



CHIA (*SALVIA HISPANICA* L.) SEED OIL A NEW SOURCE OF OMEGA-3

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Abstract

Chia seed oil (*Salvia hispanica* L.), was used as a replacement source of rich oil in omega 3. The aim of this study was to determine the physico-chemical properties, fatty acids composition, unsaponifiable matter, tocopherols and phenolic content of chia seed oil. Chia oil showed a far better overall quality, its, acid, peroxide, iodine, saponification values. Sterol fraction was found rich in β -sitosterol (6.33), stigmasterol (4.83%), campesterol (3.77%) and Δ 5-avenasterol (3.55%). the main fatty acids were identified as omega-6 fatty acid (60.93%). Chia and flax seed oils were feeding to rats for 8 weeks. The liver and kidney (aspartate amino transferase, alanine amino transferase and alkaline phosphatase activities) function testes and serum contents (total lipids, total cholesterol and low and high density lipoproteins) were measured. The result of the aforementioned measurements indicated that the feeding of chia oil didn't cause any changes in liver and kidney function and serum contents. Generally, the results suggest the chia oil is that the vegetable source with the very best content of essential fatty acids.

Keywords: Chia seed oil, fatty acids, omeg-3, biological evaluation, flax seed oil.

Introduction

The risk of disorder in heart, type-2 diabetes and colorectal cancer increases with obesity. Diet and lifestyle are often modified to stop and reduce the risks of those diseases. There's epidemiological evidence that diets that promote health are rich in dietary fiber and omega-3 carboxylic acid and low in saturated fat, trans fat and cholesterol (Hui, 2002).

Salvia hispanica L., commonly referred to as chia, may be a species of angiosperm of the Lamiaceae, native to Central and Southern Mexico and Guatemala (USNPGS, 2000). The 16th century Codex Mendoza provides evidence that it had been cultivated by the Aztec in pre-Columbian times and economic historians have suggested that it's going to be as important as maize as a food crop (Cahill 2005). Ground or whole chia seeds are still utilized in Paraguay, Bolivia, Argentina, Mexico, and Guatemala for nutritious drinks and as a food source (Kintzios, 2000). Today, chia is grown commercially in its native Mexico, also as in Bolivia, Argentina, Ecuador, Guatemala and Australia.

Chia (*Salvia hispanica* L.), a biannually vascular plant, is categorized under the *Labiatae* (*Labiatae*), super division of *Spermatophyta* and kingdom of *Plantae*, prominently grown for its seeds. Chia seed consists of protein (15-25%), fats (30-33%), carbohydrates (26-41%), high dietary fiber (18-30%), ash (4-5%), minerals, vitamins and dry matter (90-93%). The seed also contains a high amount of antioxidants (Ixaina *et al.*, 2011). The seed contains 25 to 40% oil with 60% of it comprising (Omega)-3-linolenic acid and 20% of (Omega)-6 linoleic acid. Both essential fatty acids are required by the physical body permanently health and that they can't be artificially synthesized (Mohd *et al.*, 2012). Furthermore, an omega-6/omega-3 ratio of 4:1 or less is suggested. A high ratio of omega-6/omega-3 is detrimental to health and should cause the event of chronic diseases. Improving the dietary ratio by increasing the omega-3 fatty acids is important for brain functioning and for the management of disorder, arthritis and cancer (Simopoulos and Cleland, 2003).

The amounts of A, B1, B2, B3, B6 and C vitamins found in chia seeds were 37 IU, 8.7 $\mu\text{g/g}^{-1}$, 1.7 $\mu\text{g/g}^{-1}$, 58 $\mu\text{g/g}^{-1}$, 6.9 $\mu\text{g/g}^{-1}$ and 157 $\mu\text{g/g}^{-1}$, respectively. Among the water-soluble vitamins determined, pantothenic was found at 9.40 $\mu\text{g/g}^{-1}$. Myricetin was the most flavanol (with a concentration 3 times above that of kaempferol). The antioxidants in chia seeds are polyphenols (namely myricetin, quercetin and kaempferol). Total phenolic are quantified at 47mm per 1,000 g of seeds (caffeic acid equivalents). It's known that the oxidation of chia seeds is minimal or absent, thanks to the presence of those compounds, having an excellent potential within the food industry (Ixaina *et al.*, 2011).

Chia seed may be a good source of dietary fibers containing about 5 percent soluble fiber which appears as clear mucilage when it's placed in water. These remain tightly sure to the seed and have a really large relative molecular mass, averaging 1.5 x 10⁶ Dalton (Lin *et al.*, 2004). The high viscosity of chia mucilage renders it more likely to supply desired metabolic effects than lower viscosity dietary fibers like guar or β -glucan (Wood *et al.*, 1989 and Jaddu and Yrdida 2018). Hence chia is beneficial as a dietary fiber and possesses huge potential for application in food industry (Lin 1994).

Consuming of linseed (*Linum usitatissimum* L.) is useful for human health. Flax seeds, containing about 36-40 to grease are the richest (among crop plants) source of polyunsaturated fatty acids (PUFA) essential within the human diet. PUFA are highly vulnerable to oxidation (El-Beltagi *et al.*, 2007). Linseed (*Linum usitatissimum* L.) may be a multi-purpose crop. Its' seeds containing about 36 to 40 you look after oil, have long been utilized in human and animal diets and in industry as a source of oil. Recently there has been a growing interest within the probiotic properties of flax and in its beneficial effects on coronary heart condition, some sorts of cancer and neurological and hormonal disorders (Simopoulos 2002). Flaxseed is abundant in many nutrients, like polyunsaturated carboxylic acid, protein, and lignans (Wang *et al.*, 2007). as compared with other vegetable oils, flaxseed oil is distinguished by the very best

content of α -linolenic acid, i.e. $26\pm 60\%$, which since recently has been found as especially important for human organism. Unfortunately, a high content of α -linolenic acid induces a poor oxidative stability of flaxseed oil (Rudink *et al.*, 2001). Of all lipids in flaxseed (approximately 30%), 53% are α -linolenic acid (ALA), 17% linolic acid (LA), 19% monounsaturated fatty acid, 3% octadecanoic acid and 5% hexadecanoic acid, which provides a superb n-6: n-3 carboxylic acid ratio of roughly 0.3:1 (El-Beltagi *et al.*, 2007). Therefore, the seed could also be an alternate for supplying this carboxylic acid to populations concentrated in regions of the planet where there's not large access to marine foods, which are the simplest sources of n-3 fatty acids FI (Jaddu and Yrdida, 2008). Flaxseeds are a source of the many vitamins and minerals as calcium, magnesium and phosphorus. It's of great importance, being that a 30g portion of the seed constitutes 7% to 30% of the Recommended Dietary Allowances (RDAs) for these minerals (Singh *et al.*, 2011).

A relationship between high-saturated carboxylic acid s (SFA) and low polyunsaturated fatty acid (PUFA) intake and diseases like cardiovascular diseases, diabetes, and metabolic syndrome were widely reported (Martha *et al.*, 2012). Besides, the additive effect of α -linolenic acid (ALA) and n-3 long-chain PUFA was observed to exhibit cardio-protective effects in women (Vedtolte 2011), which led to consequent human clinical studies of chia on disease risk factors. To date, four clinical trials are administered. Among these trials, only that of (Nieman *et al.*, 2009), showed no health benefits from chia seed. This difference might be thanks to the treatment durations employed and also the particular biochemical components of the dietary chia seed utilized in the varied studies. Nevertheless, later studies demonstrated the advantages of chia to human health. Today, chia remains an important element within the diet of the inhabitants of Mexico and a number of other Central American Countries, becomes an increasingly popular food and is common in supermarkets and food stores round the world.

Diet features a great effect on serum lipoprotein and serum lipid profile. Omega-3 fatty acids comprised α -linolenic acid, omega-3 fatty acid, and docosahexaenoic acid (Fernandez 2009). Alpha-Linolenic acid and eicosapentaenoic acids are related to the synthesis of prostaglandins, leukotrienes, and thromboxanes, which are involved during a wide selection of physiological activities (Craig, 2004). The cardiac and neuron protective effects of omega-3 fatty acid and decosahexaenoic acid are scientifically proven, and omega-3 fatty acids have a positive effect in controlling the harmful cardiac arrhythmias, which are caused by the sodium and calcium channel dysfunctions (Ayerza and Coates, 2004). Omega-3 and omega-6 fatty are important for the reduction of cholesterol, prevention of blood coagulation, tissue regeneration, diabetes, and cardiovascular diseases. These also regulate the system and stop some sorts of cancer¹⁹. Within the past, omega fatty acids were obtained by fish and fish product, currently most of the omega fatty acids are obtained from oilseeds (Mantizioris *et al.*, 2000)

The objective of this study was to work out the physico-chemical properties, carboxylic acid composition, unsaponifiable matter and bioactive components of oil extracted from chia (*Salvia hispanica* L.) seeds. Additionally, biological evaluation of chia oil was evaluated.

Materials and Methods

Materials: Chia and linseeds were procured from local market of Tabuk, Saudi Arabia. The seeds were air-dried at temperature (2°C) for 1 week.

All chemicals used were of analytical or HPLC grade from Merck (Darmstadt, Germany) or Sigma Aldrich (St. Louis, Mo, USA). Standards of sterols, tocopherol and phenolic compounds were obtained from Fluka Chemie (Buchs, Switzerland).

Alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, HDL-cholesterol, LDL-cholesterol and total lipids in serum were estimated by kits obtained from Boehringer Mannheim GmbH, Germany.

Methods

Oil extraction: The oil from all seeds was extracted with n-hexane using the described method by (AOAC, 2012).

Proximate analysis: The methods of the (AOAC, 2012). were used for proximate analysis. A chia Flower sample (5 grams) was used for determination of moisture content by weighing in crucible and drying in oven at 105°C, until a continuing weight was obtained. Determination of ash content was done by ashing at 550 °C for 3hr. The kjeldah method was wont to determine the protein content. The crude fiber content of the samples decided by digestion method and therefore the fat was done by Soxhlet extraction method. All determinations were wiped out triplicate.

Determination of the physico-chemical properties: The extracted chia and linseeds oil was analyzed immediately for index of refraction, color, definite quantity, peroxide value, iodine number saponification number as described in (AOAC, 2012).

Fatty acids composition: Capillary gas chromatograph (HP 6890) was used for the qualitative and quantitative determinations of fatty acids of the oil samples and reported in relative area percentages. Fatty acids were transesterified into their corresponding carboxylic acid methyl esters by shaking an answer of oil (0.1g) in heptane (2 ml) with solution methanolic potash (0.2 ml, 2N). The carboxylic acid methyl esters were identified employing a gas chromatograph equipped with DB-23 (5%-cyanopropyl–methyl poly siloxane) capillary column (60mx 0.32mm X0.25µm film thickness) and flame ionization detector. Nitrogen flow was 0.6ml/min, hydrogen and air-flow rates were 45 and 450ml/min, respectively. The oven temperature was isothermally heated 195°C. The injector and therefore the detector temperatures were 230°C and 250°C, respectively. Carboxylic acid methyl esters were identified by comparing their retention times with known carboxylic acid standard mixture. Peak areas were automatically computed by an integrator. All GC measurements for every oil sample were made in triplicate and therefore the averages were reported.

Identification of unsaponifiable matter: The unsaponifiable matters of oil samples was analyzed by an Hp 5890 gas chromatograph equipped with FID detector and DB-5 capillary column (30 m, 0.25mm (5% phenyl) -95% methyl polysiloxane, 0.25µm film thickness, 280 °C temperature injector and 300 °C temperature transfer line. The oven temperature was programmed as follows: initial temperature: 100 °C for two min, increase 10 °C /min up to 300 °C, then

hold for 20 min. The carrier gas was N₂ (2 ml/min). The identification of the various compounds was performed by comparing of its relative retention times with those of authentic reference compounds.

Determination of total phenolic content: The levels of total polyphenols of chia and flax seed oil were determined consistent with the tactic of (Gutfinger, 1981). Caffeic acid was served as a typical compound for the preparation of the calibration curve.

Tocopherol analysis: Tocopherol (α , β and δ) analysis was performed using an HPLC system consisting of a L-6000 Merck-Hitachi high pump connected to an L-4000 Merck Hitachi UV detector (Hitachi Instruments Inc., Tokyo, Japan) set at 295nm, Tocopherol contents were identified by comparing the retention times with those of pure standards as described by others (Anwar and Rashid 2007). Darmstadt, Germany) was used for data acquisition and processing.

Experimental animal: Male rats (24) of 60 days old with a mean weight of 70 g were obtained from the Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. The animals were eaten up a basal diet for 7 days as an adaptation period. The basal diet was formulated according to 24) method and consisted of casein (15%), vegetable oil (10%), cellulose (5%), salt mixture (4%), vitamin mixture (1%) and starch (65%). Water was available as required

Feeding experiment: The animals were divided into 3 groups, each group contain 8 rats to measurement the effect of feeding on basal diet containing 10 you look after chia and flax seed oil orally compared with control which contained vegetable oil. Blood samples were drawn from rat's eyes hebdomadally for 8 weeks, and then centrifuged individually to separate serum which was kept in deep- freezer until analysis.

Serum analysis: ALT, AST and AP activities were measured consistent with the methods described by (Kochmar and Moss, 1976; Bergmeyer and Horder, 1986 and Varley *et al.*, 1980) respectively. T extent of serum cholesterol, low and high density lipoproteins, total lipids and triglycerides were determined consistent with the methods outlined by (Rosell, 1991; Assmann, 1079; Frings and Dunn 1979 and Wuhelefed, 1974), respectively.

Data analysis: A minimum of three replications for every oil sample were performed with each test. The averages and variance were calculated by statistical analysis using SPSS program.

Results and Discussion

Chemical composition of chia seeds: The results of the proximate composition of chia seeds are shows in Table 1. The moisture content of the chia seed was 5.45%. The chia seed had higher values within the ash, crude fiber, protein and carbohydrate contents. The high protein content of those flour samples give a sign of their usefulness in human diet and as livestock feed. The chia seed had higher fat content of 34.53%.

Physico-chemical properties of chia and flax seed oils: Table 2 shows various physico-chemical characteristics of the extracted chia and flax seed oils. Index of refraction of chia and flax seed oils at 25 °C were 1.4765 and 1.4652, respectively. The red color at yellow 35.00 was 2.00 and 2.1 respectively. The definite quantity of chia oil (0.60% as oleic

acid) was above flax seed oil (0.30 % as oleic acid). Oils with lower values of acidity are more acceptable for edible applications. The peroxide value of chia oil was (0.83 meq.kg⁻¹ of oil) while flax seed oil were (0.85 meq.kg⁻¹ of oil) respectively. Moreover, chia oil shows higher iodine number (194.00 g I/100 g oil) than those of flax seed oil (189.3.00 g I/100 g oil) results are agreement thereupon reported by (Ixaina *et al.*, 2011 and Singh *et al.*, 2011). Saponification number and unsaponifiable matter of chia oil (194.00 mg KOH/g oil and 1.20%, respectively) were above flax seed oil shown in Table 2.

Phenolic content: Phenolic compounds are proved to be liable for antioxidant activity on many vegetable seeds oils; it's mainly thanks to their redox properties, which may play a crucial role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Osawa, 1994). Total phenolic compounds (Table 3) within the chia and flax seed oils were (840.00 and 840.00µg/g), respectively.

Tocopherols content: The info for tocopherol analysis of the chia and flax seed oils are presented in Table 3. The amount of α , γ and δ -tocopherol within the oils were 23.00, 25.00 and 890.00/g and 20.00, 21.00, 693.00µg/g, respectively. The content of α -tocopherol within the chia was in close agreement with the values reported for soybean, groundnut and palm oils (Roehlau *et al.*, 1974). It's known that the oxidation of chia seeds is minimal or absent, thanks to the presence of those compounds, having an excellent potential within the food industry (Ixaina *et al.*, 2011).

Unsaponifiable matter components of chia and flax seed oils: The hydrocarbons and sterols within the unsaponifiable matter of chia and flax seed oils are analyzed by using gas liquid chromatography. The obtained data are illustrated in Table 5. Data shows that C28 is that the major hydrocarbon in chia oil while flax seed oil that C30 is that the major hydrocarbon. The sterols profile of chia and flax seed oils is shown in Table 5. The sterol fraction of chia and flax seed oils mainly consisted of β -sitosterol (45.11% & 43.00%), Stigmasterol (19.20% & 18.00%), campesterol (16.90% & 15.20%) and Δ 5avenasterol (10.00% & 8.30%), alongside small amounts of clerosterol, 24-methylene cholesterol, Δ 7-campestanol, Δ 7-avenasterol, stigmastanol and 28.isoavenasterol, cholesterol and Brassicasterol.

Fatty acid composition of chia and flax seed oils: Fatty acid composition of chia and flax seed oil were identified by gas liquid chromatography and therefore the obtained results are tabulated in Table (4). It might be noticed that carboxylic acid "> linoleic acid is found to be the dominant unsaturated fatty acid in chia and flax seed oils, which represented about (60.93% & 56.49 %). carboxylic acid "> hexadecanoic acid was found also to be the dominant saturated fatty acid in chia and flax seed oils(6.81% & 5.87 %). The results are in agreement thereupon reported by (Mohd *et al.*, 2012 and El-Beltagi *et al.*, 2007).

Influence of feeding of chia and flax seed oils on the activity of serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase of rats:

Figures (1, 2 and 3) shows the activities of ALT, AST and AP for control rats and therefore the values were slightly increased during the entire experiment (8 weeks). Feeding on vegetable oil (control) induced significant increases in serum

ALT, AST and AP activities from the commencement and to the top of the experiment. While, feeding on chia and flax seed oils didn't cause any significant changes in enzyme activities compared with the experiment.

Influence of feeding of chia and flax seed oils on serum lipid profile

Serum total lipids: The leads to Fig. (4) show that there was non-significant differences within the total lipids for control rats throughout the entire experiment. While feeding on flax seed oil caused significant and gradual increases in serum total lipids. On the opposite hand feeding on chia oil induced non-significant rise difference in rat serum total lipids.

Serum total cholesterol and rarity lipoprotein cholesterol (LDL-C): Figures (5 and 6) shows the amount of serum total cholesterol and rarity lipoprotein cholesterol of control rats; rats feeding of chia and flax seed oils. The results for the control rats and rats feeding of chia and flax seed oils indicated that there have been no significant increases in total cholesterol levels and LDL-C during the whole experiment.

Serum high density lipoprotein cholesterol (HDL-C): the info (Fig.7) for the control rats and rats feeding of chia and flax seed oils showed non-significant changes within the levels of HDL-C during the whole experiment period (8 weeks).

Conclusion

The results show that the chia oil presents interesting physicochemical properties for the food industry. Chia oil contains 60% α -linoleic acid and quite 20% as linolic acid than other sources. The results confirm that chia oil is that the vegetable source with the very best content of essential fatty acids as omega -3. From a physiological point of view, chia oil may be a potentially interesting food ingredient thanks to its health benefits from its high levels of PUFA.

Table 1 : Chemical composition (%) of chia and flax seeds.

Ingredients	Flaxseed	Chia seed
Moisture content (%)	6.25±0.36	5.45±0.21
Protein content (%)	19.50±1.01	22.33±1.43
Oil content (%)	39.10±2.43	34.53±2.15
Fiber content (%)	17.80±1.00	20.45±1.61
Ash content (%)	4.00±0.22	3.80±0.19
Carbohydrates (%)	19.60±1.45	18.89±0.87

Data are expressed as mean±SD values given represent means of three determinations.

Table 2: Physico-chemical properties of flaxseed and chia seed oil.

Parameters	Flaxseed oil	Chia seed oil
Refractive index (25C)	1.4652±0.001	1.4765±0.001
Acid value (mg.KOH/g Oil)	1.70±0.11	1.02±0.01
Peroxide value (meq.O2/kg oil)	4.20±0.34	3.56±0.34
Iodine number (gI/100g oil)	189.3±5.67	194.50±8.30
Saponification value	189.60±6.55	194.60±9.00
Unsaponifiable matter (%)	1.10±0.09	1.21±0.06

Data are expressed as mean ± SD values given represent means of three determinations.

Table 3 : Minor components in flax and chia seeds oils.

Components	Flaxseed oil	Chia seed oil
Total polyphenols (ppm)	624±12.40	840±15.70
Total tocopherols (ppm)	725±14.50	989±16.91
α -tocopherol (ppm)	20±1.02	23±1.61
β -tocopherol (ppm)	21±1.34	25±1.55
γ -tocopherol(ppm)	693±10.35	890±14.90

Data are expressed as mean ±SD values given represent means of three determinations.

Table 4 : Fatty acids composition of flax and chia seed oils.

Name of Fatty acids	Flaxseed oil	Chia seed oil
C14:0	0.04±0.0001	0.03±0.0001
C16:0	5.87±0.31	6.81±0.31
C16:1	0.07±0.001	0.06±0.0001
C17:0	0.06±0.001	0.05±0.0001
C17:1	0.03±0.0001	0.01±0.00
C18:0	4.78±0.23	4.26±0.31
C18:1T	0.00±0.00	0.00±0.00
C18:1	18.47±1.00	7.52±0.34
C18:2T	0.00±0.00	0.00±0.00
C18:2	13.69±0.99	19.88±1.16
γ -C18:3n6	0.19±0.01	0.24±0.01
α -C18:3n3	56.38±2.15	60.69±2.98
C20:0	0.16±0.01	0.35±0.03
C20:1	0.13±0.001	0.15±0.01
C22:0	0.13±0.001	0.08±0.001
Σ Saturated Fatty acids	11.04±0.82	11.45±0.71
Σ Monounsaturated fatty acids	18.70±1.02	7.74±0.65
Σ Polyunsaturated fatty acids	70.26±4.27	80.81±5.55
Σ C18:1/C18:2	1.35±0.11	0.38±0.01
Σ C18:1/C18:3	0.33±0.01	0.12±0.01
Σ C18:2/C18:3	0.24±0.001	0.33±0.01

Data are expressed as mean ± SD values given represent means of three determinations.

Table 5 : Fractionations of unsaponifiable matter (%) in flax and chia seed oils.

Components	Flax oil	Chia oil
Hydrocarbons:		
C16	2.25±0.11	2.18±0.13
C18	3.45±0.19	3.21±0.17
C20	5.20±0.21	5.41±0.32
C22	5.81±0.25	4.60±0.25
C24	9.12±0.61	10.23±0.78
C25	7.50±0.54	6.67±0.41
C26	13.61±0.92	12.98±0.96
C28	16.33±0.97	15.45±0.99
Squaline	3.19±0.23	4.31±0.37
C30	9.35±0.65	8.57±0.64
C32	8.96±0.55	9.01±0.71
Total hydrocarbons	84.77±7.87	82.59±7.56
Sterols:		
Cholesterols	0.02±0.001	0.01±0.0001
Campesterol	3.74±0.12	3.77±0.14
Stigmasterol	3.95±0.15	4.83±0.36
β Sitosterol	5.28±0.34	6.33±0.52
Δ^5 -Avenasterol	2.24±0.18	3.55±0.31
Total sterols	15.23±0.99	17.41±1.00

Data are expressed as mean±SD values given represent means of three determinations.

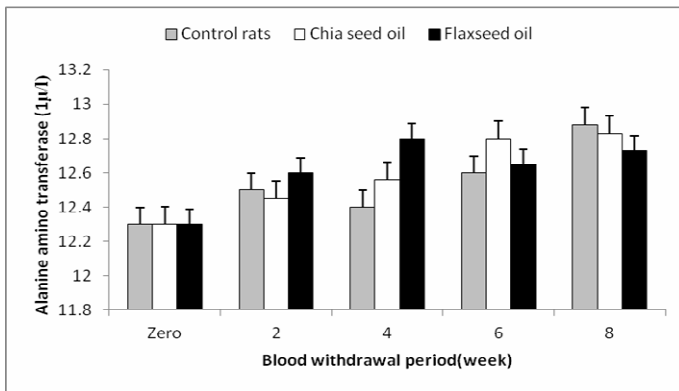


Fig. 1 : Influence of feeding of chia and flax seed oils on the activity of serum alanine amino transferase. Data are expressed as mean ± SD values given represent means of three determinations.

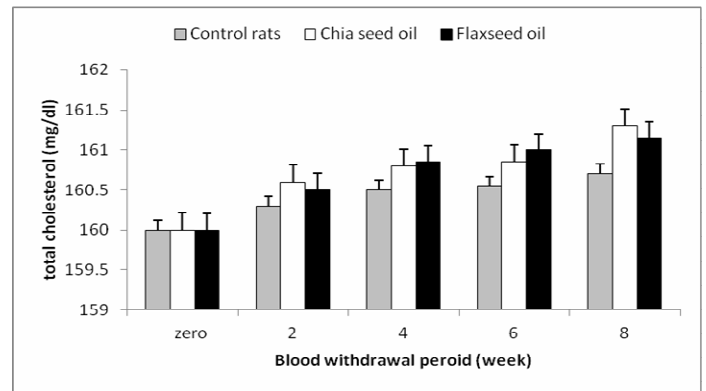


Fig. 5 : Influence of feeding of chia and flax seed oils on sera total cholesterol. Data are expressed as mean ± SD values given represent means of three determinations.

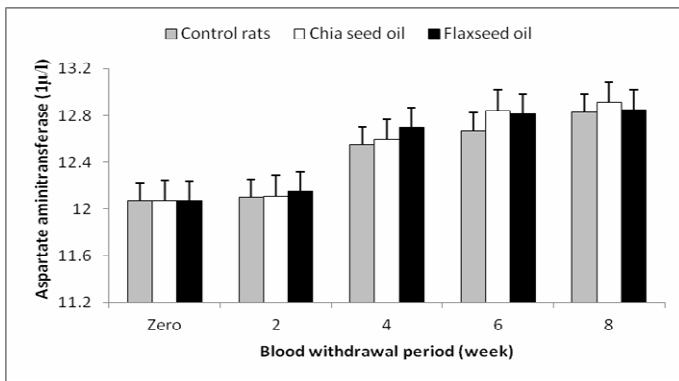


Fig. 2 : Influence of feeding of chia and flax seed oils on the activity of serum aspartate amino transferase. Data are expressed as mean ± SD values given represent means of three determinations.

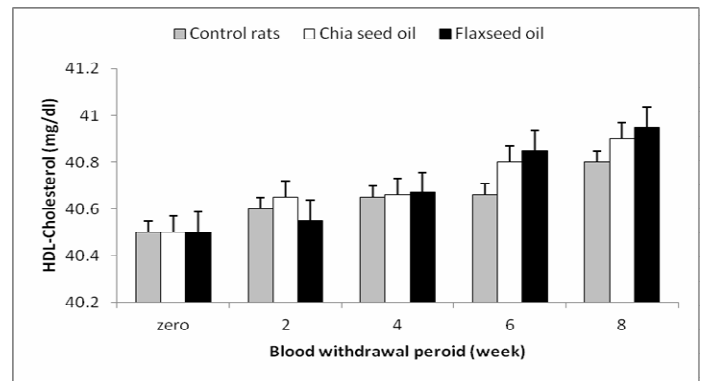


Fig. 6 : Influence of feeding of chia and flax seed oils on sera HDL-cholesterol. Data are expressed as mean ± SD values given represent means of three determinations.

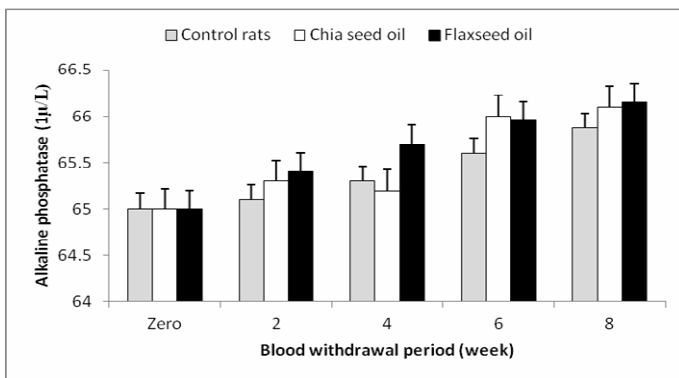


Fig. 3 : Influence of feeding of chia and flax seed oils on the activity of serum alkaline phosphatase. Data are expressed as mean ± SD values given represent means of three determinations.

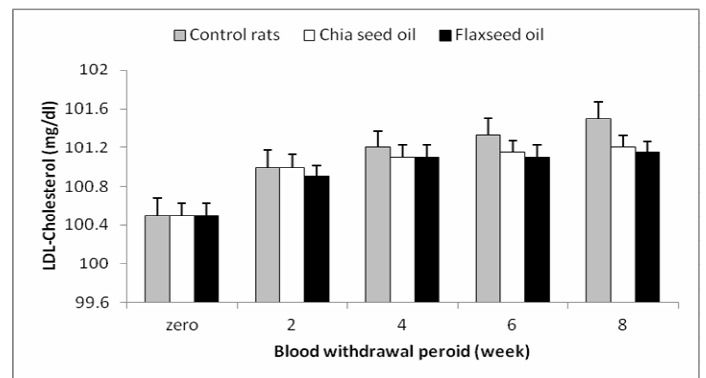


Fig. 7 : Influence of feeding of chia and flax seed oils on sera LDL- cholesterol. Data are expressed as mean ± SD values given represent means of three determinations.

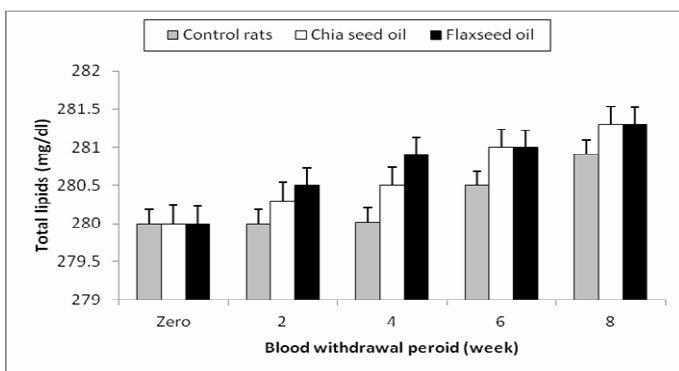


Fig. 4 : Influence of feeding of chia and flax seed oils on sera total lipids. Data are expressed as mean ± SD values given represent means of three determinations.

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