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Some Edible Fixed Oils for Management of Obesity and Related Disorders Based on Epigenetics and Metabolome Analysis

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Abstract

Background: Obesity, which is linked to dietary habits contributes to a number of metabolic disorders. Edible fixed oils viz., olive, soybean, and pumpkin seed oils are known to have a huge impact on human overall health .However, very limited work was reported about their epigenetic role in obesity and related health risks.

MiR-143p is one of the modulators involved in the regulation of adipose tissue function and adipose tissue formation (adipogenesis).

Aims: To evaluate the MiR-143p expression level changes and potential anti-obesity protective effects mediated by olive , soybean , and pumpkin seed oils intake ;to identify their chemical constitutions by GC/MS analysis ; and to explore the physiological activities of their biochemical composition on primary metabolic organs through metabolomic analysis .

Material and methods: We investigated the effect of olive , soybean, and pumpkin seed oils on the changes of total body weight, serum glucose ,lipid profile ,and subcutaneous adipose tissue MiR-143p expression levels in H.F.D induced obese rats . The preparation of unsaponifiable and saponifiable matters of the three edible fixed oils was carried out then the analysis by GC/MS technique. These edible oils were subjected to a metabolomics analysis. Moreover , the cytotoxicity of the oils and their isolated fractions have been evaluated on several human cell lines.

Results : Oil consumption did not reduce weight gain induced by high-fat feeding among treated groups. However, a significant improving effect was observed in serum glucose level and lipid profile in response to 1ml soybean and 0.5 and 1 ml pumpkin seed oil treatments Moreover the expression levels of miR-143p in the subcutaneous adipose tissue down-regulated 0.5 to 1.1 fold in soybean and pumpkin seed oils treated groups when compared to H.F.D. The major compounds of unsaponifiable matters of olive oil, soybean oil, and pumpkin oil are squalene (83.98 %), β-sitosterol (31.8%), and γ-sitosterol (42.27 %) respectively. While in saponifiable matters, the main contents are oleic acid (71.88 %) in olive oil but linoleic acid in pumpkin oil (63.25 %) and soya bean (54.02 %).

Conclusion: Both soybean and pumpkin seed oils alter expression of miR-143p in the subcutaneous fat of high-fat diet-induced obese rats, and might contribute to the regulated expression of adipocyte genes involved in obesity and associated health problems.

Keywords: olive soybean pumpkin High-fat diet MiR-143p metabolomics GC/MS

1. Introduction

Obesity is a highly prevalent chronic metabolic abnormality associated with glucose intolerance and dyslipidemia. It represents a risk factor for many chronic diseases, most notably hypertension, metabolic syndrome(MetS),diabetes mellitus (DM) type 2, cardiovascular disease (CVD), non-alcoholic fatty liver disease (NAFLD),Alzheimer's disease ,and cancer (1, 2).Edible fixed oils as dietary interventions

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have emerged as promising therapeutic tools for metabolic diseases, with little adverse side effects, however, their type and amount are important factors influencing adipose tissue function and whole-body metabolism.

Olive, soybean, and pumpkinseed oils are among plant oil varieties, that have health promoting properties(3-5).They have been correlated to particular chemical constitutions such as, polyunsaturated fatty acids (PUFA),sterols and several bioactive phytochemicals. Metabolomics as a powerful tool for sensor development has played pivotal roles in agricultural and food science research. It has the potential to introduce a series of new rapid methods for edible oil analysis. The rapid growth of metabolomics in natural products area suggests the potential of discriminative, predictive, and informative analyses to provide important information to edible oils constituents (3, 6, 7).

It has become clear that consumption of dietary pattern rich in olive oil has a profound influence on health outcomes including metabolic syndrome and diabetes. Traditionally, many beneficial properties associated with this oil have been ascribed to its high oleic content. Olive oil however, is a functional food that, besides having high monounsaturated content, contains other minor compounds that have shown antioxidant and anti-inflammatory properties. Soybean oil has numerous other health benefits such as lowering total cholesterol and LDL due to sterols and polyunsaturated fatty acids contents. Pumpkin seed oil contains high amounts of antioxidant compounds, including polyphenols and carotenoids which may help protect against inflammation and chronic diseases also the oil is rich in omega-6- fatty acid that may be associated with improved heart health and blood sugar management. Such bioactive dietary components also have the potential to regulate several cellular signaling pathways, modulate gene expression, affect transcription factors, and alter the microRNAs(miRNA) profile(8, 9).MiRNA, a class of non-coding-RNA , regulates the expression of its target gene(s) through its catalytic/regulatory functions. The miRNA is generally synthesized endogenously, but it can also be obtained through diet and can change the expression of other genes(10, 11). It targets specific mRNA, and modulates expression of the gene(s) by binding to its complementary regions; thus, silence genes by destabilizing mRNA or preventing translation of mRNA. Dietary food components can impact cellular differentiation processes, proliferator pathways, and pathophysiological conditions(12-14).Therefore ,they have the potential to affect the activity of genes associated with chronic diseases including cardiovascular diseases, obesity ,and some cancers by modulating the associated signaling pathways(15, 16).One of the transcriptional regulators that coordinates carbohydrate and lipid metabolism is miR-143-p (17).Previous research demonstrated that the expression of miR-143-3p was dysregulated in human obese patients as well as in diet-induced obese, db/db, and diabetic mice (18). Moreover , studies examining the role of miR-143-3p in adipose tissue by using gain- or loss-of-function methods have shown that miR-143-3p stimulates adipocyte development and differentiation (19).Furthermore, it increases the hepatic miR-143-3p expression which in turn affect the systemic glucose homeostasis and hepatic insulin action.(20).

2. Materials and Methods.

The experimental protocol was approved by the medical research ethics committee of National Research Centre, Giza, Egypt (ethical number; 1153042021).

2.1.Diets and Experimental Animals:

The H.F.D was prepared by adjusting the proportion of fat of normal diet based on previous studies. Fat content was 20% as sheep tallow and 1% as cholesterol. Stainless steel nipples were provided for drinking. Diet-induced obese rat model was obtained from animal house in NRC. All animal care procedures and methods were performed in accordance with the guidelines of medical research ethics committee of NRC. Forty eight male Wistar rats weighing (130-150g) were divided into eight groups under the same maintenance conditions. Oral doses of seed oils were administrated to H.F.D–treated groups daily for 8 weeks.

1- Control group: Rats in this group received normal chow diet for 8 weeks.
2- Group (H.F.D):Rats in this group received a H.F.D for 8 weeks.
3- Group (H.F.D+0.5 ml olive oil): Rats in this group received (H.F.D + 0.5ml olive oil) for 8 weeks
4- Group (H.F.D+1 ml olive oil) : Rats in this group received(H.F.D + 1ml olive oil) for 8 weeks.
5- Group (H.F.D+ 0.5 ml soybeans seed oil): Rats in this group received(H.F.D + 0.5 ml soybean seed oil) for 8 weeks.
6- Group (H.F.D+1 ml soybean seed oil): Rats in this group received (H.F.D+1 ml soybean seed oil) for 8 weeks.
7- Group (H.F.D+0.5 ml pumpkin seed oil): Rats in this group received(H.F.D + 0.5ml

bottle gourd seed oil by gavage) for 8 weeks.

8- Group (H.F.D+1ml pumpkin seed oil): Rats in this group received (H.F.D + 1ml bottle gourd seed oil by gavage) for 8 weeks.

Body weight and length (anal to nasal) measurements were recorded at 0, and after 8 weeks for all groups.

2.2. Biochemical Analysis

2.2.1. Serum glucose levels, Triglycerides and cholesterol levels, High Density Lipoprotein – Cholesterol (HDL-C) levels were determined, as instructed by the Manufacturer (Spectrum kit, Egyptian Company for biotechnology (S.A.E) Obour City, Cairo, Egypt).

2.2.2. Low Density Lipoprotein–Cholesterol (LDL-C) levels were calculated by the traditional Fried Ewald's formula:

LDL-C (mg/dl) = TC-HDLC - TG/5(21) .

2.3.MiR 143-p Expression Assay

2.3.1. RNA isolation: Total RNA was isolated from subcutaneous white adipose tissue by using miRNeasy Mini kite (Qiagen). 10 mg of adipose tissue, from three animals per group, was grinded with liquid nitrogen then placed in QIAzollysis reagent. RNA isolation was done according kite instructions.

2.3.2.RNA purification, the aqueous phase with ethanol was transferred into RNeasy Mini spin column and washed several times with different buffers. Finally; the concentration of harvested RNA was measured by Nanodrop 1000 spectophotometer (Thermo Scientific).

2.3.3. Quantitative real time PCR analysis:

miRCURY LNA miRNASybrGreen PCR kit (Qiagen) was used to evaluate the expression ofMiR-143 3p in subcutaneous white adipose tissue. Firstly; cDNA was synthesized from 10 ng of total purified RNA from the different samples according to reverse transcription reaction manual protocol. qRT-PCR was applied on mixture containing 7 µl of fresh diluted cDNA (1:59), 10 µl of Sybr master mix and 2 µl of primer, total volume was completed up to 20 µl by nuclease free water. The thermal profile of the reaction was started with initial heat activation; 95ºC for 2 minutes, followed by 40 cycles in two steps; 95ºC for 10 seconds and 56ºC for 60 seconds. Melting curve analysis was done at 60ºC and 95ºC at the end of the reaction. Experiments were done in Rotor-Gene Qiagen instrument. The expression level of MiR-143 3p was normalized by using MiR-103, as a reference gene, and calculated by $2^{-\Delta\Delta CT}$ method.

2.4. Preparation of Unsaponifiable Matter and Fatty Acid Methyl Esters (22)

2.4.1. Preparation of Unsaponifiable Matter

One ml of selected seed oils has been separately refluxed for 6 hrs with 0.5N alcoholic KOH (60ml) in a boiling water bath. The saponified fraction has been concentrated to 1/3 its volume. After cooling, the reaction mixture has been diluted with an equal volume of distilled water and exhaustively extracted with ether. The combined ethereal washed several times with distilled water until the absence of alkalinity and dehydrated over anhydrous sodium sulphate. After evaporation of ether, it has been kept at 4oC for analysis GC/MS.

2.4.2. Quantitative Determination of the Total Free Fatty Acid Content

The alkaline aqueous solution remaining after extraction of the unsap. Matter has been acidified with hydrochloric acid to liberate the fatty acids which extracted several times with ether. The combined ethereal washed several times with distilled water until the absence of alkalinity and dehydrated over anhydrous sodium sulphate, then filtered, the filtrate was evaporated then weighted to determined the percentage of the total free fatty acids in each oil.

2.4.3.Preparation of the fatty acids methyl esters.

The obtained fatty acids has been dissolved in 50 ml absolute methanol , mixed with 0.25ml sulphuric acid, refluxed for about 3 hrs, cooled, diluted with about 100ml distilled water and extracted several times with ether. The combined ethereal washed several times with distilled water until the absence of alkalinity and dehydrated over anhydrous sodium sulphate, then filtered, the filterate was evaporated and the obtained fatty acids methyl ester has been subjected to GC/MS analysis.

Conditions of GC/MS analysis of saponifiable and unsaponifiable fractions of the three edible fixed oils.

2.4.4.GC/MS of Unsaponifiable matter:

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analyses were carried out using Hydrogen as the carrier gas at a flow rate of 2.0 ml/min at a splitless, injection volume of 2 µl and the following temperature program: 50 °C for 5 min; rising at 5 °C /min to 100 °C and held for 0 min and rising at 10 °C /min to 320 °C and held for 10 min . The injector and detector were held at 280 °C,320 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 25-700

and solvent delay 6 min. The mass temperature was 230°C and Quad 150°C. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

-GC/MS of fatty acid methyl ester The GC model 7890B from Agilent Technologies was equipped with flame ionization detector at Central Laboratories Network, National Research Centre, and Cairo, Egypt. Separation was achieved using a Zebron ZB-FAME column (60 m x 0.25 mm internal diameter x 0.25µm film thickness). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 1.8 ml/min at a split-1:50 mode, injection volume of 1 µl and the following temperature program: 100 °C for 3 min; rising at 2.5 °C /min to 240 °C and held for 10 min. The injector and detector (FID) were held at 250 °C and 285 °C, respectively.

2.5. Metabolomic Analysis :

There has not been previous reported data about metabolomics analysis of these edible oils to explore their roles on a primary metabolic organ. Therefore, the metabolic variability of the different three oils analyzed by GC-Ms was explored using two unsupervised methods namely principal component analysis (PCA) and hierarchical cluster analysis (HCA).PCA algorithm is employed to achieve unbiased dimensionality reduction, providing an informative first look at the compositional differences between the samples.

2.6.Cytotoxicity:

According to Mosmann (1983)(23), Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT. This method was to determine the cytotoxic effect of extracts on normal skin fibroblast cell line (BJ1). The cells were seeded into 96-well culture plate at10x103 cells/well in fresh complete growth media which was aspirated after 24hrs. Fresh medium (without serum) was added to the cells at different concentrations of either fixed oils or Doxorubicin (positive control).The cells incubated for a further 48 h then the medium was replaced with 40ul MTT salt (2.5µg/ml).the cells were incubated for further four hours. To stop the reaction and dissolving the formed crystals, 200µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC. The absorbance was measured at 540 nm. A probit analysis was carried for IC50 and IC90 determination using SPSS 11 program.

2.7.Statistical analysis

All data were presented as mean±SD. Statistical analysis of mir-143-3p data was performed with SPSS software version 15. The comparison between group means was analyzed by one-Way ANOVA, followed by Tukey as Post-hoc test. The significant level was valued at $p<0.05$. Additionally, the metabolic variability of the different three oils was explored using two unsupervised methods namely principal component analysis (PCA) and hierarchical cluster analysis (HCA).

3. RESULTS

1- Diets and body weight change

Throughout the experimental period, and due to the higher caloric content of H.F.D, higher body weight was gained (48%) by H.F.D fed animals (group2) than control (group1) with significant value ($P<0.01$). Although, there were no remarkable changes in the body weight as well as the nasal to anus length in oil treated obese groups, little subcutaneous fat have developed compared to those fat fed group rats.

Table (1): Effect of olive, soybean, and pumpkin seed oils on total body weight change, and nose to anus distance change (cm) n=6

Groups	Mean of total body weight change(g)	Mean of nose to anus distance change (cm)
Group 1(control)	71.8±9.96	12.2±0.87
Group 2(H.F.D)	120±10.82	15.6±0.45
Group 3	123.32±9.41	16 ±0.65
Group 4	132.5±5.63	15±0.48
Group 5	139.0±5.63	14±0.45
Group 6	139.8±12.88	13.2±1.24
Group 7	138.25±24.29	14.0±1.31
Group 8	135.9±14.32	15.4±0.93

2- Biochemical Analysis

The H.F.D rats underwent about 50% significant increase in their serum glucose compared to control rats ($P<0.05$), Table (2). While H.F.D treated groups with oils displayed about 30%- 40% significant reduction compared to un-treated group (H.F.D).It also apparent from table2 that serum triglyceride and cholesterol levels did not significantly decrease in olive oil-treated groups (0.5ml, 1ml), and soybean oil -treated group (0.5ml). However, it significantly decreased in soybean oil-treated group (1ml) and pumpkin seed oil- treated groups (0.5ml and 1 ml). Additionally, a significant increment of HDL-cholesterol levels and a significant decrement in LDL- cholesterol in soybean oil- treated group (1ml) were observed compared to H.F.D. group.

Table (2): Serum glucose, triglycerides, cholesterol, HDL, LDL levels in all treated groups with olive, soybean, and pumpkin seed oils (Mean ±SEM).Letters a, b and c are statistical significance levels

Groups	Serum Glucose (mg/dl)	Serum Triglycerides (mg/dl)	Serum Cholesterol (mg/dl)	Serum HDL (mg/dl)	Serum LDL (mg/dl)
Group1	114 ±7.4	109± 9	83.5 ±15	20±1.2	30±1.4
Group2	173±10 [a]	142±25 [a]	93 ±9.3	15±1.3 [a]	43±2.3
Group 3*	120±13[ab]	149±11[a]	85±2.9	15±2.1 [a]	42±1.9
Group 4**	100±4[b]	147±26 [a]	88±10	18±1.9	49±2.5
Group 5*	113±11[b]	145±26 [a]	61±7.2[b]	13±1.0 [a]	39±2.3
Group 6**	116±4[b]	66± 7 [ab]	60±2.3[b]	11±0.9 [a]	38±1.7
Group 7*	110±8.9[b]	69 ±4 [ab]	57±3.4 [b]	19±1.1 [b]	26±1.9 [b]
Group 8**	102±17 [b]	59 ±7 [ab]	55±4.1 [b]	21±1.3[b]	23±1.6 [b]

*: 0.5 ml of seed oil**:1 ml of seed oil
a: no significant b: P<0.05 ab: P<0.01

3- MiR 143-p Expression

The up-regulation of miR-143p was evident in adipose tissue of H.F.D, and H.F.D treated group with olive oil. However, significant down regulation of miRNAwhich ranged from 0.5 to 1.1 was observed in adipose tissue of H.F.D treated groups with 0.5ml and 1ml soybean and pumpkin seed oil (Table 3& Figure 1).

Table (3): Subcutaneous miR-143-3p relative fold change in control and H.F.D fed rat groups in response to olive, soybean, and pumpkin oils oral consumption (Mean ±SEM) n=5.Letters a, b ,c ,and d are statistical significance levels.

Groups	Mir-143-3p relative fold mean
Group 1	1±0.15
Group 2	1.8±0.34[b]
Group 3	1.3±0.12[bc]
Group 4	3.4±0.11[a]
Group 5	0.5±0.15[d]
Group 6	1.05±0.27[c]
Group 7	0.98±0.16[cd]
Group 8	1.1±0.17[c]

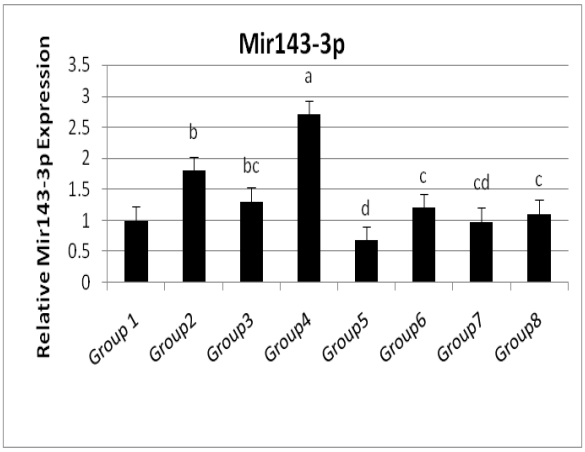


Fig.1.The effect of different doses of olive, soybean and pumpkin seed oils on the expression level of Mir-143-3p. letters a, b ,c ,and d are statistical significance levels.

GC/MS resultsof olive, soybean, and pumpkin oils Tables 4 &5 summarize the Unsaponifiable and saponifiable matters of olive oil, soybean and pumpkin oils .Olive oil has mainly terpene which is squalene 83.98 % as shown in table 4 .It is a triterpene compound formed by six isoprene units.While the majority of saponifiable matters are unsaturated fatty acids mainly oleic acid (Omega 6) 71.88 % table (5). It is also apparent that the unsaponifiable matters of soybean oil contain mainly steroids 72.23 % and terpenoids 25.23 %. The majorities of steroids are β-sitosterol 31.8%, campesterol 16.49% and stigmasterol 12.28 %. However, the saponifiable matters contain mainly unsaturated fatty acid 82.83% which consist of major metabolites as linoleic acid 54.02% and oleic acid 21.23%. Interestingly, the unsaponifiable matters of pumpkin oil contain mainly steroids 59.14 % which has γ-Sitosterol 42.27 %. γ-sitosterol is an epimer of β-sitosterol. While the saponifiable matters the majority is unsaturated fatty acid 81.76 % mainly linoleic acid (Omega 3) 63.25%.

In this study, we examined in vitro the bio-activity of three oils, soybean, olive, Pumpkin on normal skin cell line fibroblast (BJ1)forthe assessment of their toxicity on normal cell. The results indicated that, all fixed oils had no cytotoxic effect on BJ cell line.

Table (4): Percentage ratio of the identified metabolites of unsaponifiable matters of the olive, soybean, and pumpkin oils by GC/MS.

NO	Oil / Metabolites	Olive	Pumpkin	Soybean
1	Squalene	83.98	16.57	2.54
2	α-Tocopherol	1.28	000	1.11
3	β-Tocopherol	000	0.66	000
4	γ-Tocopherol	1.17	13.45	18.40
5	δ-Tocopherol	3.91	000	3.03
6	β-Sitosterol	2.51	7.94	31.8
7	γ-Sitosterol	3.21	42.27	11.66
8	Campesterol	000	0.85	16.49
9	Desmosterol	000	6.17	000
10	Stigmasterol	000	000	12.28
11	Retinoic acid	2.19	10.09	2.69
12	cis-Z-α-Bisabolene epoxide	1.21	000	000
13	Cholic acid	000	1.91	000
Total identified terpenes		83.98	16.57	2.54
Total identified terpenoids		10.30	24.29	25.23
Total identified steroids		5.72	59.14	72.23

Table (5):Percentage ratio of the identified metabolites of saponifiable matters of the olive, soybean, and pumpkin oils by GC/MS.

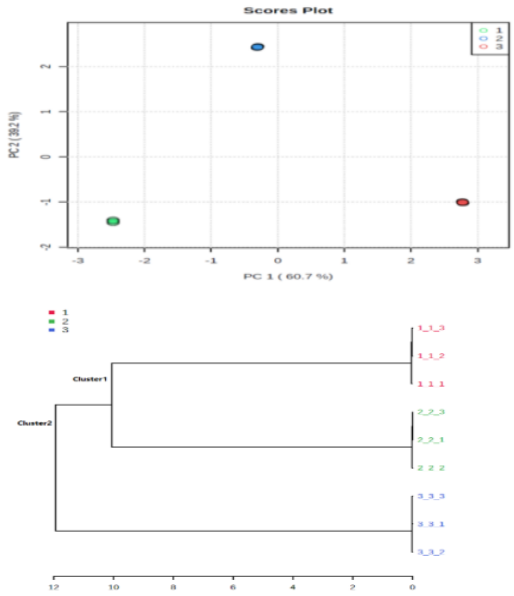
NO	Oil / Metabolites	Olive	Pumpkin	Soybean
	Myristic acid	000	0.66	000
	Pentadecanoic acid	000	0.67	000
1	Palmitic acid	12.99	9.92	10.46
2	Palmitoleic acid	0.73	000	000
3	Steric acid	2.34	5.79	4.96
4	Oleic acid	71.88	15.12	21.23
5	Linoleic acid	8.78	63.25	54.02
6	Linolenic acid	0.86	0.71	6.47
7	Arachidic acid	0.77	0.66	0.64
8	cis-11-Eicosenoic acid	0.83	0.64	0.56
9	cis-11,14-Eicosadienoicacid	000	0.67	0.55
10	EPA (Eicosapentaenoicacid)	000	0.63	000
11	Heneicosanoic acid	000	000	0.55
12	Behenic acid	0.81	0.64	0.56
13	DHA (Docosahexaenoicacid)	000	0.66	000
Total saturated fatty acid		16.91	18.24	17.17
Total unsaturated fatty acid		83.08	81.76	82.83

Table (6) : % mortality and $IC_{50}$of olive, soybean and Pumpkin oils on normal skin fibroblast cell line cell line at 1000µg/ml

Oil type	Bj1	IC_{50} (µg/ml)
olive oil	5.1%	<100
soybean oil	3.8%	<100
Pumpkin oil	4.3%	<100
-ve control	2.7%	<100
+ve control	100%	31.6

Multivariate data analysis of olive oils ,soyabean ,and pumpkin, was carried out by the established model shown in (Fig. 2A & B). It resulted in the formation of two orthogonal PCs, which explained 99.9% of the total variability among the oils samples, i.e., PC1, accounted for 60.7% of the variance versus 39.2% for PC2. The PCA score plot (Fig. 2A) showed that "olive oil" was remotely positioned, along the positive side of PC1, from the other oils samples, which could be ascribed to their variable metabolic make up. The pumpkin and soyabean oils were located along the negative side of PC1 indicating the nearly of their composition.

2A



2B

Fig.2A & B: Multivariate data analysis of olive, soyabean, and pumpkin seed oils.

Receiver Operating Characteristic (ROC) analysis for those common metabolites between two oils that showed the strongest differences between pumpkin and soyabean oils. As shown in Fig. 3 linolenic acid, stigmasterol, delta-tocopherol and EPA have AUC value: 0.0128, 0.00286, 0.00494, 0.00686 (1, 1) respectively. As shown from these results these

metabolites provided the best discriminative power two oils.

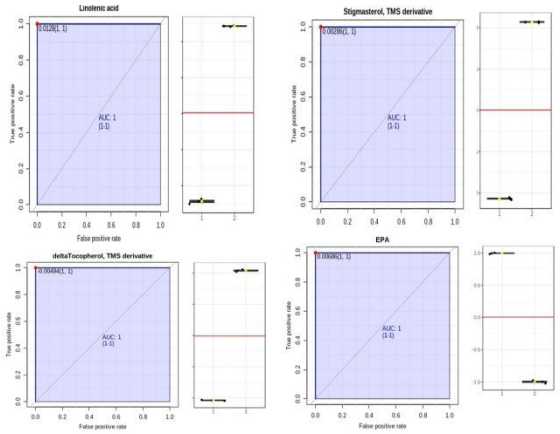


Fig 3: ROC curve analysis of potential oils related biomarkers to differentiate pumpkin and soyabean oils.

4. Discussion

Prior studies have noted that edible oils play an important role in human's food and nutrition(24).However ,The entire health is greatly impacted by its quality.

This study set out with the aim of assessing the potentially effect of olive , soybean ,and pumpkin seed oils on fat-diet induced obesity by acting on 143 p miRNA as regulator involved in adipose tissue differentiation ,and metabolism, including lipid and glucose homeostasis.

The present study demonstrated that feeding Wistar rats a H.F.D for 8weeks resulted in a 48% increase in total body weight, , increases in fasting blood glucose , total cholesterol ,and triglycerides compared to control rats similar to a previous reports by(25, 26).

The groups of rats which were given various amounts of oils did not show a significant change in total body weight promoted with the H.F.D .On the contrary, they showed a slight non significant increase. Even though these results differ from some published by Soriguer F et al., in 2009 and others (27-29) , they are consistent with those by Deol P et al,. 2010 and others who referred this to the contribution of excess energy intake by the consumed seed oils (30-32).

Although the effect of edible fixed oil on weight loss have been contradictory, it is probable that the experimental time (8 weeks) influenced the final result, adaptations of specific genes can occur which may mask the effects of the three oils.

Interestingly, our results showed that feeding rats with 0.5ml and 1.0 ml of soybean, and pumpkin seed oil despite not reducing weight, produced improvement in lipid profile including low levels of circulating cholesterol and triglycerides compared to H.F.D fed rats . One possible explanation is that the highest content of γ-sitosterol and omega 6 may act effectively in controlling the cholesterol and triglycerides blood levels in animal received pumpkin oil in the food(33-36).

However , the observed hypercholestermia in olive oil treated- group could be explained by the presence of high percentage of squalene 83.98 % which is the precursor of cholesterol(37).

Accordingly, soybean, and pumpkin seed oils could be used for the control of obesity induced dyslipidemia and hyperglycemia and there is not any observed cytotoxic effect on normal cell line.

An important finding in our study ,is that feeding rats with 0.5ml and 1.0 ml of soybean, and pumpkin seed oil showed a significant decrease in mir-143pexpression.Such decreased levels would justify a lower subcutaneous adipogenic capacity of soybean and pumpkin seed oils and, would, therefore, contribute to the decrease of fat stores observed in subcutaneous adipose tissue with higher intake of fats (sheep tallow) .

LIN XIHUA et al support the idea when observed that Hematoxylin/Eosin staining of white adipose tissues revealed that mice treated with antagomir-143-3p had smaller size of adipocytes in Inguinal white adipose tissue (iWAT)and Epididymal white adipose tissue (eWAT) , but there were not significant changes in the total body weight as well as the weight of white adipose tissues in obese mice in response to antagomir-143-3p treatment (38).

Moreover ,our finding of the adipose miR-143 level overexpression in H.F.D induced obese rats compared to control group rats is consistent with prior studies describing miR-143-3p as being upregulated in tissues of ob/ob or db/db mice, as well as diet-induced obese and diabetic mice .Moreover ,it is fairly well with Esau C et al., and Takanabe R (19, 20)who further support the idea thatmiR-143involved in adipogenesis and associated with obesity(39).Our finding, based on metabolomics approach, suggest a metabolome analysis to be applied for edible oils, identifying the profile of valuable components as authenticity biomarkers and health-promoting molecules. The obtained results in this research pointed out that GC-MS combined with chemometric analysis, such as ROC and heat map clustering, have a good predictive ability to detect the edible oils and identify qualitative and quantitative differences between individual samples from the same phenotype and the specific features of each category of oils. Therefore, these complementary data are useful not only to identify the oil category but also the quality of edible oils.

5. Conclusion and recommendation:

Either soybean or pumpkin seed oils consumption did not suppress the H.F.D-induced body weight gain but tended to minimize abnormal changes in levels of serum blood glucose, TG, TC ,HDLc, LDLc in obesity. The oils were also effective at improving the mir-143-3p expression, suggesting that it could be affecting the epigenetic regulator genes for obesity and dyslipidemia.

Their impact in prevention and/or reduction of hyperglycemia and dyslipidemia through downregulation of miR-143-3p might be carried out by their various components, such as γ-Sitosterol and poly unsaturated fatty acids mainly oleic acid (Omega 9).However, further studies are necessary to demonstrate the real physiological consequences of the epigenetics modification induced by these oils and their phenolic compounds.

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