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RESEARCH PAPER

Investigating the anti-apoptotic effect of sesame oil and honey in a novel nanostructure form for treatment of heart failure

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ABSTRACT

Objective(s): Sesame oil is a lipophilic compound and has low aqueous solubility and low oral bioavailability. It is possible to enhance sesame oil solubility in aqueous media by applying the microemulsion system in the form of oil-in-water. In this study, the anti-cholesterol and anti-Apoptotic effects of a new combination of sesame oil and honey in a microemulsion form for cardiac muscle cells Apoptosis treatment were investigated.

Materials and Methods: Two different formulations were prepared. Tween 80 was used as the main surfactant in both formulations. In the first formulation, glycerin was applied as co-surfactant. Span 80 was applied as a mixed surfactant in the second formulation.

Results: Characterization results showed that the average size of droplets of microemulsion samples were in the range of 16.6±0.1-64.6±0.2 nm with a poly dispersity index (PDI) value of less than 0.5. No turbidity and phase sedimentation were observed in certain samples in a period of 6 months after the preparation, which confirmed the high stability of samples. The in-vivo results in Wistar male rats with heart failure showed that applying sesame oil and honey in the microemulsion form caused a significant reduction in the Apoptosis level. In addition, favorable therapeutic effects for microemulsion administration was observed in comparison to the Atorvastatin drug consumption. Furthermore, the protective effect of microemulsion dosage was more obvious with increasing the oil percentage and adding honey as a hydrophilic additive.

Conclusion: Results confirmed that the new formulation containing sesame oil and honey as natural components with nano particle size could be useful for cardiac muscle cells Apoptosis treatment.

Key words: *Apoptosis, Cardiac muscle cells, In-vivo performance, Microemulsion, Sesame oil*

How to cite this article

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INTRODUCTION

Heart failure is a progressive condition that contributes to unacceptably high morbidity and mortality attributable largely to cardiac dysfunction due to cardiac structural changes [1-3].

There are an estimated 23 million people suffering from heart failure worldwide [4]. Recently, many advances have been made in therapeutic approaches of heart failure. One of the most comprehensive approaches in this field is controlling neurohormonal systems such as the sympathetic nervous system [5]. Inflammation, hypertrophy, loss of collagen, interstitial fibrosis, cardiomyocyte Apoptosis and necrosis are some of the events caused by neurohormones [6-8]. Although many drugs such as Atorvastatin have been designed to control these neurohormones, they have not yet achieved a desirable result in reducing heart failure [9-11]. So, exploration of novel and/or complement therapies still seems a necessity. In spite of progressing novel methods for designing chemical compounds, some materials with natural source can be suitable for heart failure treatment.

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Edible oils such as sesame is an appropriate candida for this aim [12]. Sesame oil is a nutritious oil. It obtained from sesame seeds. It has high nutritional value and powerful therapeutic properties. Sesame lignans such as sesaminol, episesamin, pinoresinol, sesamin, sesamolin, sesamolinol, sesamol and gamma-tocopherol isolated from the seeds of sesamum indicum and sesamum radiatum are responsible for many of the physiological and biochemical characteristics of sesame oil like blood pressure reducer [13], anti-mutagenic, anti-oxidant, anti-tumorogenic, estrogenic or anti-estrogenic, anti-diabetic, anti-cholesterol and anti-cancer properties [14-16]. Sesame oil can improve lipid profile and decrease lipid peroxidation in hypercholesterolemic patients [13]. Also, lignans in sesame oil play an important role in DNA oxidative damage prevention [17]. The unique tocopherol which can be found in sesame oil is gamma tocopherol [18]. Tocopherol is necessary to protect polyunsaturated fatty acids (PUFA) against oxidative damage in plants and animals.

Honey as a hydrophilic compound is rich in substantial enzymatic and non-enzymatic antioxidants including alkaloids, catalase, glucose oxidase, flavonoid, phenolic acid, ascorbic acid, carotenoid derivatives, maillard reaction products, organic acids, amino acids and proteins [19-30]. Honey acts against oxidant factors and prevents their combination with polyunsaturated fatty acids and stops the oxidation of lipoproteins [26]. Because of the rich anti-oxidant contain of honey and sesame oil, combination of these two materials can be introduced as an appropriate choice for heart failure treatment [12, 26]. Since the bioavailability and therapeutic efficiency of oily-nature molecules depend on their solubility in aqueous medium [31], the main problem for using sesame oil is its low absorbance in human body. Moreover, mixing the sesame oil and honey will not be effective due to their various hydrophobic-hydrophilic properties. Designing a new combination form of these materials with lower particle size can enhance their solubility and bioavailability in hydrophilic media of body. Microemulsion form of lipophilic molecules has potential to dissolve any hydrophilic compound and function as a drug nano-carrier for a wide range of drug molecules in pharmaceutical research [32]. Microemulsion is a colloidal dispersion system. It has an internal droplet size of about 10-100 nm [31]. Microemulsion system is thermodynamically stable [33-36]. Microemulsions contain oil phase, surfactant as an amphiphilic compound which can form stable connection between hydrophilic and lipophilic molecules, co-surfactant which can enhance the aqueous solubility of oily-nature matters and aqueous phase in appropriate ratios [37-39]. Among water-in-oil, oil-in-water and bi-continuous types of microemulsions, oil-in-water type is more appropriate for lipophilic molecules delivery [40, 41]. The purpose of this study was to prepare a combination of honey and sesame oil in the microemulsion form and evaluate its effects on cardiac muscle cells Apoptosis in isoproterenol induced left ventricle failure in rats. This is the first study that investigate the impact of an herbal component as drug in nanoscale on the heart failure treatment. Myocardial necrosis induced by isoproterenol (D, L-4-(2-(isopropylamino)-1-hydroxyethyl) pyrocatechol) (ISO), is a well-known standard model for studying the effects of drugs on cardiac dysfunction. Tween 80, Span 80 and edible Glycerin were selected as biocompatible surfactants and co-surfactant to prepare microemulsions based on sesame oil containing honey as a hydrophilic additive. After physico-chemical characterization of microemulsion samples, the in-vivo effects of microemulsion formulations at various oil and honey contents were explored. Calculated results were compared with obtained results from similar study that was used Atorvastatin as a chemical drug for heart failure treatment.

MATERIALS AND METHODS

Materials

Tween 80 (PubChem CID: 86289060), edible Glycerin (PubChem CID: 753) and Span 80 (PubChem CID: 9920342) were purchased from Merck Company (Germany). Sesame oil was bought from Research Fine Lab Chemicals (Yazd, Iran). Honey was provided from food company (Neyshabour, Iran). All chemicals and solvents were of analytical grade. In the experiment, fresh double distilled water was used. Isoproterenol to induce cardiac damage was obtained from Sigma-Aldrich (St. Louis, MO). 50 male Wistar rats for the in-vivo study (age = 7weeks; weight = 230 ± 20gr approximately) were obtained from Razi Maintenance and Breeding Laboratory Animals Institute (Mashhad, Iran). Standard conditions were considered for all rat groups including

12h light / 12h darkness at 21 ± 2˚C in special cages (2-3 rats per each cage) in a bed of straw. A standard pellet diet and water were freely available for animals. Before starting the study, the rats were acclimatized for a week within the aforementioned experimental condition. SPSS and Sigmaplot 12 softwares were used to provide statistical evaluation and pseudo-ternary phase diagrams, respectively.

METHODS

Construction of pseudo-ternary phase diagrams

It is possible to find out the concentration range of components by constructing the pseudo-ternary phase diagrams using the water titration method. In the first formulation, three phase diagrams were constructed with 2:1, 4:1 and 5:1 weight ratios of Tween 80/Glycerin. Three phase diagrams were provided with 8:1, 9:1 and 10:1 weight ratios of Tween 80/Span 80 in the second formulation. Based on the large area of microemulsion, optimal surfactant phase ratios were selected which yielded higher oil content. After mixing surfactant components, sesame oil was added at different ratios. After that, each mixture was titrated using double distilled water drop-wise under moderate magnetic stirring. After equilibration, the samples were assessed visually. Clear and transparent samples were classified as microemulsion, and shown as points in the phase diagram. Microemulsion area were considered the covered area by these points. The appropriate composition range was selected based on these results, and used for selection of optimal microemulsion formulations and further analysis.

Characterization of microemulsions

The average droplet size, polydispersity index (PDI) and zeta potential of microemulsion formulations were measured at 25˚C by dynamic light scattering (DLS) (Nano zeta sizer, Malvern Instruments, UK). Abbe›s refractometer (Bellingham + Stanley limited, England) and a conductivity meter (AZ 86503, Taiwan) were applied to measure the refractive index (RI) and electrical conductivity coefficient of microemulsion samples. The pH value of microemulsion samples was measured at 25˚C by a pH meter (Ciba corning diagnostics limited Sudbury, Suffolk co106xd, England).

Physical stability of microemulsion samples were investigated by a high-speed centrifuge (Sigma 3-30k) at 10000 rpm for 15 min at 25°C. Samples were assumed as physically stable when no sign of droplets conjugation, clarity and phase separation was observed. To examine the stability of samples over time, droplet size determination was performed at least 6 months after preparation. Unfavorable particle size distribution as well as a significant increase in the average droplet size and polydispersity index were assumed as signs of instability over time.

In-vivo performance studies

Isoproterenol was applied to induce cardiac damage. Isoproterenol is a synthetic β-adrenergic receptor agonist that can increase both myocardial contractility and heart rate and also causes severe stress in the necrotic lesions and myocardium in the heart muscle [42]. This induced heart failure is a reliable, reproducible and well standardized model of cardiac hypertrophy, which is used extensively in order to test new drugs or evaluate different aspects of heart failure [43].

50 male Wistar rats were randomly divided into 5 groups (G1-G5) as follows: Healthy control group (G1), ISO alone (G2), ISO plus the microemulsion formulation with lower oil content (first microemulsion formulation) (1 cc/kg) (G3), ISO plus the microemulsion formulation with higher oil content (second microemulsion formulation) (1 cc/kg) (G4) and ISO plus the microemulsion formulation containing higher oil content (second microemulsion formulation) plus honey (1 cc/kg) (G5). For the development of congestive heart failure in rats, isoproterenol was injected subcutaneously as the dosage of 0.5 mg/kg [44], daily for 10 days in all groups except G1. However dose, route of drug administration and treatment period vary among different studies. Based upon the results of similar study by Zhang et al., [44] there are two types of dose- and time-dependent responses in rats treated with isoproterenol. In rats treated with 8, 16, 32 and 64 µg/kg ISO subcutaneously, minimal reversible changes in cardiac structure and also enzymes happened whereas treating the rats with 125-500 µg/kg ISO subcutaneously, induced severe and extensive myocardial and interstitial damage. In the type I response, there were no Apoptotic or necrotic changes detected, whereas in the type II response, Apoptosis and necrosis of myocytes were obvious. As we aimed to assess the microemulsion formulations on Apoptotic

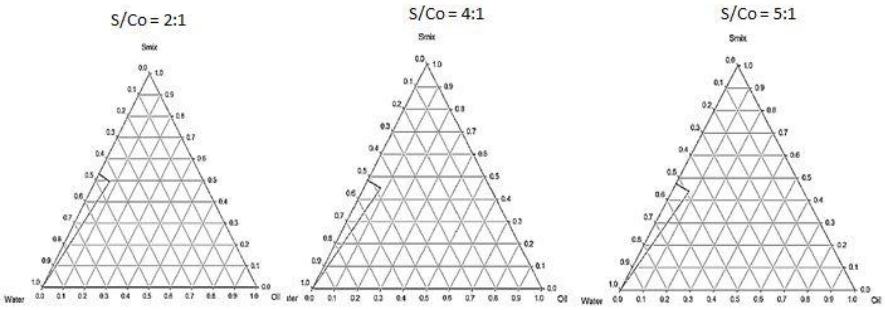

Fig 1. Pseudo-ternary phase diagrams for first microemulsion formulation (Tween 80-Glycerin-Sesame oil-water)

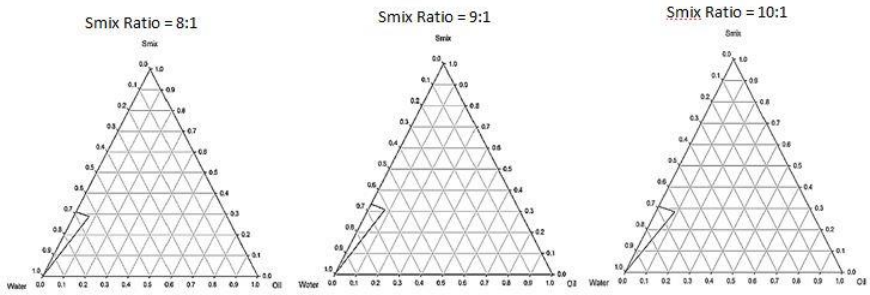

Fig 2. Pseudo-ternary phase diagrams for second microemulsion formulation (Tween 80-Span 80-Sesame oil-water)

changes in rat hearts, 0.5 mg/kg ISO was injected subcutaneously to rats. From three weeks before the isoproterenol infusion, in addition to routine diets, the rats were treated by oral solutions once daily. G1 and G2 were treated with 10 ml/kg normal saline while groups 3 to 5 were treated with 1 cc/kg microemulsion.

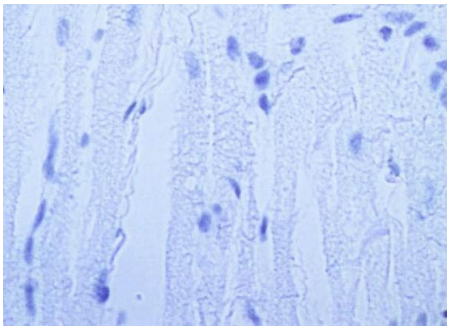

Fig 3. Health control group (G1). Normal cardiac muscle fibers have been shown (TUNEL staining × 10)

Pathological analysis

For microscopic studies, at the end of the experimental period and 24h after the final treatment, the hearts of rats were removed after euthanasia via cervical dislocation. They were stabilized in 10% buffered formalin and sent to the pathology laboratory (Medical faculty, Mashhad University of Medical Sciences). Sequence 5 micron diameter sections were provided from the left ventricle of mice for specific TUNEL staining. Histological sections were evaluated by light microscopy (ECLIPSE E200, Nikon, Japan). Apoptosis detection technique was performed based on TUNEL kit instruction (Insitu Cell death detection kit, Roche diagnostics, Mannheim, Germany). Briefly, the obtained sections were deparaffinized, rehydrated and washed in distilled water. Tissues were exposed to 20 mg/mL protein kinase K for 15 min at room temperature (Roche diagnostics, Mannheim, Germany). The endogenous peroxidase activity was blocked by incubation in 3 ml/l hydrogen peroxide/methanol for 30 min at 37˚C. Terminal deoxynucleotidyl transferase was applied to incubate the obtained sections for 60 min at 37˚C. Then digoxigenin conjugated DUTP (Deoxyuridine triphosphate) was added to 3-OH ends of fragmentized DNA molecules. Anti digoxigenin peroxidase antibodies were used for the detection of labeled nucleotides. The sections were stained by diamino benzidine on the background staining of hematoxylin. In

order to count Apoptotic cells numbers in each section, 5 microscopic fields of the left ventricle region were randomly selected and examined with ×400 magnification by light microscopy. Cells having brown stains were considered as TUNEL positive cells.

Statistic data analysis

The obtained data are presented as mean ± standard deviation (Mean±SD).

ANOVA statistical test and Tukey post hoc test were used for evaluating the differences between the two groups. A P-value < 0.05 was assumed as statistically significant.

RESULTS AND DISCUSSIONS

Construction of pseudo-ternary phase diagrams

Fig 1 and 2 show pseudo-ternary phase diagrams for two different formulations. In the first formulation, the different surfactant to co-surfactant ratios were considered to form clear microemulsions with nearly equal microemulsion area. In the second formulation, the surfactant to co-surfactant ratios of 9:1 formed clear microemulsions with slightly larger area than other ratios. On the basis of pseudo-ternary phase diagrams, microemulsion formulations prepared with 4:1 and 9:1 smix ratios from the first and second formulations, respectively, were selected for physico-chemical characterization because of more oil percentage and larger microemulsion area. No distinct oil-in-water (o/w) to water-in-oil (w/o) microemulsion conversion was observed for both formulations.

Physicochemical characterization

Samples containing the highest oil content were selected for physicochemical characterization for both microemulsion formulations. In Tables 1 to 4, components and the physicochemical analysis results of microemulsions were presented at the time of preparation and after 6 months. As tables show, the average droplet size of microemulsions was in the range of 16.6±0.1 to 64.6±0.2 nm. The particle size distribution index was lower than 0.5 for all samples. This indicated particle homogeneity and greater uniformity in droplet size distribution. Zeta potential of microemulsions was negative in the range of -10.7 to -22 mv. This negative value of zeta potential was related to the existence of hydroxyl functional groups in the surfactant phase. In the other word, oxygen atom of hydroxyl group with high electronegativity produces negative value of zeta potential. The electrical conductivity coefficient of microemulsions which determine their structural type was high and in the range of 213-311 µs/cm which indicating the formation of oil-in-water microemulsion. The refractive index of all microemulsions was around 1.39. This value was near to aqueous phase index. This confirms the formation of oil-in-water microemulsions with high transparency. In addition, oil-in-water type of microemulsions was approved with color tests. The pH value was in the range of 5.9-6.51, which was near to pH value of the body environment. Furthermore, any sign of phase sedimentation or creaming and turbidity was not observed after the centrifugation of all microemulsion samples and therefore indicated their physical stability (Tables 1 and 3). The average droplet size and PDI values of microemulsion samples did not increase significantly after 6 months and thus indicated stability over time (Tables 2 and 4). Two sample particle size distributions are presented in supporting file at the preparation and after 6 months (see supporting file, S1 & S2).

Table 1. Physicochemical characterization of microemulsions in first formulation after preparation

Sample no.	S/Co Ratio	(S+Co)* (% wt)	Oil (%wt)	Water (%wt)	Avg. Droplet Size (nm)	Zeta Potential(mV)	PDI	RI	Conductivity (µs)	pH	Phase separation after centrifuge
1	2:1	40.5	5.5	54	23.9±0.1	-22	0.310±0.012	1.3982	213	6.51	Not observed
2	4:1	36.2	5.9	57.9	64.6±0.2	-19	0.299±0.014	1.3976	246	6.51	Not observed
3	5:1	35.35	5.75	58.9	51.5±0.3	-17	0.210±0.016	1.3929	262	6.51	Not observed

(S+Co) * %wt shows the sum of surfactant and co-surfactant mass percentage

Table 2. Physicochemical characterization of microemulsions in first formulation after 6 months

Sample no.	Avg. Droplet size (nm)	Zeta Potential(mV)	PDI	Instability over 6 months
1	-	-	-	Turbidity
2	35.1±0.3	-16.5	0.412±0.013	Not observed
3	26.8±0.1	-10.3	0.371±0.016	Not observed

In-vivo Analysis Results

Fig. 3 shows the normal cardiac muscle fibers. Daily subcutaneous injection of 0.5 mg/ kg isoproterenol for 10 days resulted in major Apoptotic changes in cardiac muscle fibers in G2 (ISO alone) (Fig. 4).

In TUNEL staining, Apoptotic cells were observed in light to dark brown colors. The similar administrated dosage of microemulsions in groups G3-G5 (1 cc/kg) contained different sesame oil value.

The content of sesame oil in G3 and G4 groups was 61 and 106.32 mg/cc and 106.32 plus 106.32 mg/cc honey in G5 group, respectively. The Apoptosis level in G2 was increased significantly compared to G1 (healthy controls) (11.6±0.73 vs. 0.24±0.11, respectively; P-value<0.001). Treatment with the first microemulsion formulation supplementation (G3) resulted in reduced intensity of isoproterenol induced Apoptosis (Fig. 4-b). A significant improvement in the Apoptosis level was detected in comparison to G2 (8.1±0.28 vs. 11.6±0.73 respectively; P-value<0.05). In G4 which was treated with the second microemulsion formulation supplementation, greater reduction in isoproterenol induced Apoptosis was observed in comparison to G3 (Fig. 4-c).

The Apoptotic level in G4 was 5.18±0.29 which is significantly lower than G2 (P-value<0.01) but still is significantly higher than the healthy control group (P-value<0.01). Treatment in G5 using a microemulsion form of honey and sesame oil supplementation caused even higher decrease in isoproterenol induced Apoptosis than G3 and G4 (Fig. 4-d) and showed a significant difference with G2 (3.2±0.64 vs. 11.6±0.73, respectively; P-value<0.001).

However, a complete dissolving state of Apoptotic changes in myocardial cells of rats

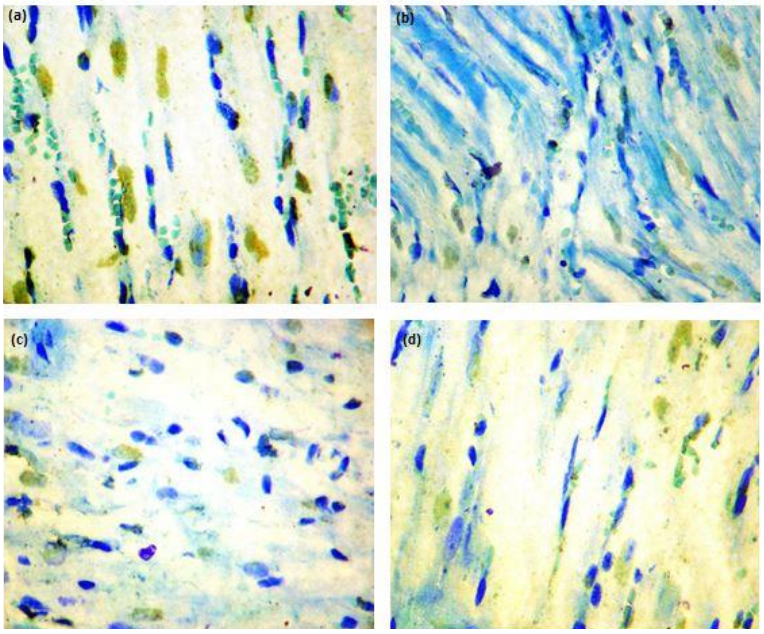

Fig 4. (a) Patient control group (G2). ISO was administered alone. Apoptotic cells observed in light to dark brown colors (TUNEL staining × 40), (b) Apoptotic changes when the low dose of sesame oil in microemulsion formulation (G3) was administrated in the dosage of 1 cc/kg (TUNEL staining × 40), (c) Apoptotic changes when the high dose of sesame oil in microemulsion formulation (G4) was administrated in the dosage of 1 cc/kg (TUNEL staining × 40) and (d) Apoptotic changes when the high dose of sesame oil in microemulsion formulation plus honey (G5) was administrated in the dosage of 1 cc/ (TUNEL staining × 40)

Table 3. Physicochemical characterization of microemulsions in second formulation after preparation

Sample no.	S/Co Ratio	(S+Co)* (%wt)	Oil (%wt)	Water (%wt)	Avg. Droplet size(nm)	Zeta Potential (mV)	PDI	RI	Conductivity (μs)	pH	Phase separation after centrifuge
1	8:1	28.8	7.2	64	16.6±0.1	-12.8	0.356±0.013	1.3916	311	6.42	Not observed
2	9:1	30.63	8.12	61.25	21.2±0.2	-10.7	0.367±0.015	1.3962	305	6.42	Not observed
3	10:1	32.43	8.61	58.96	19.6±0.2	-18.4	0.381±0.017	1.3932	297	6.42	Not observed

(S+Co) * %wt shows the sum of both surfactants mass percent

Table 4. Physicochemical characterization of microemulsions in second formulation after 6 months

Sample no.	Avg. Droplet size (nm)	Zeta Potential (mV)	PDI	Instability over 6 months
1	-	-	-	Turbidity
2	32.1±0.2	-13.4	0.248±0.019	Not observed
3	-	-	-	Turbidity

could not be achieved and its Apoptotic level was achieved as 3.2±0.64.

In accordance to observed results, it seems that the anti-oxidant capacity of both honey and sesame oil ingredients could partially protect myocytes from cellular injury and/or Apoptosis.

Also, it was observed that higher oil percentage by adding honey had better protective results in rats' myocytes. Therefore, it is strongly recommended that a higher sesame oil percentage should be used in similar experimental conditions.

To compare the performance of proposed materials in this study with a chemical drug, Atorvastatin was selected. In 2011, Doustar et al., [45] was examined the anti-Apoptotic effect of Atorvastatin in heart failure induced by isoproterenol in male Wistar rats (age = 7weeks; weight = 230 ± 20gr approximately). In their study, 50 rats were randomly divided into 5 groups (G1-G5) as follows: Healthy controls (G1), ISO alone (G2), ISO plus Atorvastatin in the dosage of 5 mg/kg (G3), ISO plus Atorvastatin in the dosage of 10 mg/kg (G4) and ISO plus Atorvastatin in the dosage of 15 mg/kg (G5).

For the development of congestive heart failure in rat, isoproterenol was injected subcutaneously as the dosage of 0.5 mg/kg, daily for 10 days in all groups except G1. A comparison between the obtained results of Atorvastatin in Dustar's study [45] and the new microemulsion form of honey and sesame oil proposed in this study, are shown in Fig. 5. As figure shows the Apoptotic variances

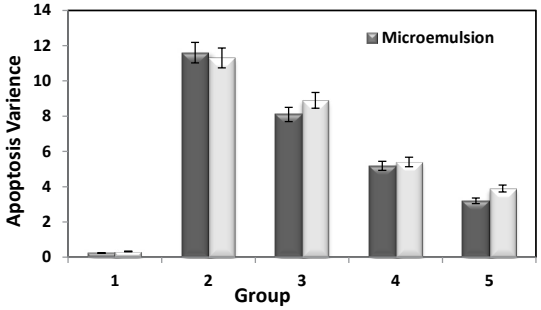

Fig 5. Comparing the anti-Apoptotic effect of honey-sesame oil in Microemulsion form and Atorvastatin drug

between healthy control group and microemulsion consumer groups are higher than in comparison to Atorvastatin consumer groups. This confirms the suitable antioxidant property of the proposed natural mixture including honey and sesame oil. So, Atorvastatin can be replaced by natural components in the microemulsion form containing nanoscale particle size.

CONCLUSIONS

In this study, the anti-cholesterol and anti-Apoptotic effects of sesame oil and honey in a new microemulsion formulation with the particle size of less than 100 nm was investigated. Therapeutic effects of this new form of natural drug was examined on male Wistar rats with heart failure. First, two different microemulsion formulation using Tween 80, Glycerin and Span 80 as surfactant, co-surfactant and second surfactant, respectively, were prepared. Then, the physicochemical characteristic properties of samples from each formulations with highest oil content were investigated. Then, the most stable microemulsions containing higher oil content were selected for in-vivo performance. In-vivo results showed that dietary supplementation of combination of honey and sesame oil in the microemulsion formulation led to decrease in the Apoptosis induced by daily subcutaneous injection of 0.5 mg/kg of isoproterenol for 10 days. Furthermore, the protective effect was more obvious with increasing the oil percentage of microemulsion. Also, adding honey as hydrophilic additive could increase the protective effects, significantly. Comparing the obtained results with Atorvastatin chemical drug results confirmed the appropriate treatment effects of this new proposed formulation. However, the combination of honey and sesame oil is one of the traditional patient feasible meals, but changing the form to the microemulsion with particle size in the nanoscale could be effectively approve treatment protocol in heart failure patients. However, double-

blind placebo-controlled clinical trials may further brighten the efficacy of this combination in heart failure patients and the mechanism involved.

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CONFLICT OF INTEREST

Author has no received research grants. The author declares that he has no conflict of interest.

REFERENCES

1. Ho K, Pinsky J, Kannel W, Levy D. The epidemiology of heart failure: the Framingham Study. J Am Coll Cardiol. 1993; 22(4): 6-13.
2. Mosterd A, Cost B, Hoes A, Bruijne Md, Deckers J, Hofman A. The prognosis of heart failure in the general population: The Rotterdam Study. Eur Heart. 2001; 22(15): 1318-1327.
3. Cowie M, Wood D, Coats A, Thompson S, Suresh V, Poole-Wilson P. Survival of patients with a new diagnosis of heart failure: a population based study. Heart. 2008; 83(5): 505-510.
4. McMurray J, Petrie M, Murdoch D, Davie A. Clinical epidemiology of heart failure : public and private health burden. Eur Heart. 1998; 19: 9-16.
5. Hunt S, Baker D, Chin M, Cinquegrani M, Feldman A. Francis GSACC/AHA Guidelines for the Evaluation and Management of Chronic Heart Failure in the Adult: Executive Summary A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1995 Guidelines for the Evaluation and Management of Heart Failure) : Developed in Collaboration With the International Society for Heart and Lung Transplantation; Endorsed by the Heart Failure Society of America. Circulation. 2001; 104(24): 2996-3007.
6. Beltrami C, Finato N, Rocco M, Feruglio G, Puricelli C, Cigola E. The cellular basis of dilated cardiomyopathy in humans. J Mol Cell Cardiol. 1995; 27(1): 291-305.
7. Narula J, Haider N, Virmani R, DiSalvo T, Kolodgie F, Hajjar R. Apoptosis in myocytes in end-stage heart failure. N Engl J Med. 1996; 335(16): 1182-1189.
8. Weber K. Extracellular matrix remodeling in heart failure: a role for de novo angiotensin II generation. Circulation. 1997; 96(11): 4065-4082.
9. Bristow M, Gilbert E, Abraham W, Adams K, Fowler M, Hershberger R. Carvedilol produces dose-related improvements in left ventricular function and survival in subjects with chronic heart failure MOCHA Investigators. Circulation. 1996; 94(11): 2807-2816.
10. Sabbah H, Stanley W, Sharov V, Mishima T, Tanimura M, Benedict C. Effects of dopamine beta-hydroxylase inhibition with nepicastat on the progression of left ventricular dysfunction and remodeling in dogs with chronic heart failure. Circulation. 2000; 102(16): 1990-1995.
11. Tang W, Francis G. Novel pharmacological treatments for heart failure. Expert Opin Investig Drugs. 2003; 12(11): 1791-1801.
12. Shahidi F, Liyana-Pathirana C, Wall D. Antioxidant activity of white and black sesame seeds and their hull fractions. Food Chem. 2006; 99(3): 478-483.
13. Chen P, Chien K, Su T, Chang C, Liu TL, Cheng H. Dietary sesame reduces serum cholesterol and enhances antioxidant capacity in hypercholesterolemia. Nutr Res. 2005; 25(6): 559-567.
14. Jeng K, Hou R, Sesamin and Sesamolin: Nature's Therapeutic Lignans. Curr Enzym Inhib. 2005; 1(1), 11-20.
15. Shittu L, Bankole M, Ahmed T, Aile K, Akinsanya M. Differential antimicrobial activity of the various crude leaves extracts of Sesame radiatum against some common pathogenic micro-organisms. Sci Res Essays. 2006; 1, 108-111.
16. Suja K, Jayalekshmy A, Arumughan C. Free radical scavenging behavior of antioxidant compounds of sesame (sesamum indicum L.) in DPPH system. J Agric Food Chem. 2004; 52(4), 912-915.
17. Kang M, Naito M, Tsujihara N, Osawa T. Sesamolin inhibits lipid peroxidation in rat liver and kidney. J Nutr. 1998; 128(6): 1018-1022.
18. Sadeghi N, Oveisi M, Hajimahmoodi M, Jannat B, Mazaheri M, Mansouri S. The contents of sesamol in Iranian sesame seeds. Iran J Pharm Res. 2009; 8: 101-105.
19. Frankel S, Robinson G, Berenbaum M. Antioxidant capacity and correlated characteristics of 14 unifloral honeys. J Apic Res. 1998; 37(1): 27-31.
20. Fahey J, Stephenson K. Pinostrobin from honey and Thai ginger (Boesenbergia pandurata): a potent flavonoid inducer of mammalian phase 2 chemoprotective and antioxidant enzymes. J Agric Food Chem. 2002; 50(25): 74722-77426.
21. Gheldof N, Wang X, Engeseth N. Identification and quantification of antioxidant components of honeys from various floral sources. J Agric Food Chem. 2002; 50(21): 5870-5877.
22. Beretta G, Granata P, Ferrero M, Orioli M, Facino R.M. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal Chim Acta. 2005; 533 (2): 185-191.
23. Inoue K, Murayama S, Seshimo F, Takeba K, Yoshimura Y, Nakazawa H. Identification of phenolic compound in manuka honey as specific superoxide anion radical scavenger using electron spin resonance (ESR) and liquid chromatography with coulometric array detection. J Sci Food Agr. 2005; 85(5): 872-878.
24. Blasa M, Candiracci M, Accorsi A, Piacentini M, Albertini M, Piatti E. Raw Millefiori honey is packed full of antioxidants. Food Chem. 2006; 97(2): 217-222.
25. Nagai T, Inoue R, Kanamori N, Suzuki N, Nagashima T. Characterization of honey from different floral sources. Its functional properties and effects of honey species on

storage of meat. Food Chem. 2006; 97(2): 256-262.
26. Perez E, AJ R.M, Vit P. Antioxidant capacity of Venezuelan honey in wistar rat homogenates. Med Food. 2006; 9(4): 510-516.
27. Küçük M, Kolaylı S, Karaoğlu Ş, Ulusoy E, Baltacı C, Candan F. Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem. 2007; 100(2): 526-534.
28. Perez R, Iglesias M, Pueyo E, Gonzalez M, Lorenz,o C.d. Amino acid composition and antioxidant capacity of Spanish honeys. J Agric Food Chem. 2007; 55(2): 360-365.
29. Bogdanov S, Jurendic T, Sieber R, Gallmann P. Honey for nutrition and health : a review. Am Coll Nutr. 2008; 27(6): 677-689.
30. Khalil M, Sulaiman S, Boukraa L. Antioxidant properties of honey and its role in preventing health disorder. Open Nutraceuticals J. 2010; 3: 6-16.
31. Dehghani F, Farhadian N, Golmohammadzadeh S, Biriaee A, Ebrahimi M, Karimi M. Preparation, characterization and in-vivo evaluation of microemulsions containing tamoxifen citrate anti-cancer drug. Eur J Pharm Sci. 2016; 96: 479-489.
32. Narang A, Delmarre S.D, Gao D. Stable drug encapsulation in micelles and microemulsions. Int J Pharm. 2007; 345(1): 9-25.
33. Chaudhary A, Nagaich U, Gulati N, Sharma V.K, Khosa R.L. Enhancement of solubilization and bioavailability of poorly soluble drugs by physical and chemical modifications: A recent review. J Adv Pharm Edu Res. 2012; 2(1): 32-67.
34. McClements D.J. Nanoemulsions versus microemulsions: terminology, differences, and similarities. Soft matter. 2012; 8(6): 1719-1729.
35. Sikarra D, Shukla V, Kharia A.A, Chatterjee D. Techniques for solubility enhancement of poorly soluble drugs: an overview. J Med Pharma Allied Sci. 2012; 1: 1-22.
36. Trivedi H, Thakur R, Ray S, Patel K. Design and Evaluation of Piroxicam Microemulsion. Int J Pharma Res Scholars. 2012; 1: 166-177.
37. Rhee Y.-S, Choi J.-G., Park E.-S, Chi S.-C. Transdermal delivery of ketoprofen using microemulsions. Int J Pharm. 2001; 228: 161-170.
38. Zhao X, Zhang Y.L.X. Enhancement of transdermal delivery of theophylline using microemulsion vehicle. Int J Pharm. 2006; 32: 58–64.
39. Wang S, Ye T, Zhang X, Yang R, Yi X. Myricetin microemulsion for oral drug delivery: formulation optimization, in situ intestinal absorption and in-vivo evaluation. Asian J Pharm Sci. 2013; 8: 18-27.
40. Dizaj S. M. Preparation and study of vitamin A palmitate microemulsion drug delivery system and investigation of co-surfactant effect. J Nanostruct Chem. 2013; 3(1): 1-6.
41. Atashafrooz Z, Dizaj S.M, Sadaghiani A.S. Cucurbita pepo oil as a drug microemulsion formulation: study of phase diagram. Nanomed J. 2014; 1: 298-301.
42. Wexler B. Myocardial infarction in young vs old male rats: pathophysiologic changes. Am Heart. 1987; 96(1): 70-80.
43. Szab J, Csaky L, Szegi J. Experimental cardiac hypertrophy induced by isoproterenol in the rat. Acta Physiol Acad Sci Hung. 1975; 46(3): 281-285.
44. Zhang J, Knapton A, Lipshultz S, Weaver J, Herman E. Isoproterenol-induced cardiotoxicity in sprague-dawley rats: correlation of reversible and irreversible myocardial injury with release of cardiac troponin T and roles of iNOS in myocardial injury. Toxicol Pathol. 2008; 36(2): 277-278.
45. Doustar Y, Mohajeri D. The anti-apoptotic effects of atorvastatin in isoproterenol induced experimental heart failure. Zahedan J Res Med Sci. 2011; 13(2): 13-19.