IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 2, Issue 4 (May. – Jun. 2016), PP 33-39
www.iosrjournals.org

Hypolipidemic, Hypoglycemic and Antiproliferate Properties of Chia Seed Oil and its blends with Selected Vegetable Oils-An in-vitro study

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Abstract: *Numerous studies conclude that inflammatory pathways predommint the pathophysiology of major metabolic syndromes like of diabetes and cardiovascular diseases (CVD). In particular, pancreatic lipase is increased during diabetes and CVD pathology. There is a gradual hepatotoxicity associated with the modern allopathic drugs though efficiently maintain the blood glucose levels but on a long run injure the vital tissues like kidney and liver. Rich content of n-3 fatty acids like α-linolenic acid (ALA) render chia (Salvia hispanica L.), as a potential candidate for metabolic disorders. In our study, chia seed oil and its blends with the selected vegetable oils such as olive oil, palmolein oil and soybean oil reduced the glucose uptake in porcine diaphragm and also reduced gluconeogenesis in hepatocytes. Further, chia seed oil reduced the lipase activity in vitro. Interestingly, our data indicated a strong cytotoxic effects of chia seed oil and combinations against Human lymphoblastic leukemic (CEM) cancer cells. Together, our results suggest a potential therapeutic role for chia seed oil and its combinations with other vegetable oils which leads to promoting it as a functional food.*

Keywords: *hypolipidemic, Gluconeogenesis, diaphragm, Anti-proliferation.*

I. Introduction

The high percentage of α-linolenic acid (ALA, n-3 fatty acid) makes chia (*Salvia hispanica L.*), a possible representative as a food medicine. Chia was a major dietary component among ancient Aztec and Mayan civilizations [1, 2]. The edible oil obtained from chia seeds is a rich source of n-3 fatty acids and antioxidants like polyphenols, chlorogenic and caffeic acids, myricetin, quercetin, kaempferol [3, 4]. Accordingly, the major therapeutic properties of chia seeds are owed to its ALA content and the antioxidants.

It has been noted that obesity is usually associated with metabolic syndromes like diabetes and CVD though cannot be generalized. These ailments are resulted from chronic and mild inflammatory reactioss affecting the multiple organ systems [5]. The compromised inflammatory state is usually attributed to higher levels of pro-inflammatory signaling from adipocytes [6]. Lipases play a pivotal role in the inflammatory status of the cells. Pancreatic lipases in particular catalyze the breakdown reactions of dietary fat into simpler fatty acids which are absorbed into the system. Increased activity of lipases is associated with diabetes and CVD [7]. Moreover, hyperglycemia due to either increased glucose uptake or increased gluconeogenesis is the key character of type II diabetes. Epidemiological studies indicate a strong association between regular consumption of n-3 fatty acids and reduced lipid abnormalities in type-2 diabetes mellitus [8]. Dietary intervention studies have indicated that n-3 PUFA rich diet alleviates the metabolic syndromes through attenuating the inflammatory status of the system [9, 10].

Owing to the abundance of PUFA fatty acids derived from ALA and antioxidants, chia seeds possess significant anti-inflammatory and antioxidant properties in vivo. Scientific reports strongly recommend the use of oral chia supplements for inflammatory related ailments, however protocols regarding extraction and effective dose, should be standardized in order to suit the human consumption. Historical citations indicate that chia was a food additive. Hence, in this study we fortified chia oil with the other edible vegetable oils like palmolein oil, olive oil and soybean oil for the biological assays. The blending with other oils was performed in order to study the effect of combination of chia seed oil with the oils containing different fatty acid composition. Fortification with the other edible oils induces a positive change in the health benefits of chia seed oil [11].

Dietary n-3 PUFA have been suggested for reduced risk of various type of cancers including melanoma via direct cytotoxicity or anti-inflammatory pathways [12]. This may be due to a collective beneficial properties of these fatty acids. However, with chia seed oil, abundance of bioactive principles along with ALA leads to a prediction that it may act as a potent anti-cancer therapeutic application with no toxicity when compared to the rigourous side effects of the current chemotherapeutic interventions.

Accordingly, our initial study the proximate parameters of the Indian chia seeds were assessed followed by physicochemical properties of the chia seed oil. Additionally, we demonstrated the anti-inflammatory properties of chia seed oil in vitro [13]. Further in this study, hypolipidemic, antidiabetic and anti-

cancer activities of chia seed oil per se as well as in combination with the selected vegetable oils were investigated.

II. Materialsand Methods

2.1. Materials:

Seeds of chia (*Salvia hispanica L.*) *were* procured locally from Hegdadevana (HD) Kote, Mysuru, Karnataka, India. The sample was authenticated by the Department of Studies in Botany, Manasagangotri, Mysore. The seeds were cleaned to get rid off impurities/damaged seeds; stored at 4 ˚C. The sound seeds were powdered and the oil was extracted [13] and used for the analysis.The selected vegetable oils such as sunflower oil, olive oil, palmolein oiland soybean oil were purchased from local market in Mysuru city, Karnataka.

2.2. Chemicals and reagents:

Insulin was procured from Torrent pharmaceuticals LTD; India, GOD-POD reagent from Aspen Laboratories, India and HBSS (Hank's Balanced Salt Solution) with sodium bicarbonate was procured from HimediaIndia. Bovine serum albumin, tris buffer, linoleic acid, lipoxidase were purchased from SRL, India. Diclofenac sodium was purchased from Cipla pharmaceuticals, India. Enzymes, all solvents and other chemicals used in the studies were of analytical grade and purchased from SDFCL, Mumbai, India.

2.3. Animals:

Animal studies were approved by the Institutional Animal Ethics Committee, University of Mysore [Approval No. UOM/IAEC/18/2012], Mysuru, as stated by prescribed guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4. Cell lines and culture

The cancer cell line used in this study was Human lymphoblastic leukemic cell lines (CEM cells) and wasprocured from the National centre for Cell Science, Pune, India. Cells were grown in RPM1 1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 U/ml of penicillin and 100 µg of streptomycin/ml and incubated at 37˚C in a humidified incubator with 5% CO_2.

2.5. Preparation of oil blends

The selection of vegetable oils for the combination studies were based on our previous study [13]. Olive oil and soybean oil are common cooking oil in western population while palmolein is a common Indian cooking oil. The oils used in this study are sunflower oil (standard), olive oil (rich in monounsaturated fatty acid), palmolein oil (rich in saturated fatty acid) and soybean oil (rich in n-6, linoleic acid). Blending of oils was conducted by taking mixture of oils in required proportions as indicated below in a beaker and was blended by using magnetic stirrer for 15 mins and used for the experiments.

The chia oil was tested either alone or in combination with other oils mixed in the ratio 1:3, 1:1 and 3:1 were prepared by using 10% DMSO and total samples tested were fourteen in number as follows: Sample 1-[Sunflower oil (SFO)], Sample 2-[Chia seed oil (CSO)], Sample 3-[Olive oil (OlO)], Sample 4-[Palmolein Oil (PO)], Sample 5-[Soybean oil (SO)], Sample 6-[CSO:OlO (75:25)], Sample 7-[CSO:OlO (50:50)], Sample 8-[CSO:OlO (25:75)], Sample 9-[CSO:PO (75:25)], Sample 10-[CSO:PO (50:50)], Sample 11-[CSO:PO (25:75)], Sample 12-[CSO:SO (75:25)], Sample 13-[CSO:SO (50:50)] and Sample 14-[CSO:SO (25:75)].

2.6. Hypolipidemicactivity:

2.6.1. Inhibiton of pancreatic lipase

Hypolipidemic activity of chia seed oil and its combinations was tested by assessing the inhibition of Pancreatic lipase activity[14]. Oil samples (S_1-S_{14}) were individually incubated with 1ml of reaction mixture containing 1Unit of lipase enzyme, 100mM phosphate buffer (pH 7.2) with triton-X-100 (0.5%). The enzyme reaction was started by adding p-nitro phenyl butyrate (5mM in acetonitrile) and monitored at 340nm. A control tube was maintained without any oil sample and the results were expressed as percent activity.

2.6.2. Inhibition of glucose-6-phosphate dehydrogenase activity

Glucose-6-phosphate dehydrogenase activity was measured by monitoring reduction of NADP at 340nm [15]. Oil samples (S_1-S_{14}) were individually incubated with 1ml of reaction mixture containing 0.5 Unit of enzyme, 50mM TrisHCl (pH 7.4) and 0.1mM Glucose-6-phosphate. The enzyme reaction was started by adding 0.15 mM NADP and monitored at 340nm. A control tube was maintained without any oil sample and the results were expressed as mean nmol NADP/ min from triplicate analyses.

2.6.3. Inhibition of malic dehydrogenase activity

Malic dehydrogenase activity was measured by monitoring reduction of NADP at 340nm [16]. Oil samples (S_1-S_{14}) were incubated with 1ml of TrisHCl (100mM, pH 7.4) containing 0.5 Unit of Malic dehydrogenase enzyme, L-malate (10mM) and $MnCl_2$ (2mM). The enzyme reaction was started by adding 2mM NADP and monitored at 340nm. A control tube was maintained without any oil sample and the results were expressed as mean nmol / min from triplicate analyses.

2.7. Antidiabetic studies

2.7.1. Glucose uptake assay by porcine diaphragm

Porcine diaphragm system was used for glucose uptake assay [17]. Porcine diaphragm was obtained from the local slaughter house and thoroughly washed using ice cold saline to remove the blood stains. Clean diaphragm was used to investigate the inhibitory effects on the glucose uptake process by oil samples (S_1-S_{14}) dissolved in DMSO at 20 and 40 µl concentrations respectively. Diaphragm weighing around 100 mg was suspended in a 24 well culture plate containing 0.5 ml saline, and 0.2% glucose was added to each well to initiate the reaction. DMSO alone served as vehicle control. Further, insulin (0.4 units) was added to each well to enhance the glucose uptake by the diaphragm and the final volume was maintained at 2 ml using saline. Culture plates were incubated for 30 min at 37˚C (with 100% O_2). The quantity of glucose formed in the culture plate was assayed using the GOD-POD kit according to the instructions of the manufacturer.

2.7.1. *In-vitro* gluconeogenesis assay in isolated rat liver slices

Adult male albino rats (fasted overnight) were euthanized by cervical dislocation according to the university animal ethics committee directive (letter no. UOM/IAEC/18/2012). The liver excised was washed in ice cold saline and stored on ice. Slices of livers were cut as previously described [18, 16] and were weighed using a digital balance. Approximately 100 mg slices were placed in the wells of 24well plates containing HBSS medium and pyruvate (10mmol/l). Oil samples (S_1-S_{14}) were dissolved in DMSO at different concentrations (20, 40 and 80 µl) were introduced into the wells. DMSO treated plates served control and insulin (1mmol/l) was used as standard. The culture plates were incubated at ambient temperature (37 ˚C) for 60 min. Aliquots of spent HBSS were collected from the culture plates at timely intervals (0, 30 and 60 min). The extent of glucose formed was estimated using the GOD-POD kit according to the instructions of the manufacturer.

2.8. Antiprolifirate property

2.8.1. Cell viability assay (MTT assay)

The cytotoxic effect of the oil samples against the CEM leukemic cells ($5x10^5$ cells) was assessed using 3-(4, 5 dimethyl-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) assay [19]. Cells in a 96 well plate were incubated with the samples (prepared in 10% DMSO) in triplicates and control groups were incubated with only vehicle. After 48 h treatment, the cells were incubated with MTT (0.5 µg/ml) for 2h at 37˚C.The medium was discarded and the blue MTT formazan precipitate formed in the viable cells is solubilized by the addition of 100 µl DMSO. The suspension is placed in microvibrator for 5 min and absorbance was measured at 540 nm using multimode reader (Varioskan Flash Multimode, Thermo scientific, USA).

2.9. Statistical Analysis:

The data obtained were analyzed using Graph pad software prism 5.1 and excel software. The data were expressed as mean±standard deviation and all experiments were compared with control and performed in triplicates.

III. Results And Discussion

3.1 Hypolipidemic activity:

3.1.1. Enzyme activity: Pancreatic lipase

Hypolipidemic activity of oil samples was assessed by estimating their inhibitory effect on the pancreatic lipase activity in vitro. Pancreatic lipase activity usually increases with diabetes and CVD leading to increased absorption of fatty acids from dietary fat[14, 20] inducing an increase in the free saturated fatty acids.Interestingly, SFO, OlO and SO individually inhibited the lipase activity on a concentration dependent manner (Fig 1). Though, chia seed oil and palmolein oil individually did not affect the lipase activity but in combination of both oils there was significant inhibition (S_9 to S_{11} : 9-41%). Further, the lipase inhibitory activity of SFO, OlO, SO per se were not compromised when in combination with chia seed oil. Our previous reports indicated that chia seed oil has potent anti-inflammatory activity *in-vitro* [13].This suggests that chia seed oil does not alter the hypolipidemic activity of the other selected vegetable oils when blended together. Therefore, in addition to the health benefits of ALA of chia seed oil the hypolipidemic activity of the oils in the blend are also unaffected indicating that chia seed oil is a safe adjuvant.

3.1.2. Enzyme activity: Malic dehydrogenase and glucose-6-phosphate dehydrogenase

Malic dehydrogenase (MDH) and glucose 6 phosphate dehydrogenases (G6PDH) activity in serum usually decrease with the severity of diabetes [15]. Hyperglycemia is associated with the decreased activity of these enzymes [21]. Reduced MDH activity results in reduced malate/succinate shuttle mechanism leading altered carbohydrate dependent energy metabolism [22]. In this study, the oil samples used affected the MDH activity in vitro to a variable extent (Table 1).There was a marginal increase in the activity in S_2 and S_3 samples (only 11%). However, there was a significant increase in G6PDH activity among S_2 and S_3 (14%). Interestingly rest of the samples had no significant effect on the activity levels in vitro. The activity levels of glycolytic enzymes in the hepatocytes are rigid to alter as it is the ultimate energy production machinery. However, in diabetic conditions these activity levels are compromised forcing alternate pathways for utilizing the glucose. Normal activity with oil samples suggest a less harmful treatment for diabetic dysfunctions though these results call for further studies in vivo.

3.2. *In-vitro* hypoglycemic effects

3.2.1. Effect on glucose uptake by Porcine diaphragm

Being a striated muscle sheath, diaphragm provides an excellent system to study the glucose disposal among the animal tissues. *In-vitro* glucose uptake assay using porcine diaphragm showed increased utilization of glucose by different oil samples (Fig 2). The effect on glucose uptake was predominent among S_2 and S_{6-8} (upto 30%) suggesting the hypoglycemic effect of chia seed oil per se and in combination with olive oil. Interestingly other combinations did not alter the glucose uptake significantly. However, the hypoglycemic effects of the oil combinations were markedly enhanced (upto 50%) in presence of insulin. This indicates at a possible interaction of oil combinations with insulin and hypoglycemic machinary.

***3.2.2. In-vitro* gluconeogenesis studies on isolated rat liver slices:**

The oils (S_{1-14}) were tested for their hypoglycemic activity by estimating their effect on the gluconeogenesis in rat liver slices (Fig 3).Positive control Insulin (1mmol/l) as anticipated had an inhibitory effect (82%) on gluconeogenesis with only 18% glucose production. S_2 and S_3 (Chia and Olive oils) showed upto 35% inhibition of gluconeogenesis individually. The effect of other vegetable oils was marginal. Interestingly, though S_4 and S_5 did not alter the glucose production when exposed individually, in combination with chia seed oil reduced the gluconeogenesis upto 21%. There was a marked and significant inhibition of gluconeogenesis in chia combination with olive oil (S_{6-8}= upto 41%).

Gluconeogenesis is an essential pathway in restoring the blood glucose levels. Hence, inhibition of gluconeogenesis in hepatocytes is a promising approach for an antidiabetic drug [23]. Though the individual oil samples(S_2 and S_3) demonstrate marked inhibition, their combinations had no different effects (S_{6-14}). In addition, palmolein and soybean oils did not affect the gluconeogenesis however, they did not alter the beneficial effects of chia when in combination. Therefore, this indicates that the blending of less healthy but commercially cheaper oils with chia seed oil would increase the efficiency in terms of their hypoglycemic effects.

3.3. MTT assay for assessing cell viability

MTT is a most accepted assay to estimate the viability of the cells in vitro [24].Among all the oil samples (S_1-S_{14}) tested for anticancer effect, S_2 demonstrated highest cytotoxic efficacy (upto 90%) followed by S_3 and S_5 (Fig 4). Though comparatively the cytotoxic effects of palmolein oil (S_4) was marginally lesser when compared to other oil samples, but in combination with chia seed oil (S_{9-11}) the cytotoxic property seems to be enhanced. Same is true with S_5.Interestingly, the anti cancer activity of these oil combinations suggest a potent food adjuvant role for chia in the futuristic diet.

IV. Figures And Tables

Fig. 1: The hypolipidemic actitivity of different oil samples (S_1-S_{14}) as assessed by inhibition of pancreatic lipase activity

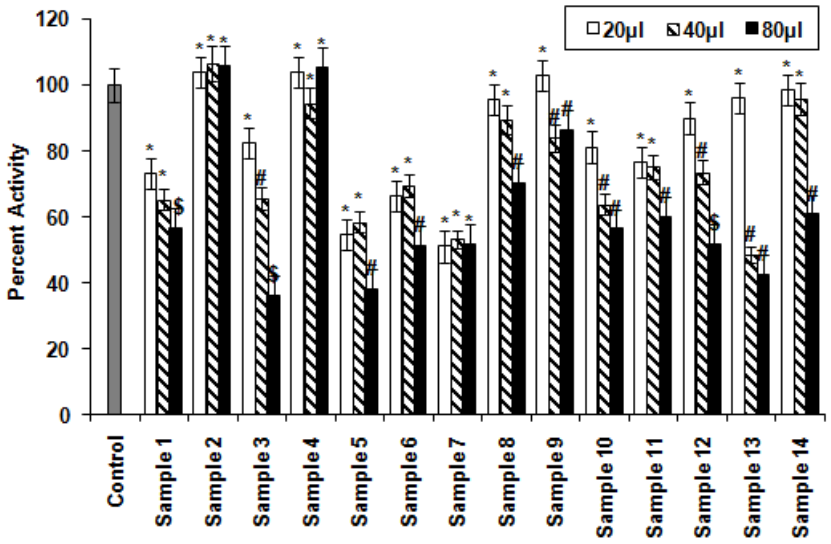

Fig. 2. 1. Effect of oil samples on glucose uptake (S_1-S_{14}) in porcine diaphragm

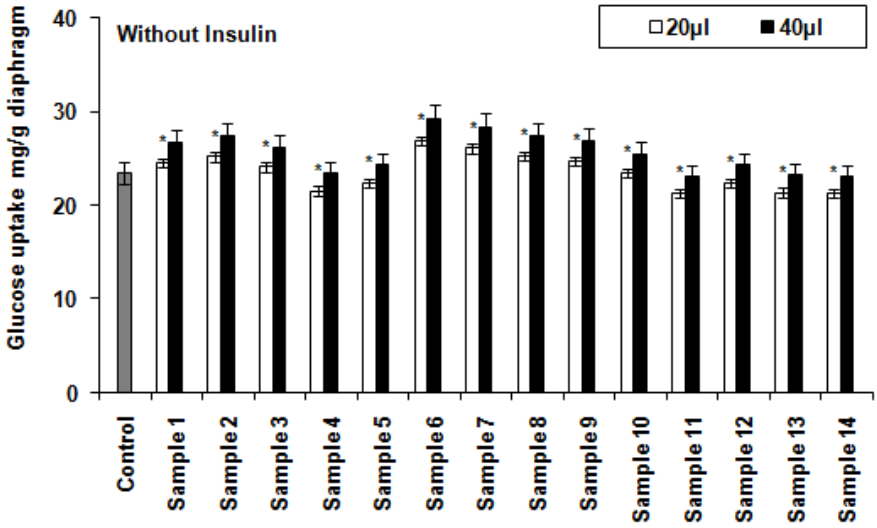

Fig. 2. 2. Effect of oil samples on glucose uptake (S_1-S_{14}) in porcine diaphragm

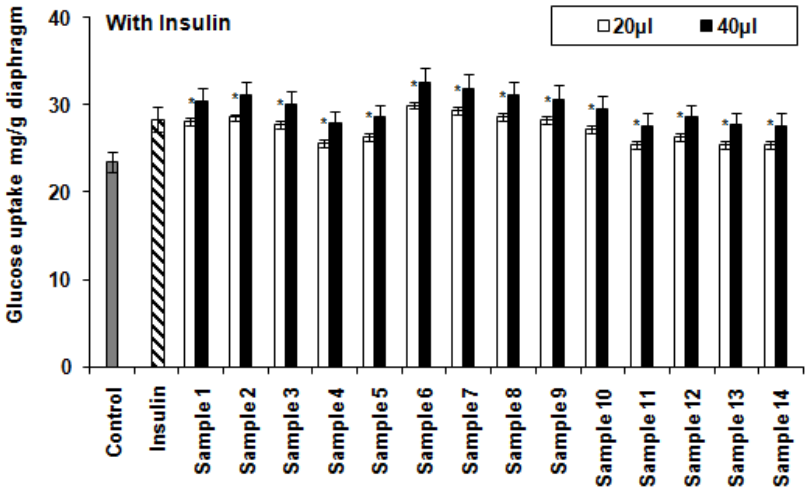

Fig. 3. The hypoglycemic effects of chia seed oil alone and in combination (S_1-S_{14}) on gluconeogenesis in rat liver slices.

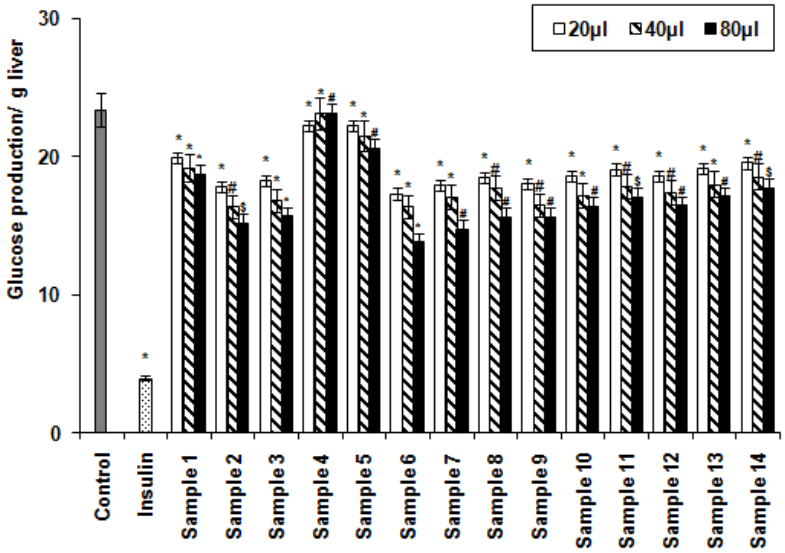

Fig. 4. Effects of oil samples against viability (or proliferation) of Human CEM leukemic cells *in-vitro* (48h exposure).

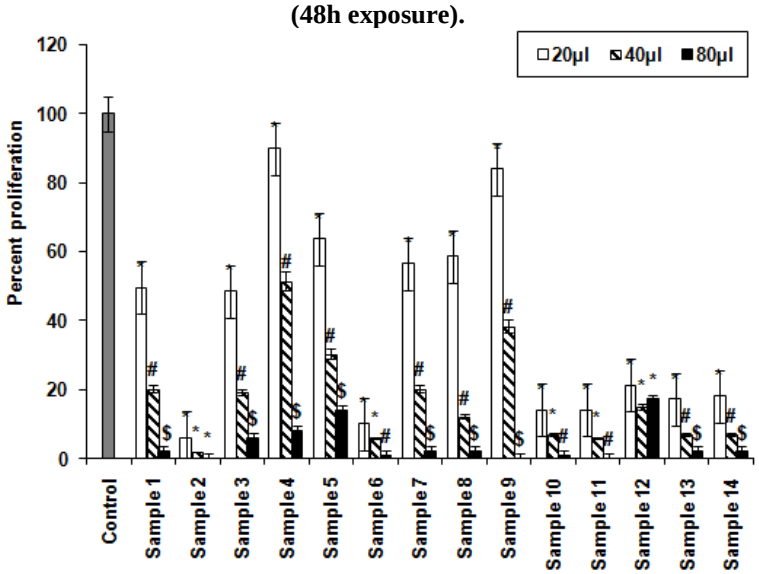

Table 1. Inhibitory effects of oil samples on malic dehydrogenase and glucose 6 phosphate dehydrogenase activity.

	Malic dehydrogenase (Mean nmol/ min ± SD)	**Glucose-6-phosphate dehydrogenase (Mean nmol/ min ± SD)**
Control	5.44 ± 0.18	7.62 ± 0.11
Sample 1	5.33 ± 0.21	7.46 ± 0.14
Sample 2	6.09 ± 0.22	8.53 ± 0.13
Sample 3	6.13 ± 0.15	8.58 ± 0.19
Sample 4	5.28 ± 0.26	7.39 ± 0.17
Sample 5	5.49 ± 0.27	7.69 ± 0.18
Sample 6	5.33 ± 0.23	7.46 ± 0.21
Sample 7	5.23 ± 0.26	7.32 ± 0.23
Sample 8	5.44 ± 0.19	7.61 ± 0.22
Sample 9	5.33 ± 0.21	7.46 ± 0.17
Sample10	5.55 ± 0.13	7.77 ± 0.24
Sample11	5.38 ± 0.27	7.54 ± 0.18
Sample12	5.66 ± 0.16	7.92 ± 0.21
Sample13	5.49 ± 0.25	7.69 ± 0.18
Sample14	5.33 ± 0.23	7.46 ± 0.13

V. Conclusion

Chia seed oil and the selected vegetable oils were assessed individually and in various combinations for the *in-vitro* hypolipidemic, hypoglycemic and antiproliferative properties. Studies suggest that oils alone have prominent biological effects and the efficiency is modulated considerably in their combinations. However, antiproliferative effects of chia seed oil and its blends were highly significant. Chia seed oil and its blends with vegetable oils could contribute as a dietary source to the prevention of degenerative diseases, such as diabetes mellitus, cancer and coronary artery diseases. Thus, it can be concluded that the blending of other vegetable oils with chia seed oil enhances the biological acitivity and health benefits of those oils. Hence, combination of dietary oils with chia seed oil is recommended.

Acknowledgement

The author Mr. RamziAbdulrashed expresses his thanks to IBB University, Yemen for the financial assistance and to Scintilla BIO-MARC pvt. Ltd, Bangalore, India for providing cell culture facilities.

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