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Improvement of cardiovascular Diseases by Pine (Pinus) Nut Oil and its Alternatives

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***Abstract*- Pine nut oil and its alternatives are rich in fatty acids and important minerals. AAS was used to identify copper, zinc and iron, calcium, sodium, magnesium and potassium in oils. HPLC technique was applied to identify fatty acids and vitamin K. Then 5 groups of rats each having 5 rats were taken. One group was a controlled group with basic diet. Other four group were given high cholesterol diet for 20 days to increase their total cholesterol level (TC), low lipoprotein cholesterol level (LDL-C), high lipoprotein cholesterol level (HDL-C) and triglycerides (TGs). After 20 days, each group was fed basic diet with oil of 15mL. Second group was fed with 15mL/day pine nut oil (PNO), third group with 15mL/day pumpkin seed oil (PSO), fourth group was fed with 15mL/day walnut oil (WO) and fifth group was fed with 15mL/day sunflower oil (SO). After 4 weeks of this diet, the results were obtained that show the significant results. The present results revealed the decrease in low density lipoprotein cholesterol level, triglyceride, total cholesterol and increase in high density lipoprotein.**

Keywords: Pine nut oil, walnut oil, pumpkin seed oil, sunflower oil, LDL-C, HDL-C, triglyceride, total cholesterol.

I. INTRODUCTION

Pine nut oil and its three alternatives pumpkin seed oil, walnut oil and sunflower oil are rich source of carbohydrates, fats, protein, vitamins, minerals and water. Pine nut oil has been used for many years for healing illnesses and also for cooking. Pine nut oil is rich in vitamin E, vitamin K, copper, iron, manganese and especially monounsaturated fatty acids: the pinolenic acid. It is a rich source of carbohydrates like starch, sugars dietary fibers, fats like saturated, monounsaturated, polyunsaturated (linoleic acid, linolenic acid), protein, vitamins like thiamine, riboflavin, niacin, vitamin E, vitamin K, minerals like calcium, copper, iron, magnesium, potassium, zinc, manganese, phosphorus and water in dried form [1].

Pumpkin seed contains carbohydrates: sugars and dietary fibers, fats: saturated, monounsaturated, polyunsaturated, vitamins: Thiamine, riboflavin, niacin, vitamin C, vitamin E, vitamin K, folate, minerals: calcium, iron, magnesium, manganese, phosphorus, potassium, sodium, protein, zinc and water [2]. It contains antioxidants, high fiber, fatty acids, zinc and magnesium that reduce high blood

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pressure and high bad-cholesterol level. It contains antioxidants to reduce inflammation. It contains antioxidants to reduce inflammation. It is beneficial for hair growth, skin health, eye health, urinary health, sexual health status, pregnancy, insomnia prevention, improve immune system, and improve digestion and weight loss [3].

Walnut oil contains high percentage of alpha-linolenic acid that reduces inflammation and improves blood fat composition. Fatty acids in walnut oil lowers bad-cholesterol level, reduces inflammation and improve blood vessel function [4]. Sunflower oil contains fatty acids: oleic acids and linoleic acids are in high quantity, vitamins: vitamin E, vitamin k etc [5]. Sunflower oil good to control the bad-cholesterol level in the blood and prevent from heart diseases. These oils are examined for treatment of cardiovascular disease [6].

Some important unsaturated fatty acids which are very effective for fats release are alpha-linolenic acid, linoleic acid and oleic acid. Alpha linolenic acid ($C_{18}H_{30}O_2$) is unsaturated fatty acid and is named as omega-3. Its intake is beneficial for heart diseases. It shows the effects on heart by reducing cardiac attack, reduces clot formation (thrombosis), decreases triglyceride level, reduce the blood pressure and inflammation too. Linoleic acid ($C_{18}H_{32}O_2$) is a polyunsaturated fatty acid and named as omega-6. It reduced the total cholesterol level and LDL level that causes blood pressure, and affects the heart muscles [7]. Oleic acid ($C_{18}H_{34}O_2$) is also unsaturated fatty acid and named as omega-9 because double bond is present at carbon number 9. It is also useful for reducing the blood pressure, improving HDL level and decreases the LDL level in blood [8]. Vitamin K as a whole is an antioxidant and control the calcium deposition in the walls of arteries. Vitamin K1 deficiency causes the bleeding in gum, nose bleeds, anemia and coronary heart disease [9]. Likewise, some metals are present in the oils that show the effects on the heart. Calcium ions works as an electrolyte and beneficial for blood cells and circulatory system. Studies revealed that the calcium ion is used for the contraction of all the muscles by passing the electrical signals from one cell to other cell [10]. Sodium is present in the blood and blood cells and maintains the volume of the blood in the body. When body looses the balance of sodium in the blood, high or low blood pressure causes [11]. Potassium controls the blood pressure and bad cholesterol. If its concentration is lower in the blood it may lead to disturbance of heart beat and may causes diseases [12]. Magnesium acts as guard for entrance and exit of calcium in muscle cells. Due to the electrolytes in blood magnesium concentration decreases in the blood and may cause cardiovascular disease and hypertension [13]. Copper in the blood also affects the heart. Low level of copper level causes the high cholesterol level

and high blood pressure. Due to its deficiency, change the structure and function of blood cells [14]. Zinc regulates the calcium in the heart cells and works as antioxidant. If the zinc amount decreases then calcium releases in excessive amount that cause the heart failure [15]. Iron is an important element of hemoglobin that forms the blood. When the iron level decreases, the blood level also decreases and oxygen intake becomes low then heart beat becomes irregular. Sometimes angina causes or heart failure occurs [16].

A. Material and methods

Pine nut oil was purchased from SAC manufacturer Karachi, pumpkin seed oil and walnut oil were purchased from local market in Lahore. While sunflower oil was purchased from Bio Hunza manufacturers, Islamabad, Pakistan. Nitric acid, Sulfuric acid, Ethanol and Potassium hydroxide were purchased from sigma Aldrich Company.

B. Experiment

Some effective metals, vitamin and unsaturated fatty acids present in oils were detected by different techniques in PCSIR Lahore. While the experiments on rats and their results identifications were done in University of veterinary and animal sciences, Lahore, Pakistan.

C. Metals detection through AAS:

Sample preparation:

In determination of Cu, Fe, Zn, Ca, Mg, Na and K, the sample was prepeared to run in AAS. 3ml of sample oil was taken in beaker and added 10ml HNO_3 through pipette in it. Then heated sample for few minutes till fumes start evaporation. Then 10ml HNO_3 was added in solution. Again fumes formed on heating. Then 20ml of distilled water was added in the beaker. When sample started boiling, 50ml warm distilled water was added. Then remove from sand bath and blown out the lamp. Then the sample was filtered through the filter paper. Then 5ml filtrate was taken and put the water of 100ml volumetric flask. These samples were determined through AAS.

AAS technique:

AAS was run by inserting the sample and required conditions. The prepared sample was added in the injector that was injected in the sample cell. Then process was started by pressing the buttons to start the automated instrument. The source hollow cathode lamp emitted the rays of specific range. The column contains sample medium and reference medium with chopper and mirrors for separation of rays. The rays came from source, reached to the beam splitter that split the rays and sent them to the reference cell and sample cell. Then beams passed through the sample cell and the reference cell that contained the sample and standard. The sample absorbed some specific rays and transmitted

others. While all the rays of reference cell were transmitted as it is. Then rays reached to beam splitter that sent the emitted light one by one towards the Monochromator came from sample medium and reference cell. Then Monochromator sent the rays one by one to the amplifier that amplifies the rays and help to separate all the rays from other rays appeared on graph. Then these rays moved towards detector that is attached to the computer. The detector after reading the rays gave the results on the computer in the form of peaks. Graph contained calibration curve that explained the amounts of metals present in the oils.

D. Fatty acids and vitamin K detection through HPLC:

Sample preparation:

2g of all liquid oil samples were taken in 250 mL round bottom flask separately and dissolved them in 50ml of 0.5M KOH alcohol solution. Then all oil samples were put in water bath and heated with occasional stirring at 80°C for 1 hour. After heating, all oil samples were cooled in 500ml flask. Then 25ml of sulfuric acid was added to make solution lightly acidic.

E. HPLC technique:

Lipoprotein test:

This test was done on rats. Twenty five male rats of weighing 190±10gm and 10 to 16 weeks old were purchased and placed in controlled environment of light, temperature and humidity. Rats were fed with the diet containing nutrients required for good health.

Basel diet: The diet contained 3% fat, 2% cholesterol and basic nutrients. They were placed in light for 12 hours and 12 hours in dark.

Normal Diet: They were fed with basic diet and water for a week before experiment. After one week, the low density lipoprotein cholesterol and high density lipoprotein cholesterol level were measured. The all required cholesterol level, triglyceride level; HDL-C and LDL-C levels were in controlled range as before. Then rats were divided into five groups and each has five rats. First group of rats was taken as controlled group and fed with basic diet and pure water.

Hypercholestermic diet given to rats: All other four groups of rats were fed with high fat and cholesterol containing diet for twenty days. After twenty days, the heart beat of rats were high and unequal. Then blood was tested to check the levels of TC, TGs, LDL-C and HDL-C. The level of LDL-C was increased and HDL-C level was decreased in high quantity. Then these rats were kept for a week in standard conditions for stabilizing the blood cholesterol level. After one week, the

cholesterol levels were again checked and resulted in high levels as before one week. Then these rats were taken for the experiment. The all other four groups were taken as positive.

Hypercholestermic rats groups fed with different oils: The second group was fed with addition of 15mL pine nut oil in basic diet three times a day. Third group was fed with addition of 15mL pumpkin seed oil in basic diet. Fourth group was fed with addition of 15mL walnut oil in basic diet and fifth group was fed with addition of 15mL sunflower oil in basic diet three times a day. After 4 weeks, again blood tests were done. The results obtained were different due to the linolenic acid and linoleic acid quantities in all four oils.

II. Results and Discussion:

A. *AAS result:*

AAS gave the results for the detection of minerals in the oils that were affecting the heart muscles and their regulations of ions to make smooth muscles and blood circulation.

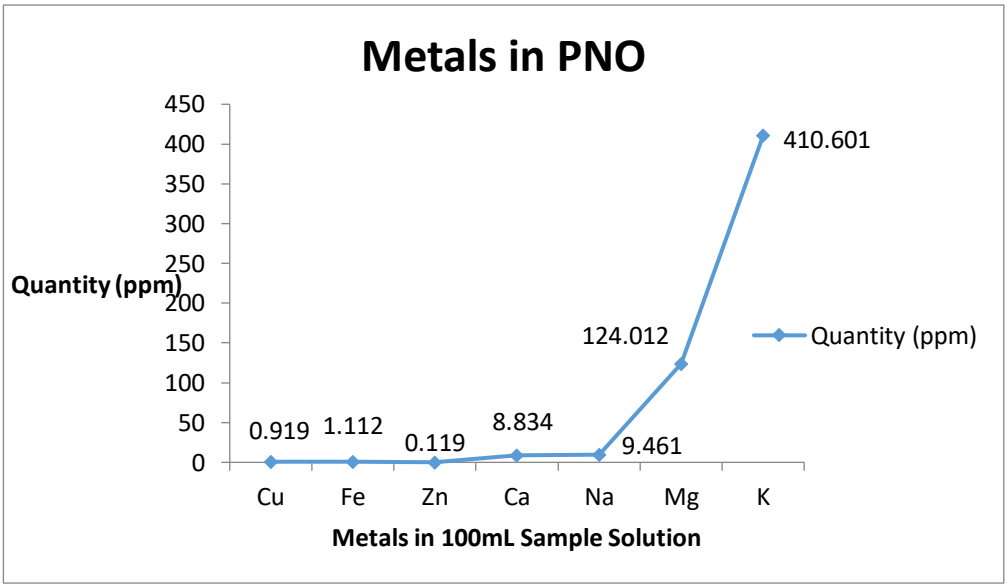

Fig. 1 Metals in PNO

In fig.1 100mL solution of oil, copper was 0.919ppm, iron was 1.112ppm, zinc was 0.119ppm, calcium was 8.834ppm, sodium was 9.461ppm, while magnesium was 124.012ppm and potassium was 410.061ppm in quantity.

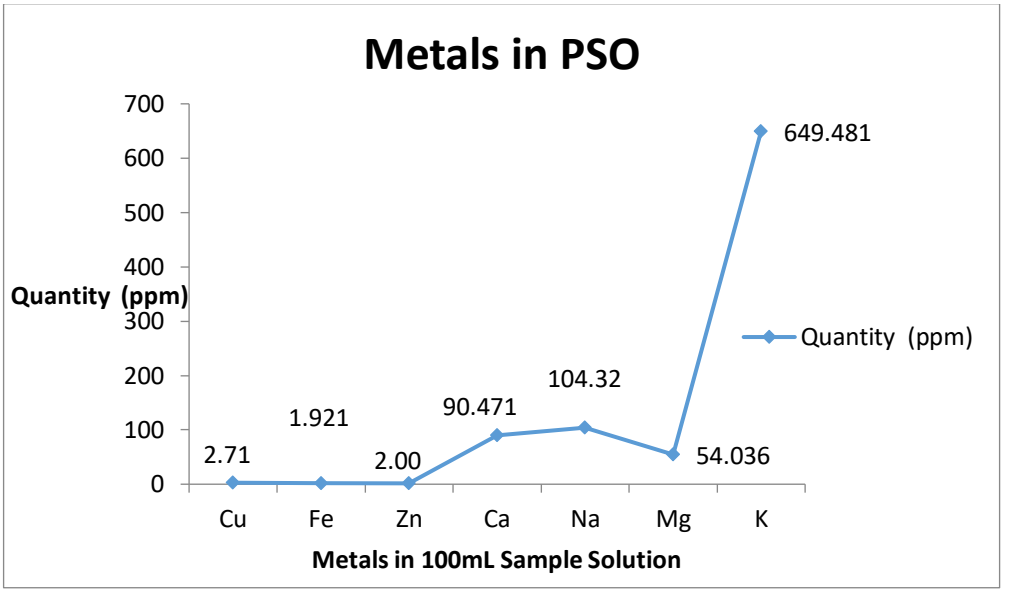

Fig. 2 metals in PSO

In fig. 2 AAS experiment was applied on 100mL pumpkin seed oil sample solution and resulted in the graph of the detected metals quantities; Copper 2.71ppm, iron 1.921ppm, zinc 2.00ppm, calcium 90.471ppm, sodium 104.32ppm, magnesium 54.036ppm and potassium 649.481ppm.

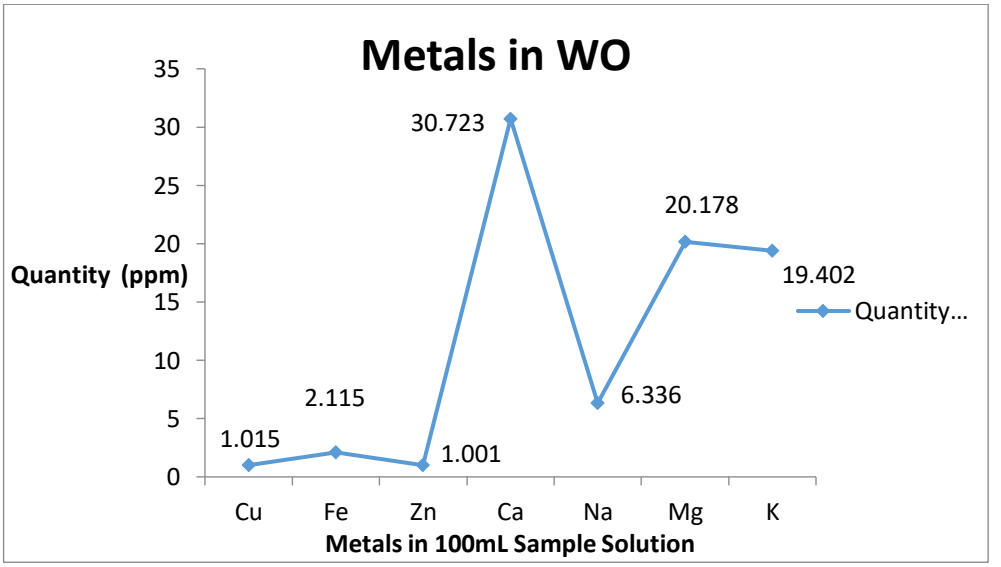

Fig. 3 Metals in WO

After AAS experiment on 100mL sample solution following quantities of metals were obtained and shown in fig. 3. Copper 1.015ppm, iron 2.115ppm, zinc 1.001ppm, calcium 30.723ppm, sodium 6.336ppm, magnesium 20.178ppm and potassium 19.402.

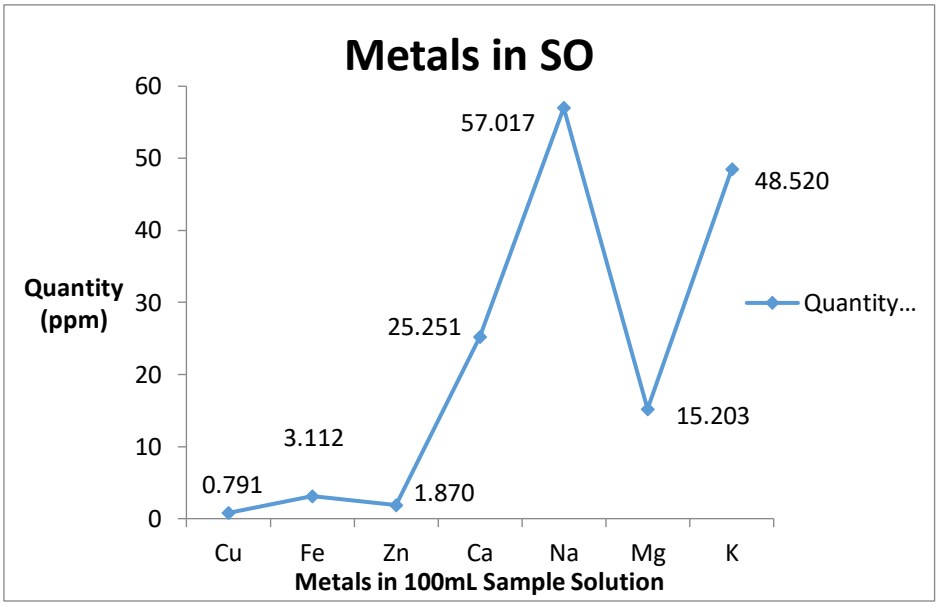

Fig. 4 Metals in SO

The results were obtained in parts per million that are: calcium 0.791ppm, iron 3.112ppm, zinc 1.870ppm, calcium 25.251ppm, sodium 57.017ppm, magnesium 15.203ppm and potassium 48.520ppm and shown in fig. 4.

B. HPLC result:

Oleic acid ($C_{18:1}$):

The quantity of oleic acid present in the pine nut oil, pumpkin seed oil, walnut oil and sunflower oil was identified by HPLC and shown in fig. 5. PNO contains 28.3, PSO contains 30.5, WO contains 20.2 and SO contains 43.9 oleic acid.

Linoleic acid ($C_{18:2}$):

Linoleic acid is present in the pine nut oil, pumpkin seed oil, walnut oil and sunflower oil. PNO contains 44.5, PSO contains 46.5, walnut oil contains 54.6 and sunflower oil contains 42.8 linoleic acid. These quantities are expressed in figure 6.

Alpha-linolenic acid ($C_{18:3}$):

Different values of linolenic acid are detected from the oils by HPLC. PNO contains 17.0, PSO contains 6.4, WO contains 15.0 and SO contains 3.5 alpha-linolenic acid as expressed in fig. 7.

Vitamin K (Phylloquinone):

Pine nut oil, pumpkin seed oil, walnut oil and sunflower oil has different values of phylloquinone measured by HPLC and expressed with the graph in figure 8. PNO contains 49.52, PSO contains 84.01, WO contains 3.68 and SO contains 1.82.

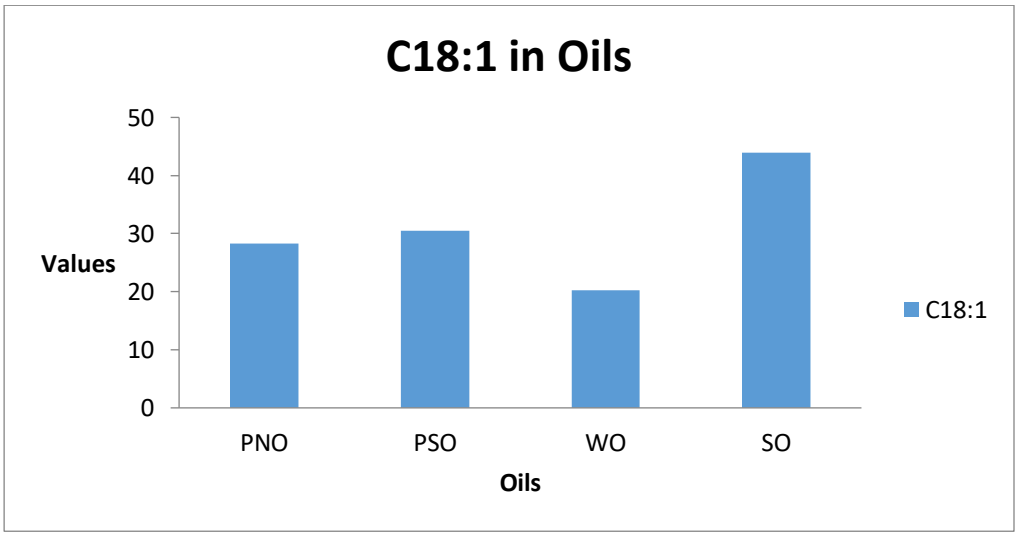

Fig. 5 Oleic acid in Oils

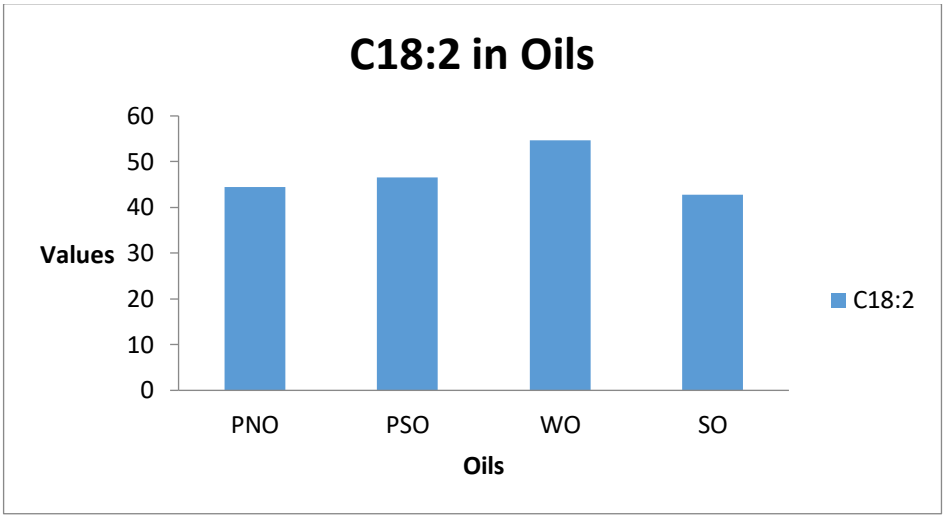

Fig. 6 LA in Oils

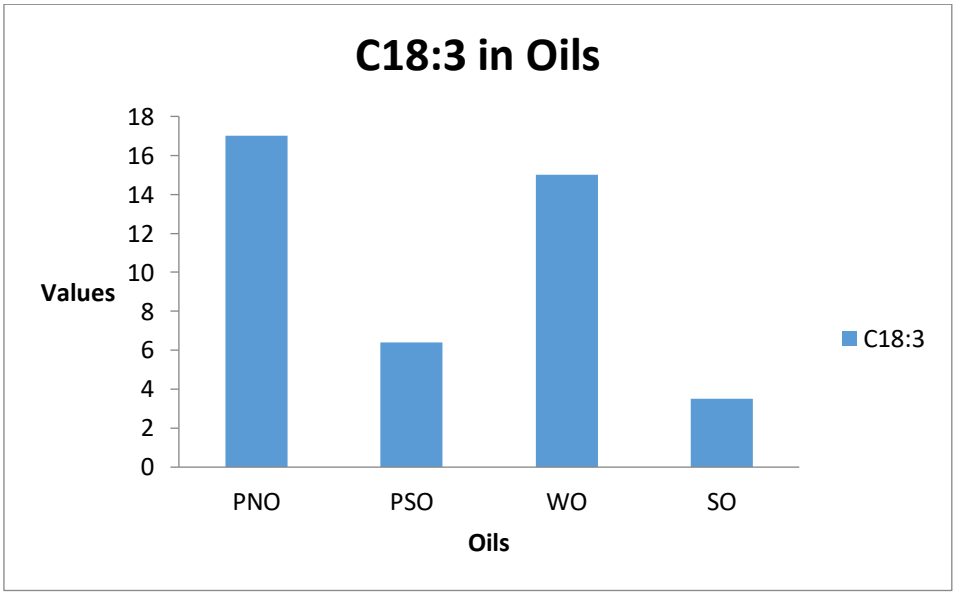

Fig. 7 ALA in Oils

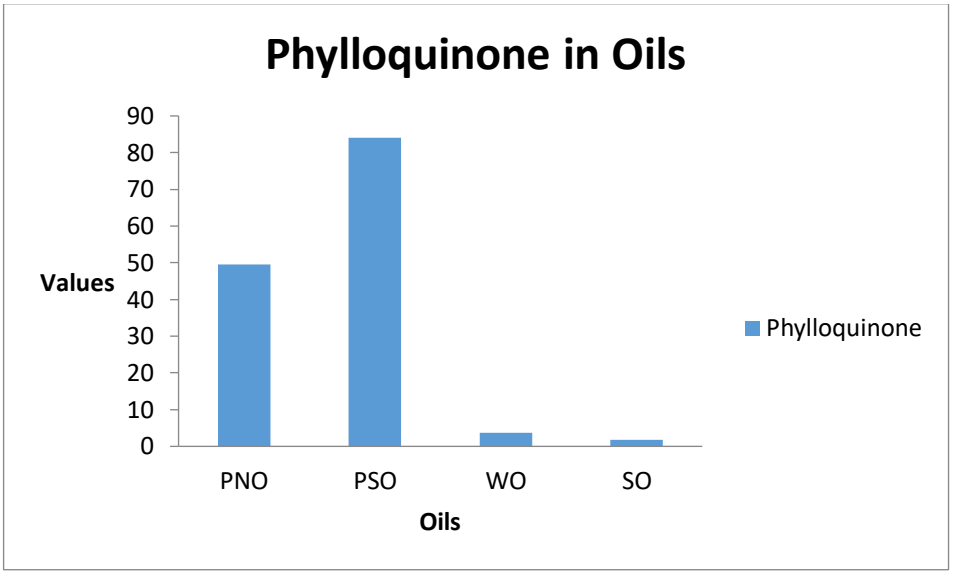

Fig. 8 Phylloquinone in Oils

C. Lipoprotein result:

After 4 weeks of this diet, the results were obtained that show the significant results. TC of controlled group 110.20±5.80, positive group 150.38±8.72, PNO 113.75±2.31, PSO 116.78±5.56, WO 115.27±9.29, SO 119.74±8.23, HDL-C of controlled group 48.49±3.62, positive group 40.86±2.25, PNO 50.24±1.71, PSO 47.89±0.74, WO 49.18±3.39, SO 41.42±6.26, LDL-C of controlled group 77.05±9.89, positive group 120.71±4.39, PNO 78.40±2.16, PSO 81.19±5.44, WO 72.30±9.91, SO 91.90±3.51, TGs of controlled group 80.67±5.43, positive group 96.63±6.62, PNO 79.35±3.52, PSO 86.25±4.20, WO 70.80±4.11, SO 75.03±5.02 . The comparison of these oils results are shown in table 1 and graphically in figure 9.

Table 1 Comparison of Lipoprotein test results of all Oils

Groups	TC mg/dL	HDL-C mg/dL	LDL-C mg/dL	TGs mg/dL
Controlled group	110.20±5.80	48.49±3.62	77.05±9.89	80.67±5.43

Positive group	150.38±8.72	40.86±2.25	120.71±4.39	96.63±6.62
PNO	113.75±2.31	50.24±1.71	78.40±2.16	79.35±3.52
PSO	116.78±5.56	47.89±0.74	81.19±5.44	86.25±4.20
WO	115.27±9.29	49.18±3.39	72.30±9.91	70.80±4.11
SO	119.74±8.23	41.42±6.26	91.90±3.51	75.03±5.02

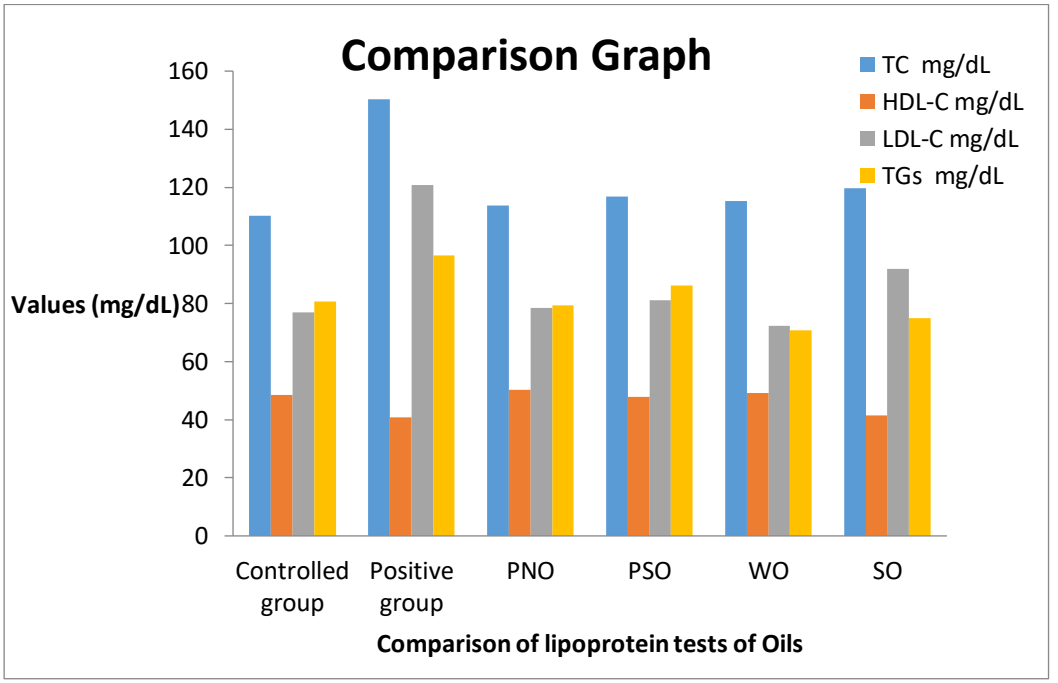

Fig. 9 Comparison of Lipoprotein test of all Oils

III. Discussion:

The present study is to know the effects of pine nut oil, pumpkin seed oil, walnut oil and sunflower oil at the different levels on the rats having high fats level. After 4 weeks of this diet, the results were obtained that show the significant results. TC of controlled group 110.20±5.80, positive group 150.38±8.72, PNO 113.75±2.31, PSO 116.78±5.56, WO 115.27±9.29, SO 119.74±8.23, HDL-C of controlled group 48.49±3.62, positive group 40.86±2.25, PNO 50.24±1.71, PSO 47.89±0.74, WO

49.18±3.39, SO 41.42±6.26, LDL-C of controlled group 77.05±9.89, positive group 120.71±4.39, PNO 78.40±2.16, PSO 81.19±5.44, WO 72.30±9.91, SO 91.90±3.51, TGs of controlled group 80.67±5.43, positive group 96.63±6.62, PNO 79.35±3.52, PSO 86.25±4.20, WO 70.80±4.11, SO 75.03±5.02.

This study revealed the major finding of decrease in LDL-C level and improvement of HDL-C level in blood causes the decrease in blood pressure; relax the muscles of arteries and heart. The heart beat became smooth and walls of vessels became wide by dissolving the fats deposited in them.

IV. Conclusion and Future research direction:

The present results revealed the antioxidant and anti-inflammatory effects of pine nut, pumpkin seed, walnut and sunflower oil. These oils show effect on the cholesterol levels causes the walls of vessels fat free and makes the heart vessels wide and blood flow easily and smoothly. These oils help to reduce the risk factors of cardiovascular diseases.

These oils can be mixed in their different percentage compositions and their composition results will be examined not just for heart diseases but can also be studied for other major organs like stomach.

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