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A comparative study between the effect of mesotherapy against a dietary mixture on blood lipids in obese experimental animals

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Abstract:
This study aimed to compare the effect of mesotherapy and a nutritional mixture (coffee, soybean, and oats) on blood lipids among obese rats. Thirty-six male albino rats, divided into two main groups; 1st group was weighing (140±10g) ,used as a negative control group and kept on a standard diet along the trial, while second group (30 rats) were weighing (230±10g), fed on a high-fat diet (20% fat) for 21 days, then divided into five groups (6 rat in each). The 1st was control positive group; the second fed 2% of the nutritional mixture, the third fed 4 % nutritional mixture, the fourth injected with 2.5 ml mesotherapy, and the fifth injected with 5 ml mesotherapy. The nutritional mixture was made from coffee, soybean, and oats, then dried and administered as powder, while mesotherapy was administered by daily injection. At the end of the experiment, serum total cholesterol (TC), triglycerides (TG), HDL, VLDL, and LDL were determined. The results showed that positive control rats had higher LDL, VLDL, TG, and T.C than the negative control group and other groups. In parallel, the HDL value of the negative control group was significantly the highest. Rats fed 4% of the natural products were more effective in reducing LDL, TG, and TC than rats fed 2% of natural products. Injection with 5% of mesotherapy was more effective in reducing LDL, VLDL, AI, TG, and TC. In conclusion, injection with 5 ml mesotherapy and feeding 4% natural products showed hypolipidemic effects among experimental animals.

Keywords*: Mesotherapy, Lipid profile, Experimental animals.*

Introduction

Obesity refers to the increase of excess body fat due to an energy imbalance. When energy intake exceeds energy expenditure, the surplus energy is stored in the body in form of fat, leading to adipose tissue enlargement, dyslipidemia, and fatty liver, and is linked to other metabolic diseases such as diabetes mellitus, cardiovascular disease (CVD), and certain

types of cancer (Lumeng and Saltiel, 2011). More recently, it has been reported, that people are more likely to become obese due to restrictions on physical activity caused by the COVID-19 pandemic (Jia et al, 2021). Zhu et al, (2021) reported that during the COVID-19 outbreak people spent most of their time at home, resulting in decreased physical activity and increased food intake, which was closely related to weight gain. However, obesity is a modifiable factor by regulating food consumption as well as physical activity (Zhu et al, 2021).

Mesotherapy is a medical technique developed in 1952 by the renowned French physician Dr. Michel Pistor for the management of pain and vascular disorders in France (Matarasso and Pfeifer, 2005). Mesotherapy injection contains many ingredients that have an effective role in the treatment of obesity such as aqua, soybean extract, deoxycholate, carnitine, Cynara scolymus, leaf extract, organic silicon, caffeine, and theophylline (Atiyeh et al, 2008).The results of the experiments confirmed that the safe rate of mesotherapy injection should not exceed 10 cm per session in order to prevent skin swelling and burns (Lecoz,2009). Some nutritional mixtures affect the treatment of obesity such as soybean, coffee, and oat. Zheng et al, (2004) declare that caffeine and chlorogenic active compounds in coffee that help improve and reduce body weight gain and coffee had an anti-effect of obesity. Coffee (Coffea) contains a large number of important phenols and acids such as chlorogenic acid (Yashin et al., 2009). Coffee contains many antioxidants which prevent body fat and increase the antioxidant capacity for the cells and this protects the cells from damage (Addicot et al., 2009). However, similar studies carried out on rats found that Arabic coffee (Mahmoud et al., 2013), and moderate amounts of Turkish coffee dark roasting (Ismail et al., 2015) have hypolipidemic effects on serum total cholesterol, LDLc, and total blood lipids of experimental animals. Practical research has proven that drinking coffee in abundance 8 cups daily were more likely than others leads to liver cancer (Lai et al, 2013).The oats (Avena sativa) attenuated hyperglycemia and diabetes, prevented obesity, abdominal fat and improved liver function by inhibiting lipogenesis in animal models. Consequently, a daily oat supplement can act as an effective adjuvant for the treatment of metabolic disorders in humans (David and Kataz,2001).Published data revealed that consumption of oat reduced body weight, BMI, body fat, and the waist-to-hip ratio. AST, and ALT both showed decrements in patients with oat consumption, and researchers concluded that consumption of oat reduced obesity (Butt et al, 2008). Consuming oats at a rate of more

than 150 grams per day leads to digestive disorders such as feeling bloated, stomach cramps and constipation (Kemppainen etal,2009). Soybeans (Glycine max) are an important dietary source because they contain high-quality proteins and are rich in bioactive compounds such as isoflavones and saponins (Chatterjee et al, 2018). Quantity intake of soy foods is equivalent daily to 25 and 100 mg total isoflavones and between 8 and 50 g soy protein (Erdman et al, 2004). Soy is the only legume that contains the nine essential amino acids in the correct proportion for human health (Hoogenkamp, 2005). Soybean affects obesity by lowering body weight, fasting blood glucose levels, and hepatic fat accumulation in animal models (Velasquez and Bhathena,2007). Soybean polysaccharides and genistein prevent high fat-induced body weight gain, dyslipidemia, oxidative stress, and inflammation in mice (Lu et al, 2019). The main objective of this study is to compare the outcome of mesotherapy injections against the natural product (coffee, oat, and soybean) on blood lipids among obese experimental rats.

Materials and methods

Materials:

The main materials used in this study were mesotherapy injection called meso shape obtained from Meso Static Company, and natural product (coffee, soybean, and oats as powder) obtained from authorized suppliers in Egypt. These natural products have been mixed in equal proportions at room temperature, then it was added to animals' diet in the form of powder into two concentration 2% and 4%.

Experimental animals

Thirty-six adult male albino rats were obtained from Medical Insects Research Institute, Doki, Cairo, Egypt. Rats were housed in wire cages under normal laboratory conditions and were fed a standard diet for one week as an adaptation period. Diet was introduced to rats in special food cups to avoid scattering of food. Also, water was provided to rats by glass tubes projecting through the wire cages from an inverted bottle supported to one side of the cage. Food and water were provided ad-labium and checked daily. A standard diet was prepared from fine ingredients according to Macia, (2006), and it was formulated to fulfill the dietary requirements given by AIN, 1993 .

Induction of obesity in rats:

Rats were kept for four consecutive weeks on a high-fat diet to induce obesity. High fat diet prepared from fine ingredients per 100g according to the following composition: fat 30% (tallow 15% + corn oil 15%); casein 12%; salts mixture 4%; vitamins mixture 1%;

fiber 5%; DL-methionine 0.3; choline chloride 0.2%; bile acid 0.2 and corn starch up to 100g according to Moss,(1982). The targeted rats were fed a high-fat diet for 21 consecutive days.

Experimental design

All rats were fed on a basal diet for one week for adaptation, then the rats were divided into two main groups :The first group was used as a negative control group (-ve) and kept on a standard diet along with the trial.The second group (30 rats) was fed on a high-fat diet (20% animal fat) for three consecutive weeks. Then bodyweight of all rats in this group was recorded then divided accordingly into five equal subgroups (6) rats in each group. The first subgroup was the control positive group (+ve). The second subgroup (2% NM) fed a standard diet plus 2% of the nutritional mixture. The third subgroup fed a standard diet plus a 4 % nutritional mixture (4% NM).The fourth subgroup fed a standard diet and injected daily with 2.5 ml/100 g mesotherapy (2.5 Mes). The fifth subgroup fed a standard diet and injected daily with 5 ml/100 g mesotherapy (5 Mes).By the end of the experiment, body weight gain (BWG), food intake (FI), and feed efficiency ratio (FER) were recorded. Feeding and growth performance were carried out by determination of body weight gain (BWG%) and Feed efficiency ratio (FER) according to Chapman et al., (1959) using the following formulas:

$$BWG\% = (\text{Final weight-Initial weight})/(\text{Initial weight}) \times 100$$

$$FER = (\text{Body weight gain (g)})/(\text{Food intake (g)})$$

Lipid profile assay:

Blood samples were collected after twenty-eight days of the experiment from control and obese rats after eight hours fasting after the experiment. The experiment comprised of the rats that were given ether anesthetized when scarified. Blood samples were taken in the tubes from the hepatic portal vein then centrifuged directly for ten minutes at 3000 rpm to separate the serum. Later, it was carefully transferred after aspiration into clean tubes and stored at -20°C frozen for analysis .

Total lipid, total cholesterol, triglyceride, and high-density lipoprotein (HDLc) were determined according to (Fassati and Prencipe 1982). While low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were calculated according to the equation given by Lee and Nieman (1996) as follow:

$$VLDL = TG/5$$

$$LDL = TC - (HDL+VLDL).$$

Statistical Analysis

Data were statically analyzed using a computerized Costat program by one-way AVOVA and follow-up test - LSD. The results are presented as mean ±SD. Differences between treatments at (P< 0.05) were considered significant (Steel and Torri,1980)

Results and Discussion

Table (1) showed the effect of natural product (soybean, coffee and oat) and mesotherapy injection on initial body weight (IBW), final body weight (FBW), body weight gain (BWG), food efficiency ratio (FER) and food intake (FI) of normal and obese rats. The statistical analysis showed that there were a significant a differences between positive control rats and negative control rats. The mean values of obese rats' groups were significantly lower (P<0.05) than positive control group in FBW, BWG, FER and FI. However, the body weight gain of positive control group (14.7±0.8 g/21 days) was significantly (P<0.05) higher than corresponding values of other groups, which ranged from 3.27±0.3 g/21 days for negative control group to -12.9±0.8 g/21 days for rats injected with 5 ml of mesotherapy. Simultaneously, the FER of positive control group (3.19±0.78) were significantly the higher among all experimental groups. No significant differences was found in relation to EBW, FBW, B.W.G, FER and FI values in rats that feed on natural product (2%) and rats that feed on natural product (4%). Injection rats with (5%) of mesotherapy decrease F.W, WG, FER in percent change of control positive group by -23.6, -18.77, -225.3 respectively. Pimentel et al., (2004) recommended that there are relationship between obesity and consumption of coffee which induce weight loss. Coffee is the main source of caffeine which reduce the obesity , boosts energy, increases gastric acid secretion

reduces insulin sensitivity, reduces fat absorption by 22% Because it increases energy consumption and may prevent diseases caused by oxidative injuries (Frary et al., 2005).

Villani et al., (2000) found that, the rationale for carnitine supplementation as a weight-loss agent is based on the assumption that regular oral ingestion of the substance increases its intracellular concentration. This would increased fat oxidation and gradual reduction of the body’s fat reserves. Artichoke is full of natural bioactive components, that is, caffeic acid, chlorogenic acid, cynarin, and luteolin. These components it has a small percentage of calories, so it contains fiber ,improves metabolism, helps increase bone density during the slimming process, the body gets rid of toxins and excess fluids during the slimming process,stimulates the production of gastric juices, which facilitates the

slimming process ,helps feel full for long periods of time Zapolska-Downar, et al., (2002) ;Wang, et al., (2003); (Juzyszyn, et al., (2008).

Table (1): Effect of natural product (soybean, coffee and oat) and mesotherapy injection on body weight parameters and food intake of normal and obese rats.

Variables	(-ve)	(+ve)	2% NP	4%NP	2.5 Mes	5 Mes	LSD
IBW(g)	$142.3^{d}\pm1.7$	$159.1^{c}\pm1.4$	$161.8^{ab}\pm1.7$	$162.6^{a}\pm1.9$	$161.6^{ab}\pm1.03$	$160.1^{bc}\pm1.1$	1.8
FBW(g)	$147^{c}\pm1.8$	$182.8^{a}\pm1.6$	$168.3^{b}\pm1$	$169.8^{b}\pm3.6$	$148.1^{c}\pm1.1$	$139.5^{d}\pm2$	2.39
BWG(%)	$3.27^{b}\pm0.3$	$14.7^{a}\pm0.8$	$4.02^{b}\pm1.3$	$4.4^{b}\pm1.7$	$-8.3^{c}\pm0.8$	$-12.9^{d}\pm0.8$	1.43
FER(g)	$0.59^{b}\pm0.07$	$3.19^{a}\pm0.78$	$0.96^{b}\pm0.22$	$1.13^{b}\pm0.46$	$-2.4^{c}\pm0.7$	$-4^{d}\pm0.5$	0.67
FI(g)	$7.8^{a}\pm0.7$	$7.8^{a}\pm1.1$	$6.5^{b}\pm1.04$	$6.5^{b}\pm1.04$	$5.8^{bc}\pm1.1$	$5.16^{c}\pm0.7$	0.99

Values are expressed as mean ± SD. Means in the same raw subscribed with different letter indicate significant differences between these values. N P: natural product. Mes: mesotherapy

Table (2) showed the effect of natural products (soybean, coffee, and oat) and mesotherapy injection on lipids profile in normal and obese rats. The results showed that positive control rats had higher LDL, VLDL, T.G, and T.C than the negative control group and the rats' groups which fed natural products and which injection by mesotherapy. The HDL value was higher. Rats that ate 2% of the natural product were no different from rats that ate 4% of natural product in VLDL and AI. However, feeding rats with 4% of natural product was more effective in reducing LDL, T.B, and T.C than rats feeding with 2% of natural product. The group of rats injected with 2% or 4% doses of mesotherapy gave the same result in HDL. Injection rats by 4% of mesotherapy were more effective in reducing LDL, VLDL, AI, T.G, and T.C .The results of previous research inform us that the oat lowered the levels of total cholesterol, LDL-cholesterol, and triglycerides in rats Kalra and Jood, (2000). Higdon and Faris, (2006) studied the effect of coffee on human health and they found that the coffee improved blood lipids especially total cholesterol and triglyceride, and increase HDL. Soy protein has been reported to affect obesity by lowering body weight, hepatic fat accumulation in animal models and prevent high fat-induced body weight gain in rats Velasquez and Bhathena, (2007). Mesotherapy is capable of causing local fat reduction by two distinct mechanisms, fat ablation, and lipolytic stimulation. It also reduces cellulite Caruso et al.,(2008).

Mesotherapy is not an effective alternative treatment modality for body contouring because it contain artichoke extract, soybean extract and natural silicon, these substances

improve lymphatic drainage and stimulate the formation of collagen fibersBranda et al .,(2005).

Table (2): Effect of natural product (soybean, coffee and oat) and mesotherapy injection on lipids profile of normal and obese rats.

Variables	(-ve)	(+ve)	2% NM	4%NM	2.5 Mes	5 Mes	LSD
HDL(mg/dl)	$63.7^{a}\pm4.7$	$35.7^{d}\pm1.0$	$39.5^{c}\pm1.4$	$45.8^{b}\pm1.7$	$44.8^{b}\pm2.1$	$48.0^{b}\pm2.6$	3.03
LDL(mg/dl)	$16.9^{d}\pm5.2$	$96.4^{a}\pm7.5$	$54.9^{b}\pm3.0$	$38.5^{c}\pm2.0$	$50.7^{b}\pm6.6$	$34.0^{c}\pm3.9$	6.48
VLDL(mg/dl)	$17.4^{d}\pm0.6$	$48.46^{a}\pm3.7$	$31.21^{b}\pm1.1$	$29.3^{b}\pm0.4$	$24.7^{b}\pm0.9$	$23.3^{c}\pm0.3$	2.04
AI(mg/dl)	$54.0^{e}\pm0.1$	$4.03^{a}\pm0.3$	$2.1^{b}\pm0.1$	$1.45^{b}\pm0.1$	$1.65^{c}\pm0.2$	$1.17^{d}\pm0.1$	0.24
TG(mg/dl)	$86.9^{f}\pm3.0$	$250.6^{a}\pm2.4$	$156.1^{b}\pm5.6$	$146.9^{c}\pm2.1$	$125.5^{d}\pm2$	$116.6^{e}\pm1.8$	3.24
TC(mg/dl)	$98.0^{e}\pm2.0$	$180.6^{a}\pm6.6$	$125.6^{b}\pm3.6$	$113.6^{c}\pm2.1$	$120.6^{b}\pm5.0$	$105.4^{d}\pm3.0$	5.12

Values are expressed as mean ± SD. Means in the same raw subscribed with different letter indicate significant differences between these values.

Conclusion

The selected natural products and mesotherapy injection in this study were effective in protecting rats against obesity. These results supported that natural products and mesotherapy injection are able to losing weight in obese rats but the proportion of mesotherapy injections should not exceed 10 cm per session so as not to cause local burning or swelling of the skin. Therefore, it is recommended to add this food products in moderate quantities in the daily diet.

Conflict of interest

The authors declare that they have no conflict of interest concerning the publication of this article. This article is extracted from a Master's thesis submitted to the Department of Nutrition and Food Science, Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt.

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دراسة مقارنة بين تأثير الميزوثيرابي وخليط غذائي على نسبة الدهون في الدم في حيوانات التجارب المصابة بالسمنة

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قسم التغذية وعلوم الأطعمة، كلية الاقتصاد المنزلي، جامعة المنوفية، شبين الكوم، مصر

الملخص العربي

تهدف هذه الدراسة إلى مقارنة تأثير العلاج بالميزوثيرابي وخليط غذائي (القهوة وفول الصويا والشوفان) على دهون الدم للفئران البدينة. تم تقسيم ٣٦ فئران ذكور إلى مجموعتين رئيسيتين. تم استخدام المجموعة الأولى وزنها(140±10جرام) كمجموعة ضابطة سالبة تغذت علي الوجبة النموذجية طوال التجربة، في حين تم تغذية المجموعة الثانية (30 فأر) تتراوح اوزانهم (230± 10جرام)على نظام غذائي عالي الدهون (20٪ من الدهون) لمدة 21 يوما ، تم قسمت خمس مجموعات (6 فئران في كل منها). الأول كانت مجموعة ضابطة موجبة. والثانية تغذت على 2٪ من الخليط الغذائي، والثالث تغذت على 4 ٪ من الخليط الغذائي، والرابعة حقنت بـ 2.5 مل ميزوثيرابي، والخامسة حقنت بـ 5 مل ميزوثيرابي. تم صنع الخليط الغذائي من القهوة وفول الصويا والشوفان، ثم جفف وتم إعطاؤه للفئران في صورة مسحوق، في حين كان يتم إعطاء الميزوثيرابي عن طريق الحقن اليومي. في نهاية التجربة، تم تقدير كوليسترول الدم، والدهون الثلاثية والليبوبروتينات منخفضة الكثافة وعالية الكثافة والمنخفضة جدا في الكثافة. أظهرت النتائج أن فئران المجموعة الضابطة الموجبة كانت لديها الكوليسترول في الدم، والدهون الثلاثية والليبوبروتينات منخفضة والمنخفضة جدا في الكثافة أعلى من المجموعة الضابطة السالبة وغيرها من المجموعات. وبالتوازي مع ذلك، قيمة الليبوبروتينات عالية الكثافة عند المجموعة الضابطة السالبة هي الأعلى بشكل معنوي. حدث نقص بين الفئران التي تم تغذيتها على 4٪ من المنتج الطبيعي في مستوى الكوليسترول في الدم، والدهون الثلاثية والليبوبروتينات منخفضة الكثافة عن الفئران التي تغذت على خليط 2٪. أيضا كان الحقن بـ 5٪ من الميزوثيرابي أكثر فعالية في الحد من كوليسترول الدم، والدهون الثلاثية والليبوبروتينات منخفضة والمنخفضة جدا في الكثافة. الخلاصة كان للحقن بـ 5 مل ميزوثرابي وتغذية الفئران بـ خليط 4٪ من المنتجات الطبيعية آثار واضحة في انقاص مستوى دهون الدم بين حيوانات التجارب.

الكلمات الرئيسية: الميزوثيرابي ، دهون الدم، حيوانات التجارب.