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Extracted Oil from Flax Seed Inhibited only Thrombin and Collagen Induced Washed Platelet Aggregation

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ABSTRACT

The current study deals with the antioxidant and antiplatelet properties of extracted oil from flax seed. Flax seed oil inhibited the aggregation of thrombin and collagen-induced washed platelets, and the percentage of inhibition was found to be 45% and 30 %, respectively. Whereas, it did not alter the ADP, epinephrine, arachidonic acid and PAF inducing washed platelet aggregation. Flax seed oil also exhibit antioxidant property which is identified by using cyclic voltammetry. Fascinatingly, the extracted oil found to be non-toxic in nature as it devoid of haemorrhagic and edema inducing property in experimental mice.

KEYWORDS

Flax, Seed, Thrombin, Collagen, Platelet

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INTRODUCTION

Flax seed is being cultivated majorly for the purpose of oil, thus it is generally called as oil seed or linseed. It is to mention that, flax seed stores less quantity of carbohydrates (28%) and protein (21%), while highest amount of lipids (41%) in addition to vitamins and minerals. The key fatty acids identified so far includes about 73% of polyunsaturated fatty acids, 18% of monounsaturated fatty acids and 9% of saturated fatty acids[1]. Thus, flax seed is commercially available in powder and oil forms. Flax seed oil was found to represent plenty of health benefits, besides it has been widely used in food preparation and other bakery products. Flax seed oil contains Alpha Linolenic Acid (ALA) OR Omega-3 fatty acid[2]. In the body, ALA catabolically converted into Eicosapentaenoic and Docosahexaenoic acid. Moreover, flax seed oil is naturally high in exogenous anti-oxidants like tocopherols and beta-carotene[3]. Fatty acids from flax seed do have curative efficacy on kidney disease, hypertension, heart disorder, stroke, Alzheimer's disease and alcoholism. In addition to above all, it is also used as creams (ointments) in severe pain. Flax seed oil is also used to treat Staphylococcus aureus infection as an antimicrobial substance[4]. Moreover, flax seeds are very useful in the treatment of gout and rheumatic swellings as an emollient[5]. Flax seed oil mixed with lime water could be potentially used in the management of burns, gonorrhoea and wounds[6]. The oil from the seeds removes biliousness and bad blood; useful for internal wounds and kills ringworm[7].

MATERIALS AND METHODS

The chemical substances used were of analytical grade. For the preparation of Platelet Rich Plasma (PRP) fresh human blood was collected from healthy donors.

Extraction of Oil from flax seed

Flax seeds were bought from the local market of Tumakuru. Oil was extracted from the flax seeds using Soxhlet extraction method by the hexane solvent. The oil finally obtained was termed as FSO (Flax Seed Oil).

Preparation of PRP and PPP

PRP and PPP was prepared using the method of Ardle and Han[8]. The platelet concentration of PRP had been adjusted to 3.1×10^8 platelets/ml with the help of PPP. The prepared PRP was used within 2hr for the process of aggregation. All the above arrangements were made using plastic materials or siliconized glass wares.

Preparation of washed platelets

Washed platelets were prepared by the method of Born[9]. Acid citrate dextrose buffer 1.5 ml was taken in plastic centrifuge tube, 9ml of blood was added and centrifuged for 15min at 30g. The supernatant PRP was transferred into plastic tube and kept in an incubator at 37°C for 15min and then it was centrifuged for 20min at 4500g. The supernatant was treated with tyrode albumin buffer at pH 6.5 . After mixed,and centrifuged at 4500g for 20min. The supernatant was suspended in tyrode albumin buffer and centrifuged again for 20min at 4500g. Finally, obtained suspension was treat with tyrode albumin buffer (pH 7.35) and further used for platelet aggregation study.

Platelet aggregation

Platelet aggregation was assayed according to the method of Born by using optical lumi system. Briefly, FSO (0–30µl) was pre-incubated with PRP in 0.25ml reaction mixture. The aggregation process was initiated with the addition of agonists and followed for 6min.

Determination of antioxidant property using cyclic voltammetry

Antioxidant property of FSO was done according to the method of Korotkova[10]. The experiments were carried out using electrochemical CHI 660c. Cyclic voltammetry was applied to characterize the reducing ability of FSO. Cyclic voltammetry Extract behavior was carried on an electrode made of carbon paste. In order to assess the effect of different scan levels on anodic oxidation of the extract, the concentration was maintained at 2mg in 1M potassium chloride. Crude extract behavior has been observed by varying the sweep rate from 50 to 300mV s-1. Scanning rate effects and concentration effects were analyzed and plots were linear, suggesting that the overall electrode cycle was found adsorption.

Edema inducing activity

Edema inducing activity was performed according to the method of Vishwanath[11]. Mice with different doses were injected separately into the right foot pads (0 to 100µl) of FSO in 20µl saline. The 20µl saline alone to the left foot pads served as control. After 1hr mice were anesthetized by inhalation of diethyl ether. Hind limbs were removed and weighed at the ankle joint. Weight gain was calculated as the ratio of edema, which is equivalent to the weight of edematous leg X 100/weight of normal leg. MED is nothing but the amount of protein required to induce an edema ratio of 120%.

Haemorrhagic activity

Haemorrhagic activity was performed as described by Kondo[12]. Briefly, different concentration of FSO (0-100µl) was individually injected (intradermal) into

groups of four mice in 30µl saline. Group receiving saline alone acts as a negative test and a venom group (2MHD) as positive control. After 3hr, mice were anesthetized by inhalation of diethyl ether. Dorsal patch of the skin surface was carefully removed and control mice were examined for hemorrhage against saline injected mice.

Statistical analysis

The data are presented as mean ± SD. Statistical analyses were performed by Student's T-test. A substantial variance among the groups were considered if $P < 0.01$.

RESULTS

FSO inhibits only thrombin and collagen induced washed platelet aggregation

FSO showed specificity in agonists induced platelet aggregation inhibition. Specifically FSO inhibits only thrombin and collagen induced washed platelet aggregation. And the inhibition percentage was found to be 45% (Fig.1) and 30% (Fig.2) respectively. While, it did not alter the ADP, epinephrine, arachidonic acid and PAF inducing washed platelet aggregation.

FSO exhibits antioxidant property

Cyclic voltammetry is a tool for analyzing the electrochemical potential of the substance. The antioxidant property of FSO is identified using cyclic voltammeter (Fig.3). Anodic peak current (Epa) has been found to rise linearly with FSO (1µl to 10µl) concentration increase. It also found that the anodic peak potential (Epa) and half peak potential (Epa/2) are moved towards more positive values. This indicates that the FSO could be scavenging oxygen-free radicals.

Non-toxic property of FSO

FSO is devoid of hemorrhage and edema inducing properties in the experimental mice up to the concentration 200µl (Fig.4).

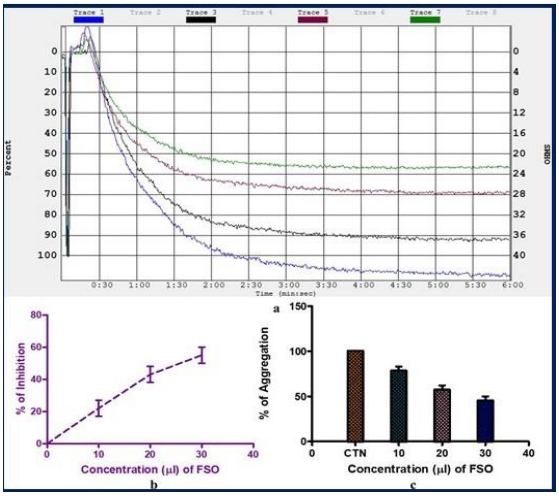

Figure 1 Inhibition of Thrombin induced washed platelet aggregation by FSO

(a) Traces of platelet aggregation: Trace 1 (Thrombin 2µM); Trace 2 (Thrombin 2µM+10µl of FSO); Trace 3 (Thrombin 2µM+20µl of FSO); Trace 4 (Thrombin 2µM+30µl of FSO). The values reflect three experiments independent of each other.

(b) Dose dependent platelet aggregation inhibition%

(c) Dose dependent platelet aggregation%.

DISCUSSION

Flax seed oil found to have immense therapeutic applications due to the presence of ω-3 fatty acid/α-linolenic acid[13]. Flax seed oil has less quantity of saturated fatty

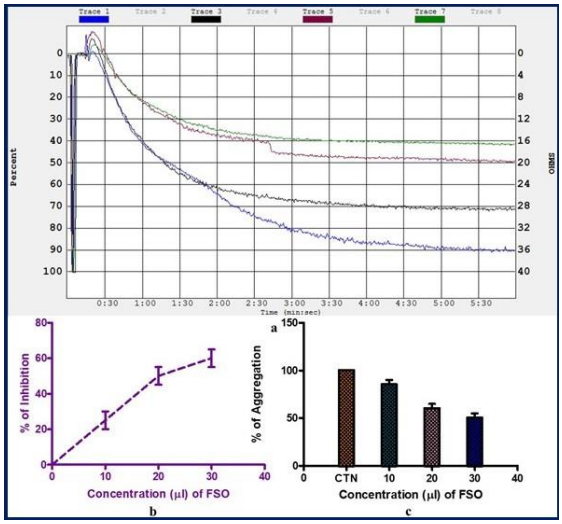

Figure 2 Inhibition of Collagen induced washed platelet aggregation by FSO

(a) Traces of platelet aggregation: Trace 1 (Collagen 5µM); Trace 2 (Collagen 5µg+10µl of FSO); Trace 3 (Collagen 5µg+20µl of FSO); Trace 4 (Collagen 5µg+30µl of FSO). The values reflect three experiments independent of each other. **(b) Dose dependent platelet aggregation inhibition% (c) Dose dependent platelet aggregation%.**

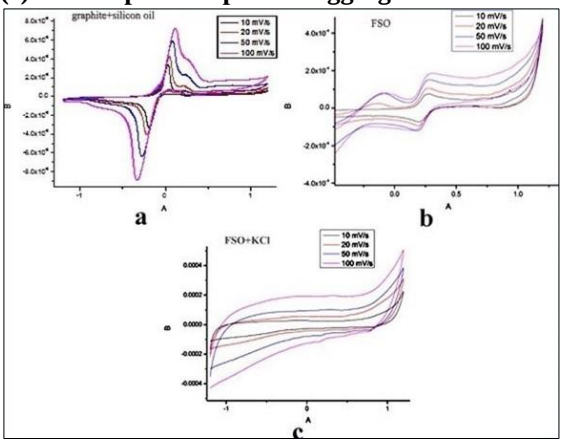

Figure 3 Antioxidant potential of FSO by CV method

(a) Chromatogram signal of graphite with silicon oil obtained from CV data. (b) Chromatogram signal of FSO alone (c) Chromatogram signal of FSO + KCl.

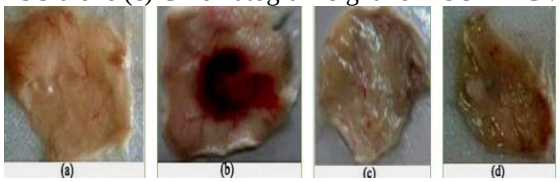

Figure 4 Dose-dependent haemorrhagic activity of FSO

(a) Saline, (b) positive control 2 MDH venom, (c) 50µl and (d) 100µl of FSO were injected independently (intradermal) into mice in a total volume of 150µl

acids, reasonable quantity of monounsaturated and polyunsaturated fatty acids[14]. In addition, flax seed oil contains α-linolenic, oleic, linoleic, palmitic and stearic acids. Flax seed oil withholds several anti-oxidants such as tocopherols and beta-carotene[15]. In our previous study we have reported the role of cysteine protease extracted from flax seed buffer extract on thrombotic disorder[16]. In the present study, an effort was made to demonstrate the role of flax seed oil on platelet function. The preliminary screening of extracted oil was described in our previous study[17]. Optimal and stable thrombus formation requires multiple platelet signalling pathways[18]. Genetic variants due to mutation in the platelet receptors requires protein signalling and synthetic pathways[19]. Patients with platelet granules and aspirin-like deficiencies are the characteristics of mild mucocutaneous bleeding[20]. In addition, unusual clot formation due to unwanted platelet plug in the arteries and veins leads to thrombosis. Fascinatingly, FSO exhibit mild antiplatelet property by inhibiting thrombin and collagen induced washed platelet aggregation without altering the ADP, epinephrine, arachidonic acid and PAF inducing washed platelet aggregation. Thus, FSO could be useful in the treatment of thrombotic disorders.

Uncontrolled way of biological oxidation leads to production of Reactive Oxygen Species (ROS), in excess they could cause deleterious effects to the internal tissue results in diabetes, cancer, arthritis and cardiovascular diseases[21]. While, the latter is implicated in hyper activation of thrombotic agents[22]. Thus, mitigation of the level of ROS helps in the better management of stress related diseases. FSO, exhibited antioxidant property authenticates the presence of ω-3 fatty acid/α-linolenic acid and other unsaturated fatty acids[23]. In addition, FSO did not damage RBC cells and devoid of edema inducing activities strengthened its observed therapeutic potential.

CONCLUSION

In conclusion, the FSO has demonstrated antiplatelet and antioxidant activity. The presence of fatty acid/α-linolenic acid (ALA) and other unsaturated fatty acids could be the cause for observed activities by the FSO. Hence, further characterization of FSO helps in the better understanding of its therapeutic efficacy.

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Conflict of interest

The authors declared no potential conflict of interest over the authorship and publishing.

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