

Effect of Sage, Sesame and Olive Oils Alone and in Combination with Sage on Weight Reduction and Fasting Blood Sugar in Obese Non-Alcoholic Fatty Liver Albino Rats.

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Abstract

The aim of this study was to investigate the effects of sage, sesame and olive oils alone and in combination with sage on daily feed intake (FI), body weight gain% (BWG%), organs weight to body weight ratio, peritoneal fat pad ratio, serum blood glucose and leptin hormone in nonalcoholic fatty liver disease (NLFLD). A total of 72 adult male albino rats of Spragu-Dawley Strain weighing $200 \pm 10g$ were used in this study. The rats were divided into two main groups. The first group (8 rats) was fed on basal diet (BD) and used as a control negative group (-ve). The second main group (64 rats) was fed on high fat diet (HFD) for (8) weeks to induce non-alcoholic fatty

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liver disease (NLFLD). NLFLD rats were divided into (8 subgroups). One of them (8 rats) was fed on (HFD), used as a positive control group (+ve). The other (7 subgroups) fed on modified (HFD), one of them by adding 5% sage. The other subgroups were fed on modified (HFD) by replace 20% sheep tallow (ST) to 20% sesame oil (SO), 20% olive oil (OO) and mixed 10% (SO) with 10% (OO) without or with sage for (8 weeks). At the end of experiment, rats were anesthetized by ether before sacrificing after fasting overnight, liver, kidney and peritoneal fat pad were then taken and weighted. Blood samples were collected, left to clot, the serum was separated, glucose and leptin level were determined. The obtained results revealed that, feeding obese (NLFLD) rats on modified high fat diet (MHFD) containing 5% sage or by replacing 20% (ST) to 20% (OO), 20% (SO) or mixed 10% (SO) with 10% (OO) plus or without 5% sage significantly decreased body weight gain%, relative weight of liver, kidney and peritoneal fat pad%, serum fasting blood glucose and leptin hormone. Our results revealed that, mixed 10% (SO) with 10% (OO) plus 5% sage may represent a potentially therapeutic provide the greatest activity decrease in BWG%, organs weight / body weight %, peritoneal fat pad%, fasting blood glucose and leptin hormone level of obese nonalcoholic fatty liver disease (NLFLD) in rats.

Introduction

Non-alcoholic fatty liver disease affects 10-35% of adult population worldwide (**Bellentani and Marino, 2009**). When we consider the management of nonalcoholic fatty liver disease (NAFLD), two aspects should be considered. One is that can be a part of metabolic syndrome (**Lonardo et al., 2015**). About 80% of patients with metabolic syndrome have NAFLD (**Antunes and Bhimji, 2017**). Although the prevalence of NAFLD is 20 – 40% in the general population, about 70% of type 2 diabetes mellitus (**Leite et al., 2009**) and 85% of patients with morbid obesity (BMI \geq 40) have NAFLD (**Fabbrini et al., 2009**). In the general population, 80% of patients with NAFLD are overweight and 20% of NAFLD patients have normal weight as per ultrasonography (**Bellentani and Tiribelli, 2001**).

High fat diets (HFD) are well known to increase body weight, body fat and induce insulin resistance in rodent models (**Samuel et al., 2004**). On the other hand, **Yki-Jarvinen, (2005)** reported that, dietary fat content modifies liver fat in overweight non diabetic subjects. Decreasing total fat consumption and shifting to mono-unsaturated fatty acids MUFAs found in olive oil (20-40%) of total energy or n-3 PUFAs found in fish oil (2g/d) could lead to a decrease in postprandial lipidemia and steatosis (**Capanni et al., 2006**). Sesame oil, which is extracted from sesame seeds, contains both monounsaturated fatty acids and polyunsaturated fatty acids. Sesame oil contains vitamin E and other antioxidant compounds

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known as lignans. Lignans acts as compounds important for antioxidant activity since they help the absorption of vitamin E and other phytochemical (**Sankar et al., 2006**).

Herbal treatment of many diseases including hepatopathy is increasing in many countries. Some plants have been shown to have protective antioxidant effects and are therefore hepatoprotective. Such plants include sage "*Salvia officinals* L". (**Carla et al., 2009**). *Salvia officinals* L (Sage) has been reported to have a wide range of biological activities, such as antioxidants, antibacterial, hypoglycemic and anti-inflammatory properties. Recent studies have found that the sage has positively physiological effects on heart, liver, kidney and testes (**Chien et al., 2011**). Therefore, this study aimed to investigate the effects of sage, sesame and olive oils alone and combination the oils with sage on daily feed intake (FI), body weight gain% (BWG%), organs weight to body weight ratio, peritoneal fat pad ratio, serum glucose and leptin hormone in obese Non-Alcoholic Fatty Liver Albino Rats.

Materials and Methods

Material: Casein, vitamins, minerals, cellulose, and choline chloride were purchased from El- Gomhoria Company, Cairo, Egypt. Soy, sesame and olive oils were obtained from Agricultural Research Center, Giza, Egypt. Sage was obtained from the National market of Agricultural herbs and medicinal plants, Cairo, Egypt. **Kits** for

biochemical analysis were obtained from Alkan for pharmaceutical and chemical Dokki, Egypt.

Rats: Seventy – two adult male albino rats (Spragu Dawley Strain) were purchased from Helwan farm of experimental animals, Ministry of Health and population, Helwan, Cairo, Egypt.

Methods:

Experimental Design:

After acclimation on a basal diet (BD) for one week, rats were classified into two main groups. The first main group (n=8) fed on BD as a control negative group (-ve). The second main group (64) rats were fed on high fat diet (HFD) to induce nonalcoholic fatty liver disease according to *(Zarghani et al., 2016)*. After 8 weeks' body weight gain %, serum lipid; cholesterol and triglycerides were determined for first and second main groups to insure the induction of obesity and fatty liver, then the second main group fed on (HFD) for 8 weeks and then were divided into eight subgroups:

- The first group fed on (HFD) all over the experimental period, as a control positive group (+ve).
- The second group fed on HFD containing 5% sage.
- The third and fourth groups fed on modified high fat diet (MHFD) by replace 20% (ST) to 20% olive oil (OO) or 20% sesame oil (SO), respectively.
- The fifth and sixth groups fed on modified high fat diet (MHFD) by replace 20% (ST) to (10% OO and 10%SO) or (10% OO and 10%SO) plus 5% sage, respectively.

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-The seventh and eighth groups were fed on modified high fat diet (MHFD) by replacing 20% (ST) to 20% (SO) plus 5% sage and 20% SO plus 5% sage, respectively.

Biological Evaluation:

Daily feed intake (FI) and body weight gain% (BWG%) were checked once a week. At the end of the experimental period (8 weeks) daily feed intake (FI), body weight gain (BWG %) and organs weight / body weight were specified according to the method of (**Chapman et al., 1959**). Animals were fasted overnight, and then anesthetized by very light ether before sacrificing. Blood samples were collected from hepatic portal vein of each rat. Serum were carefully separated by centrifugation of blood sample. Then kept frozen at - 20°C Until the analysis is done. Liver, kidneys and peritoneal fat pad were taken from rats by careful dissection, washed in saline solution (0.9%) and lastly dried using filter paper and independently weighed.

Biochemical analysis:

Determination of serum leptin was done according to (**Guillaume and Bjorntorp 1996**) and serum glucose according to **Trinder (1969)**.

Statistical Analysis:

Results of biochemical analysis and biological evaluation of each group were statistical analyzed, mean, standard error and one-way ANOVA test using SAS package with level of significant $p < 0.05$ (**SAS, 2004**).

Results and Discussion

Effects of sage, sesame and olive oils alone or in combination on feed intake and body weight gain % in obese nonalcoholic fatty liver rats.

Table (1) illustrate the effects of high fat diet (HFD) alone or with modification by adding 5% sage or by replacing (20%) (ST) to 20% olive oil (OO), 20% sesame oil (SO), 20% mixed (OO) with (SO) (1:1 W/W) without or with 5% sage on feed intake (FI) and body weight gain % (BWG%). Results revealed that the (-ve) group fed on basal diet (BD) recorded a significant increase ($p < 0.05$) in FI, as compared to the control (+ve) fed on (HFD). While (+ve) control group which fed on (HFD) with modification by adding 5% sage, or by replacing 20% sheep tallow (ST) to 20% (SO), 20% (OO) or 20% from mixed (SO) plus (OO) (1:1 W/W) with or without 5% sage, our results revealed that, there is no significant difference in FI between (+ve) control group and all groups which fed on (HFD) with different modifications. However, the caloric intake of albino rats fed on (BD) (-ve) group was lower, as compared to the (HFD) groups. We also found that no significant difference in (FI) between (+ve) control group fed on (HFD), as compared to the other groups fed on modified (HFD).

Concerning body weight gain % (BWG%) Table (1) shows that (-ve) group fed on (BD) and (+ve) group fed on (HFD) recorded (19.260 ± 0.708 vs 29.724 ± 1.084 , respectively). Results revealed a significant increase ($P < 0.05$) in BWG % of the (+ve) group, as

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compared to the (-ve) group. However, the caloric intake of albino rats (-ve) group fed on (BD) was lower as compared to the (HFD) groups. Concerning groups which fed on modified (HFD) by adding 5% sage or by replacing 20% sheep tallow (ST) to 20% (OO), 20% (SO), 20% mixed from 10% (SO) plus 10% (OO) (1:1 W/W) with or without 5% sage. Results revealed a significant decrease ($P < 0.05$) of all treated groups fed on modified (HFD), as compared to the (+ve) group fed on (HFD). On the other hand, our results revealed that the best results as induced decrease in BWG% recorded by (+ve) group (NAFLD) fed on modified (HFD) by replacing 20% (ST) to 20% from mixed 10% (OO) with 10% (SO) plus 5% sage, followed by the (+ve) group fed on modified (HFD) by replacing [20% (ST) to 20% (OO)] plus 5% sage. Our results revealed that the combination between 10% (OO) with 10% (SO) plus 5% sage induced high potential effects on reducing BWG % in (NAFLD) fed on (HFD). Our results revealed that sage species might potentially provide natural treatment for the cure of obesity.

In this concern (*Hamidpour et al., 2014*) suggested that salvia species, in addition to treating minor common illness, might potentially treatment for relief of many diseases such as depression, dementia, obesity, diabetes, lupus, heart disease and cancer, concerning the effect of (SO) our results are at the same line with (*Hareeri et al., 2017*) who found that oral intake of sesame oil in the treated doses 4 and 8 ml / kg / body weight caused a significant ($p < 0.05$) decreased in BWG %, as compared to (+ve) control group.

Concerning the effects of MUFA in olive oil promoted benefits on weight reduction. in this concern (*Kaippert et al., 2015*) suggested that diet high in MUFA promoted benefits on weight loss and body composition in women with obesity. Olive oil causes weight reduction, decreases in the serum triglycerides (*Wani et al., 2015*).

Effects of sage, sesame and olive oils alone or in combination on peritoneal fat pad % and some organs weight / body weight% in obese nonalcoholic fatty liver rats.

Table (2) illustrate the effects of (HFD) alone and modified (HFD) by adding 5% sage or by replacing 20% (ST) to 20% from (SO), (OO) or mixed 20% from (1:1 w/w) (SO) plus OO without or with 5% sage on peritoneal fat pad %(PFP %) and some organs weight/body weight% (OW/BW %).

Results revealed that PFP%, liver and kidney weight for (-ve) group fed on (BD) recorded a significant decrease ($p < 0.05$), as compared to the (+ve) control fed on (HFD), on the other hand, our results revealed that all groups fed on modified (HFD) by adding 5% sage or replacing 20% (ST) to 20% from (SO), (OO) or 20% mixed between them (1:1 w/w) without or with 5% sage induced a significant decrease ($P < 0.05$) in PFP % & (OW / BW%) when compared with +ve control group. The best results recorded by (+ve) obese (NAFLD) group which fed on modified (HFD) by replacing 20% (ST) to 20% from mixed (SO) with (OO) (1:1 w/w) plus 5% sage.

In this respect (*Ben khedher et al., 2018*) suggested that sage had efficacy in the prevention of lipid accumulation within 3T3-L1 adipocytes in consistent with it reducing body weight gain (related

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adipose tissue hypertrophy) in (HFD) mice treated with low dose of sage. On the other side, our results are at the same line with (*Han et al., 1999*). *Han et al., (2000)* suggested that olive oil causes weight reduction, decreases the serum TG and significantly reduces the accumulation of fat in liver. On the other hand, (*Hareeri et al., 2017*) demonstrated that oral intake of (SO) caused a significant ($p < 0.05$) decreased in BWG% and liver relative weight when compared to positive control group.

Effects of sage, sesame and olive oils alone or in combination on serum leptin hormone and blood glucose levels in obese nonalcoholic fatty liver rats.

Results presented in table (3) illustrate the effects of (HFD) alone and modified (HFD) by adding 5% sage or by replacing 20% (ST) to 20% from (SO), (OO) or mixed from (1:1 w/w) (SO) plus (OO) without or with 5% sage on leptin hormone and glucose levels in obese albino rats suffering from (NAFLD). Our results revealed that, serum leptin hormone and glucose levels in control (+ve) group fed on (HFD) were (10.678 ± 0.65 and 142.387 ± 4.888), while control (-ve) group fed on (BD) recorded (2.206 ± 0.150 and 77.856 ± 3.688 , respectively). Statistical analysis showed a significant increase ($p < 0.05$) in serum leptin and glucose levels of (+ve) control group fed on (HFD), as compared to (-ve) control group fed on (BD).

On the other hand, our results revealed that all groups fed on modified (HFD) by adding 5% sage or by replacing 20% from (ST) to 20% from (SO), (OO) or mixed 20% from (1:1 w/w) (SO) plus (OO)

without or with 5% sage, recorded a significant decrease ($p < 0.05$) in serum leptin hormone and glucose levels in obese albino rats (NAFLD). The best results induced by the group fed on modified (HFD) by replacing 20 (ST) to 20% mixed from (1:1 w/w) (SO) with (OO) plus 5% sage.

Our results are at a harmony with **(Taha et al., 2014)** who reported that supplementation with (SO) possess a better improving potential for hyperlipidemia, serum glucose, adiponectin, thyroid hormones, hepatic lipid profile. Concerning sage effect on blood glucose level **(Eidi et al., 2005)** suggested that aqueous extract of sage has been found to exhibit insulin – like activities. In this respect **(Ben khedher et al., 2018)** was found a marked improvement in insulin sensitivity by sage extract concern in olive oil a MUFA rich diet improves post prandial glucose, lipid and glyp-1 responses in insulin – resistant subject. On the other hand, our results are at the same line with **(Frederich et al., 1995)** who reported that the increase in leptin resistance as a result of consuming (HFD) in rodent, on the other side fat mass loss triggers a decrease in serum leptin level may reflect restoration of leptin sensitivity **(Pilcova et al., 2003)**.

Conclusion

In conclusion was drawn that replacing by 20% from saturated fatty acid sources sesame and olive oil (1:1 w/w) plus 5% sage, ranged somewhere between them rise the effects of either component alone and induced the greatest improvement in case of

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obese (**NAFLD**) fed on (HFD). The present study showed that preliminary phytochemical analysis to sesame, olive oils and sage revealed the presence of flavonoids, phenolic compounds are of therapeutic value can be modifies liver fat patients in obese (NAFLD).

Table (1): Effects of Sage, Olive and Sesame Oils Alone or in Combination on Feed intake and Body Weight Gain% in Non-Alcoholic Fatty Liver Albino Rats.

Groups	Parameters	Feed Intake (g/day/each rat)	BWG%
Control (-ve) group, fed on basal diet (BD).		21.200 ^a ± 1.303	19.260 ^g ± 0.708
Control (+ve) group, fed on high fat diet (HFD).		18.800 ^b ± 0.836	29.724 ^a ± 1.084
Fed on modified high fat diet containing	5% sage	19.400 ^b ± 0.961	26.833 ^b ± 1.082
	20% olive oil "OO"	19.800 ^b ± 0.570	26.398 ^{b c} ± 0.855
	20% sesame oil "SO"	19.300 ^b ± 0.543	25.723 ^c ± 0.606
	10% OO plus 10% SO	19.600 ^b ± 0.418	23.740 ^d ± 0.559
	20% OO plus 5% sage	19.600 ^b ± 0.961	23.244 ^{d e} ± 0.360
	20% SO plus 5% sage	19.280 ^b ± 0.766	22.519 ^e ± 0.963
	10% OO plus 10% SO plus 5% sage	19.540 ^b ± 0.594	21.068 ^f ± 0.525

OO: Olive Oil **SO:** Sesame Oil These results are (Mean ± SD)

BWG%: Body weight gain%. **LSD:** Least significant differences (P≤0

Mean values in each column with same letters are not significantly different.

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Table (2): Effects of Sage, Olive and Sesame Oils Alone or in Combination on Peritoneal Fat Pad and Some Organs Weight/Body Weight% in Obese Albino Rats Suffering from Non-Alcoholic Fatty Liver Disease.

Groups	Parameters	Organs weight / body weight %		PFP %
		Liver	Kidney	
Control (-ve) group		2.615 ^e ± 0.046	0.511 ^f ± 0.023	1.288 ^h ± 0.022
Control (+ve) group, fed on high fat diet (HFD).		3.389 ^a ± 0.026	0.784 ^a ± 0.015	2.178 ^a ± 0.046
Fed on modified high fat diet containing	5% sage	3.231 ^b ± 0.057	0.687 ^b ± 0.032	1.966 ^b ± 0.037
	20% olive oil "OO"	2.929 ^c ± 0.102	0.651 ^c ± 0.038	1.856 ^c ± 0.042
	20% sesame oil "SO"	2.829 ^d ± 0.064	0.606 ^{d,e} ± 0.025	1.776 ^d ± 0.047
	10% OO plus 10% SO	2.843 ^d ± 0.045	0.611 ^d ± 0.010	1.696 ^e ± 0.039
	20% OO plus 5% sage	2.790 ^d ± 0.077	0.600 ^{d,e} ± 0.009	1.617 ^{f,g} ± 0.032
	20% SO plus 5% sage	2.697 ^e ± 0.083	0.595 ^{d,e} ± 0.022	1.678 ^{e,f} ± 0.053
	10% OO plus 10% SO plus 5% sage	2.664 ^e ± 0.035	0.575 ^e ± 0.012	1.577 ^g ± 0.086

OO: Olive Oil **SO:** Sesame Oil These results are (Mean ± SD)

BWG%: Body weight gain%. **LSD:** Least significant differences (P≤0

Mean values in each column with same letters are not significantly different.

Table (3): Effects of Sage, Olive and Sesame Oils Alone or in Combination on Serum Leptin and Glucose in obese rats suffering from Non-Alcoholic Fatty Liver Disease.

Parameters		Leptin mg/dl	Glucose mg/dl
Groups			
Control (-ve) group		2.206 ^g ± 0.150	77.856 ^h ± 3.688
Control (+ve) group, fed on high fat diet (HFD).		10.678 ^a ± 0.654	142.387 ^a ± 4.888
Fed on modified high fat diet containing	5% sage	8.656 ^b ± 0.662	127.234 ^b ± 3.515
	20% olive oil "OO"	7.194 ^c ± 0.437	117.949 ^c ± 2.542
	20% sesame oil "SO"	6.678 ^d ± 0.285	111.309 ^d ± 2.459
	10% OO plus 10% SO	6.094 ^e ± 0.129	104.298 ^e ± 1.553
	20% OO plus 5% sage	5.644 ^e ± 0.132	97.209 ^f ± 2.484
	20% SO plus 5% sage	5.048 ^f ± 0.162	91.275 ^g ± 2.034
	10% OO plus 10% SO plus 5% sage	4.986 ^f ± 0.303	87.403 ^g ± 3.874

OO: Olive Oil **SO:** Sesame Oil These results are (Mean ± SD)

BWG%: Body weight gain%. **LSD:** Least significant differences (P≤0

Mean values in each column with same letters are not significantly different.

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تأثير المرمرية، وزيت السمسم والزيتون منفردا أو مع المرمرية علي
إنقاص الوزن ومستوى سكر الدم في فئران الالبينو البدنية المصابة بتدهن
الكبد غير الكحولي

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المستخلص العربي

الهدف من هذه الدراسة هو معرفة تأثير المرمرية "المريمية" وزيت السمسم و الزيتون منفردا ومختلطا مع المرمرية على المأخوذ اليومي من الطعام والنسبة المئوية للزيادة في الوزن ونسبة وزن الأعضاء منسوبا لوزن الجسم ونسبة دهون الاحشاء الداخلية ونسبة جلوكوز الدم وهرمون اللبتين في الفئران المصابة بالسمنة والكبد الدهني غير الكحولي ومن أجل ذلك . تم في هذه الدراسة تم استخدام عدد ٧٢ فأرا بالغا من نوع الالبينو من فصيلة الاسبراج داولي اوزانهم 200 ± 10 جم. تم تقسيم الفئران الي مجموعتين رئيسيتين. المجموعة الرئيسية الأولى (٨ فئران) تم تغذيتها علي غذاء اساسي واستخدمت كمجموعة ضابطة سالبة. المجموعة الرئيسية الثانية (٦٤ فأرا) تم تغذيتها علي غذاء عالي الدهون لمدة ثمانية اسابيع لإحداث مرض تدهن الكبد غير الكحولي وبعد ذلك. تم تقسيم الفئران المصابة بتدهن الكبد غير الكحولي الي (٨ مجموعات فرعية).

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مجموعة منهم (٨ فئران) تم تغذيتها علي غذاء عالي الدهن واستخدمت كمجموعة ضابطة ايجابية "مصابة". المجموعات السبع الفرعية الاخرى تم تغذيتها علي نظام غذائي معدل عالي الدهن. مجموعة منهم تم تغذيتها علي غذاء عالي الدهن تحتوى علي ٥% مرمرية. المجموعات الفرعية الاخرى تم تغذيتها علي غذاء عالي الدهن معدل تم باستبدال ٢٠% دهن الغنم بـ ٢٠% زيت سمسم، ٢٠% زيت زيتون وخليط " ١٠% زيت سمسم مع ١٠% زيت زيتون " بدون أو بإضافة المرمرية لمدة (٨ اسابيع). في نهاية فترة التجربة، تم ذبح الفئران بعد صيام طوال الليل، ثم تم فصل الكبد والكلي ودهون الاحشاء الداخلية وتم وزنهم. تم تجميع الدم، ثم فصل السيرم، تم تقدير مستوى الجلوكوز والليبتين. أشارت النتائج المتحصل عليها الي ان تغذية الفئران البدينة المصابة بتدهن الكبد غير الكحولي علي غذاء عالي الدهن المعدل الذي يحتوى علي ٥% مرمرية أو استبدال ٢٠% دهن الغنم بـ ٢٠% زيت زيتون، ٢٠% زيت سمسم، أو خليط " ١٠% زيت سمسم مع ١٠% زيت زيتون" مع أو بدون ٥% مرمرية أحدثت تناقصا معنويا في النسبة المئوية للزيادة في الوزن، الوزن النسبي للكبد والكلي ، النسبة المئوية لوزن دهون الاحشاء الداخلية ، مستوى الجلوكوز في سيرم الدم وهرمون الليبتين كما اشارت النتائج الي ان استخدام خليط من ١٠% زيت سمسم مع ١٠% زيت زيتون بالاضافة الي ٥% مرمرية قد يمثل علاجا محتملا يحدث اكبر انخفاضا في النسبة المئوية للزيادة في الوزن، الوزن النسبي للاعضاء، النسبة المئوية لوزن دهون الاحشاء الداخلية، مستوى الجلوكوز في سيرم الدم وهرمون الليبتين في الفئران البدينة المصابة بتدهن الكبد غير الكحولي.