrate anti-inflammatory effects in vivo. However, there is a limitation of data about the cancer preventive role of fatty acids however they have been demonstrated to preserve the muscle mass and function in chemotherapeutic subjects [3]. The n-3 fatty acids like eicosapentaenoic acid and docosahexaenoic acids have been found to reduce the risk of breast cancer [4]. A new concept of a combination of chemotherapy and nutrition therapy is emerging.

Polysaturated fatty acids have been claimed to enhance the membrane dynamics. Hence, the concentration of the former is usually associated with the mitochondrial function. Several reports suggest a strong anticancer property for polysaturated fatty acid (PUFA) in vivo and in vitro [5]. Accordingly, we selected chia (Salvia hispanica) a vegetarian PUFA source and set out to study its biological properties using the accepted in vitro models.

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RESULTS AND DISCUSSION

Many experimental evidence suggests that inflammatory pathways predominmate the pathophysiology of major metabolic syndromes such as diabetes and cardiovascular diseases (CVD). It has been noted that obesity and comorbidities such as diabetes and CVD are resulted from chronic, low-grade inflammation impacting multiple organ systems [22]. The compromised inflammatory state is usually attributed to higher levels of proinflammatory signaling from adipocytes. Dietary intervention studies have indicated that n-3 PUFA rich diet alleviates the metabolic syndromes through attenuating the inflammatory status of the system [23,24]. Hence, it was interesting to study the anti-inflammatory properties of Indian chia seeds in an in vitro setup. Even though chia is not used as a major food component in today’s diet globally, owing to the high content of alpha linolenic acid (ALA), chia is speculated to become an excellent dietary adjuvant shortly. Hence, in this study, we fortified chia oil with the other major edible vegetable oils such as PO, OIO, and SO for the anti-inflammatory assays. It was assumed that fortification with the other edible oils may replicate the changes in the beneficial effects of CSO when it is used in association with the regular diet [23]. Fortification with the other vegetable oils is a mandatory method of activity assessment in case of not so frequently used food products like chia since this method reveals the complementary effects of these dietary components [26]. In this study, we examined the anti-inflammatory property of CSO per se and in synergy with the other vegetable oils employing in vitro LOX assay.

In vitro anti-LOX activity

The anti-inflammatory property of CSO was assessed by estimating inhibition of LOX activity in vitro (Fig. 1). Enzymes like LOX actively participate in the inflammatory reactions in vivo. These are autocalytic enzymes which are activated by numerous factors. Therefore, studying the inhibitory effects on LOX reveal a better picture of the biological activity of the oil blends. For this purpose, the crude extract of LOX was prepared from soybeans. Incubation with CSO alone significantly inhibited the LOX activity in a concentration-dependent manner (up to 63%). The LOX inhibitory properties of the other oils individually were not different than that of CSO. However, in combination groups, CSO slightly increased the inhibition among blends of soybean and PO.

Previous reports suggest that blending of different oils resulted in better storage and improved antioxidant properties in vitro [27]. Moreover, health benefits from functional foods as chia seeds are usually conspicuous on a chronic supplementation. The results of our study are in contrast with a few reports about anti-inflammatory properties of ALA and CSO among in vivo systems.

Fig. 1: Effects of oil samples on the activity of lipoxygenase in vitro.

Values are mean±standard deviation (n=3 replications for each sample and concentration) analyzed by one-way ANOVA followed by post-hoc Tukey’s test. Data are pooled from three independent experiments (*, # and $ indicate significant difference at p<0.05)
In vitro anticancer property against CM leukemic cells

MTT is a most accepted assay to estimate the viability of the cells in vitro [28]. Among all the oil samples tested for anticancer effect, chia oil demonstrated highest cytotoxic efficacy (up to 90%) followed by olive and SOs (Fig. 2) against the human CM cells. The IC50 of CSO was 5.32 µl (Table 1) which was slightly increased in the blends of palmolein and SOs (up to 12.07 µl). Although comparatively the cytotoxic effects of PO was marginally lesser when compared to other oil samples, but in combination with CSO, the cytotoxic property was enhanced. Similar results were observed among SO alone and blend with chia oil. Contrast to MTT assay, in trypan blue assay; the oil samples demonstrated a slightly different trend of anticancer activity (Fig. 3). Based on trypan blue assay, CSO alone inhibited CM cells proliferation significantly (up to 67%), and this effect was similar to OIO alone. Palmolein and SOs individually did not affect the cancer cells. However, CSO in blending with PO and SO the anticancer activity was observed up to 50%. As evidenced from the data, the enhanced anticancer activity among these blends is totally owed to CSO.

In vitro anticancer property against HeLa cells

Further, the cytotoxic property of CSO alone and in combinations was assessed in HeLa cells also. HeLa cells are the human cervical cancer cells and are typical of their kind. HeLa cells are the oldest used human-derived cancer cells for the research purpose. These cells are widely employed to assess the anticancer activity of possible potential drug compounds [29]. In addition, these cells are also employed to study the mechanistic pathways of those active principles. As anticipated, CSO per se significantly inhibited proliferation of HeLa cells as evidenced by the MTT assay (Fig. 4). The IC50 of CSO was 11.46 µl (Table 1). Interestingly, the cytotoxic effects were similarly significant in combination groups with OIO, however, was not up to the extent of CSO alone. Further, though individually palmolein and SO did not demonstrate anticancer activity, but in combination with CSO, they showed significant inhibition of cancer cell proliferation. The IC50 concentration of olive, palmolein and SOs individually were 72.46, 167.79, and 126.26 µl; however, the values decreased significantly in combination with the chia oil in their blends and were 18.7, 30.3, and 24.18, respectively (Table 1). In addition, the trypan blue assay confirmed the results obtained in MTT assay. In trypan blue assay, CSO alone inhibited HeLa cell proliferation significantly (Fig. 5). In combination, chia oil enhanced the activity of palmolein and SO marginally. Evidently, the results obtained from trypan blue assay were similar to that of MTT assay.

In vitro anticancer property against MCF-7 cells

The anticancer activity of CSO was confirmed using the breast cancer cells, namely, MCF-7. The MCF-7 breast cancer cells represent the metastatic cells with the highest virulence and are experimentally assessed in HeLa cells also. HeLa cells are the human cervical cancer cells and are typical of their kind. HeLa cells are the oldest used human-derived cancer cells for the research purpose. These cells are widely employed to assess the anticancer activity of possible potential drug compounds [29]. In addition, these cells are also employed to study the mechanistic pathways of those active principles. As anticipated, CSO per se significantly inhibited proliferation of HeLa cells as evidenced by the MTT assay (Fig. 4). The IC50 of CSO was 11.46 µl (Table 1). Interestingly, the cytotoxic effects were similarly significant in combination groups with OIO, however, was not up to the extent of CSO alone. Further, though individually palmolein and SO did not demonstrate anticancer activity, but in combination with CSO, they showed significant inhibition of cancer cell proliferation. The IC50 concentration of olive, palmolein and SOs individually were 72.46, 167.79, and 126.26 µl; however, the values decreased significantly in combination with the chia oil in their blends and were 18.7, 30.3, and 24.18, respectively (Table 1). In addition, the trypan blue assay confirmed the results obtained in MTT assay. In trypan blue assay, CSO alone inhibited HeLa cell proliferation significantly (Fig. 5). In combination, chia oil enhanced the activity of palmolein and SO marginally. Evidently, the results obtained from trypan blue assay were similar to that of MTT assay.

Table 1: IC50 concentrations of oil samples (µl) and standard drug compounds (ng) calculated from the MTT assay among the cancer lines

| Samples  | CM cells (µl) | HeLa cells (ng) | MCF-7 cells (ng) |
|----------|--------------|-----------------|-----------------|
| Sample 1 | 9.90±0.10    | 15.06±0.15      | 10.42±0.10      |
| Sample 2 | 5.32±0.05    | 11.46±0.11      | 17.24±0.17      |
| Sample 3 | 9.71±0.10    | 72.46±0.72      | 10.30±2.41      |
| Sample 4 | 49.50±0.50   | 167.79±0.68     | 51.00±2.50      |
| Sample 5 | 13.75±0.14   | 126.26±1.26     | 252.00±2.50     |
| Sample 6 | 5.56±0.06    | 18.70±0.19      | 64.94±0.65      |
| Sample 7 | 11.51±0.12   | 20.33±0.20      | 11.00±1.00      |
| Sample 8 | 12.07±0.12   | 23.35±0.23      | 51.10±5.00      |
| Sample 9 | 5.82±0.06    | 30.30±0.30      | 33.70±0.34      |
| Sample 10| 5.82±0.06    | 61.32±0.61      | 79.62±0.80      |
| Sample 11| 11.11±0.11   | 126.26±1.26     | 257.00±2.50     |
| Sample 12| 6.35±0.06    | 24.18±0.24      | 26.23±0.26      |
| Sample 13| 6.04±0.06    | 39.23±0.39      | 45.60±0.46      |
| Sample 14| 15.63±0.16   | 57.06±0.57      | 72.46±0.72      |
| Standard | 10.42±0.10   | 10.20±0.10      | 10.31±0.10      |

Values are mean±SD (calculated from three independent experiments using the Prism software). SD: Standard deviation, MTT: (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), CM: Chronic myelogenous leukemia, CO: Chronic myelogenous leukemia.

Fig. 2: Effects of oil samples against viability (or proliferation) of human chronic myelogenous leukemia cells in vitro (48 hr exposure). Values are mean±standard deviation (n=3 replications for each sample and concentration) analyzed by one-way ANOVA followed by post-hoc Tukey’s test. Data are pooled from three independent experiments (*, # and $ indicate significant difference at p≤0.05).

Fig. 3: Effects of oil samples against human chronic myelogenous leukemia cells in vitro (trypan blue exclusion method). Values are mean±standard deviation (n=3 replications for each sample and concentration) analyzed by one-way ANOVA followed by post-hoc Tukey’s test. Data are pooled from three independent experiments (*, # and $ indicate significant difference at p≤0.05).
Dietary PUFAs have been attributed numerous health benefits if consumed on a regular basis. Scientific reports strongly suggest the beneficial effects of chia on human health owing to its high PUFA content. Interestingly in an isolated study, feeding hens with chia seeds resulted in eggs with highest ALA content when compared to hens fed with linseed or rapeseed [30]. In addition, rats fed on chia seed rich diet demonstrated a decrease in low-density lipoprotein and serum triglycerides, in contrast, high-density lipoprotein and n-3 PUFA levels were elevated [31]. It was also observed that no adverse effects were observed on the rat’s thymus and IgE serum level. In a similar study, pigs and rabbits fed with chia seeds resulted in an increased of PUFA content, flavor and aroma in the meat fats [32]. At present, a major part of dietary PUFA is obtained from marine sources. However, the psychological stigma of people about biomagnification of some heavy metals and pesticides in addition to the disapproving odor, hinder them from consuming the fish based supplements. Moreover, a typical organoleptic characteristic such as flavor and smell from marine sources were not found in chia making it a desirable vegetable source for PUFA since ALA is converted enzymatically to PUFA in vivo [31]. In addition, global conscience about the vegetarian diet has compelled the food industry to watch out for vegetable sources of the n-3 PUFA. Thus, the excellent biological activities prove a better market presence for chia and make chia a prime candidate as a health supplement for improving the food quality.

Our study clearly demonstrates the potent anti-cancer property of CSO as estimated with reduced MTT reduction among CM leukemia, HeLa, and MCF-7 cells. In addition, the anti-inflammatory property of CSO and its blends was confirmed with the inhibition of LOX in vitro. Our results provide evidence that CSO alone or in combination with the vegetable oils proves to be a healthy synergistic supplement or an adjuvant for this diet. The supplementation with CSO is suggested for the modern lifestyle and the unhealthy diet to delay or prevent the incidence of degenerative disorders among humans.

ACKNOWLEDGMENT
The author Dr. Ramzi Abdulkhaleq Gazem expresses his thanks to IBB University, Yemen, for the financial assistance and to Scintilla BIO-MARC Pvt.Ltd, Bengaluru, India, for cell culture assistance.

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