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# Anti-obesity and body weight reducing effect of *Docynia indica* (Wall.) Decne fruit extract fractions in experimentally obese mice

Nguyen Thi Thanh Loan[1], Hoang Thi Minh Tan[2], Vu Thi Hanh Tam[3], Chu Luong Luan[3], Le Mai Huong[4], Do Ngoc Lien[3,*]

[1]*Ha noi National University of Education, 136 Xuan Thuy, Hanoi, Vietnam*
[2]*Hanoi Pedagogical Univerisity No.2; Xuan Haa, Vinh Phuc, Vietnam*
[3]*Hanoi University of Science, VNU, 334 Nguyen Trai, Hanoi, Vietnam*
[4]*Institute of Natural Product Chemistry, VAST, 18 Hoang Quoc Viet, Hanoi, Vietnam*

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**Abstract**. The objective of the present study was to assess anti-obesity and body weight reducing effect of *Docynia indica* (W.) Decne fruit extract fractions base on experimentally obese mice model.

Male mice (*Mus musculus*, Swiss strain of 4 weeks of age, weighed at 14-15g) purchased from National Institute of Hygiene and Epidemiology (NIHE) were housed in a temperature (25 ± $2^0$C) and humidity (80% ± 10 %) under a 12h light/12 h dark cycle. Diets and tap water were provided *ad libitum.* Experimental mice were divided into 5 groups in which, the first group was fed with normal pellet diet (ND) (Standard chow from NIHE), and other groups were fed high fat diet (HFD) containing 58% calories as fat for a period of 28 days [1,2] The obese mice were treated daily with extract fractions from *Docynia indica* fruit for 14 days

The HFD mice exhibited significant increase in body weight to be 44.4% as compared to ND group at that time ($p \leq 0.05$). Besides, the HFD mice showed significant increase in blood lipid parameters such as total cholesterol(TC), triacylglycerols(TG), Low Density Lipoprotein cholesterol (LDL-c) and glucose concentrations to be 67.3%, 46.2 %, 28.6 % and 18.3 %, respectively as compared to ND mice. Our investigations show that anti-obesity and body weight reducing effects in obese mice treated orally daily with 650 mg/kg of extract fractions from *Docynia indica* (W.) Decne fruit were demonstrated. The obtained results indicate that the body weights of HFD mice daily treated by different extract fractions were reduced by 9.5 % (for ethyl acetate extract), 3.8 % (for chloroform extract) and 8.9 % (for total ethanol extract) as compared to the control( obese mice untreated )($p \leq 0.05$)).

Besides,the blood lipid parameters of HFD mice treated with ethylacetae extract fraction of *Docynia indica* fruits for 14 days were decreased clearly, such as TC decreased 10.3%,TG decreased 31.6% LDL-c decreased 28.6%, especially blood glucose level in obese mice decreased 14.3% compared to control ($p \leq 0.05$).

*Keywords:* Anti-obesity, body weight reducing effect, *Docynia indica* (W.) Decne, experimentally obese mice.

* Corresponding author. Tel.: 84-4-38582179.
E-mail: liendn@vnu.edu.vn

## 1. Introduction

Obesity can be considered as an abnormal state of lipid metabolism and increasing in developed and even developing countries. The fact that the obesity explosion significantly increases the risk of developing various live-threatening diseases, including type II diabetes, hypertension, coronary heart diseases stroke and certain cancers. The worldwide prevalence of obesity in adults is currently estimated to exceed 1.7 billion [3]. Most of the medications listed by United States Food and Drug Administration (FDA) were only approved for short-term use because side-effects of drug to patients usually were exposed [4]. One of the novel strategies for anti-obesity is to use the natural products from traditional medicinal plants in form of plant extracts or functional food [5]. However, investigations of pharmacological mechanisms of natural compounds from traditional remedies were limited [6].

For a long time, the *Docynia indica* (W.) Decne fruits were used to prepare traditional remedies for treatment of in and infectious diseases and digestive, but its anti-obesity effect is not studied yet [7]. In this report the anti-obesity effect of *Docynia indica* (W.) Decne fruit extract was studied on experimentally obese mice.

## 2. Materials and methods

- *Docynia indica* (W.) Decne fruit were collected from plants grown in Van Chan – Yen Bai province.

- Characterization of some secondary compounds from *Docinia indica* fruit extracts was carried out by specialized techniques as specific colour reactions: shinoda, vanilin/HCL, etc. and thin layer chromatography on silica gel sheets (Merck Flufolien 60 F254) with solvent system: Toluene: ethyl acetate: acetone: formic acid (5:3:1:1) ratio [8].

- Quantification of total phenolic compounds was carried out by Folin-ciocalteau technique [9].

- Male mice (*Mus musculus*, Swiss strain of 4 weeks of age, weighed at 14-15g ) purchased from National Institute of Hygiene and Epidemiology (NIHE) were housed in a temperature ($25 \pm 2^0$C) and humidity (80% ± 10 %) under a 12h light/12 h dark cycle. Diets and tap water were provided *ad libitum.* Experimental mice were divided into 5 groups in which, the first group was fed with normal pellet diet (ND) (Standard chow from NIHE), and other groups were fed high fat diet (HFD) containing 58% calories as fat for 28 days [1, 2]. The obese mice were treated orally daily with the extract fractions of *D.indica* fruit for 14 days.

- Determination of some blood biochemical parameters such as: glucose, cholesterol, triacylglycerol, HDL-c and LDL-c concentrations was carried by Olympus Analyzer AU400, (Japan).

- The obtained data were calculated by biological statistics with MS-excel.

## 3. Results and discussion

*Compositions of natural compound from the extracts of D. indica*

Using the solvents such as ethanol, n-hexan, chloroform, ethyl acetate and water. The process of natural product extraction from *Docynia indica* (W.) Decne fruit was designed. The scheme of extraction processes was presented in the Fig.1.

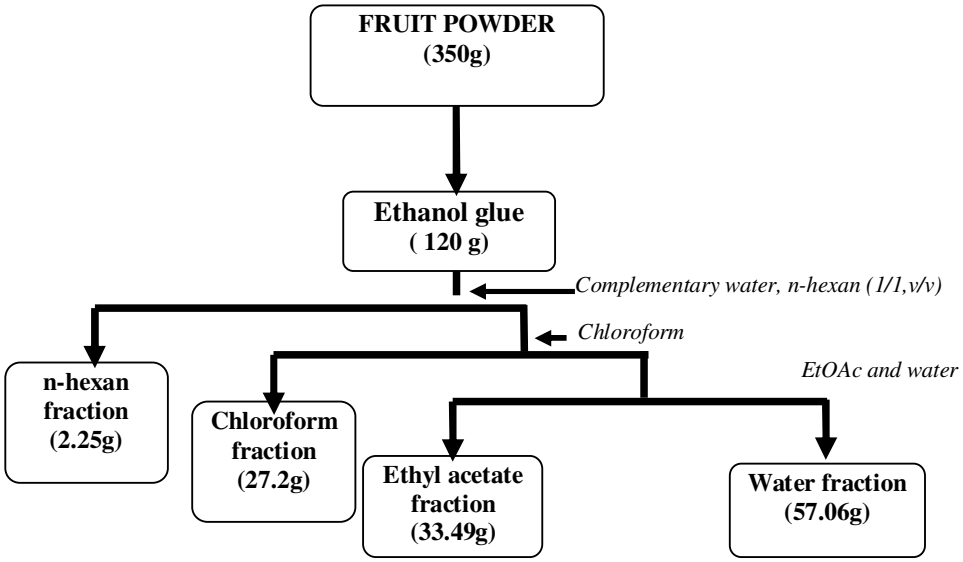


Fig.1. Process of isolation of natural products from *Docynia indica* (W.) Decne fruit.

After the extract fractions were concentrated in vacuum to prepare dry fraction concentrates. In order to analyses the composition of extraction fractions we used the specific reactions. The results were represented in the table 1.

Table 1. Characterizations of natural products from *Docynia indica* (W.) Decne fruit extract fractions by specific reactions

| Compounds | | Specific reaction | Fractions | | | |
|---|---|---|---|---|---|---|
| | | | Ethanol | n-hexan | Chloroform | Ethyl acetate |
| Phenolic | Flavonoid | Shinoda | ++ | + | + | ++ |
| | | $H_2SO_4$ | ++ | - | + | +++ |
| | Tannin | Lead Acetate | ++ | + | ++ | ++ |
| | | Gelatin/NaCl | - | - | - | + |
| | Other Polyphenol | 10% NaOH | ++ | ++ | + | ++ |
| | | 5% $FeCl_3$ | + | - | ++ | ++ |
| Glycoside | | Keller-killian | +++ | + | ++ | + |
| Alkaloid | | Dragendroff | ++ | + | + | +++ |

*(+) positive level*

The results listed in the Table 1 show that the compositions of natural products from *Docynia indica* (W.) Decne fruit extract fractions consist of phenolic, glycoside and alkaloid compounds. However, phenolic compounds such as flavonoids, tannin, catechin were found principally in the ethyl acetate and ethanol fractions.

In order to determine relatively the composition of natural compounds we used the technique of thin layer chromatography on Silica gel sheets (Merck, Flufolien 60.F254) with solvent system Toluene/ ethyl acetate/

acetone/ formic acid (5:3:1:1 ratio). The results show that *Docynia indica* (W.) Decne fruit extract fractions were composed from 6 to 12 bands of natural products (Fig.2).

Using the technique of Singleton et al (1999) for quantification of phenolic compounds, we have determined total phenolic contents of *Docynia indica* (W.) Decne fruit extract fractions. (Table 2)

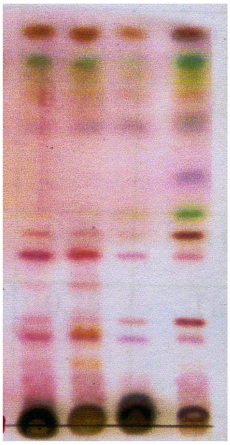


Fig. 2. Thin layer chromatography on silica gel sheet by solvent system: Toluene-ethyl acetate-acetone-formic acid (5:3:1:1).
(1) Total ethanol fraction; (2) n-hexan fraction;
(3) Chloroform fraction; (4) Ethyl acetate fraction

Table 2. Total polyphenol content determinened by Follin- Ciocalteau technique

| Fractions | Total polyphenol, mg) | % Dry concentrate |
|---|---|---|
| EtOH concentrate | 0,4992 | 4,99 |
| n-hexan concentrate | 0,2142 | 2,14 |
| $CHCl_3$ concentrate | 0,2722 | 2,72 |
| EtOAc concentrate | 0,6662 | 6,66 |
| Water concentrate | 0,0782 | 0,78 |

The results listed in the Table 2 show that highest concentration (mg) of phenolic compounds from 350gr of dry concentrate determined in the ethanol (EtOH) fraction is 0.4992mg (4.99% of dry concentrate). The other fractions such as ethyl acetate and chloroform fractions is also expressed the high concentrations of phenolic compounds, from 0.2722 mg (2.72% of dry concentrate) to 0.66mg (6.66% of dry concentrate) respectively.

*Designing the model of experimentally obese mice*

The mice of 4 weeks of age, *Mus musculus* Swiss strain, weighed at 14-15 g were divided into 6 lots (6 mice/lot repeated three times)

+ Lot 1: control, mice were fed with normal standard diet (ND)

+ Other lot fed with high fat diet (HFD).

The composition of HFD calculated by National Institute of Nutrition was represented in the Table 3

Table 3. Composition of high lipid diet

| Composition | % |
|---|---|
| Carbohydrate | 30 |
| Lipid | 35 |
| Casein | 25 |
| Cholesterol | 3 |
| Vitamin and minerals | 3 |
| Other compounds | 4 |

First, the body weight of experimental obese mice was determined in comparison with control mice (fed standard diet). The obtained result was represented in the Fig.3

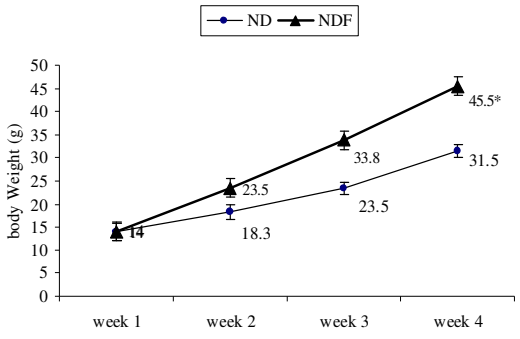


Fig. 3. A diagrammatic representation of body weights increase of experimentally obese mice fed with high fat diet for 28 days.

The results obtained from the model of experimentally obese mice in the figure 4 show that the body weights of experimentally mice fed with high lipid diet for 16 days increase clearly from 125% to 225% compared to the first day. It is clear that, the body weight of HFD fed mice fed increases 44.44% in comparison with the ND fed mice ($p< 0.05$) (fig 3)

In order to demonstrate the disorder of lipid metabolism of experimentally obese mice we have proceeded to analyze blood lipid compositions in experimentally obese mice compared to control mice. The results show that:

- The blood total cholesterol concentration of obese mouse increases 67.3% compared to ND mice

- Blood LDL of obese mouse blood increases especially 28.6% compared to ND mice

- Blood HDL-c of obese mouse decreases 21.4% compared to ND mice

- The blood glucose concentration of obese mouse increases 37,7 % compared with to ND

Therefore, the blood lipid parameters indicated that the experimentally obese mice suffered from lipid metabolism disorder.

*Oral treatment of obese mice with D.indica extracts*

In order to treat the obese mice, we have designed the experimental schema for daily repeated oral administration (14 days) of *Docynia indica* (W.) Decne fruit extract fractions (650mg fraction concentrates /kg).

The experimental schema was presented in the following:

+ Lot 1: Control mice fed with standard nomal (ND) diet and drinking water

+ Lot 2: Obese mice treated with ethanol fraction (650mg/kg)

+ Lot 3: Obese mice treated with chloroform fraction (650mg/kg)

+ Lot 4: Obese mice treated with ethyl acetate fraction (650mg/kg)

+ Lot 5: Obese mice treated with metformin (500 mg/kg)

The results obtained show that the effect of daily repeated oral administration of *Docynia indica* (W.) Decne fruit extracts was prove clearly on decrease of body weight fig. 5.

The body weight of all the obese mice treated with *Docynia indica* (W.) Decne fruit extracts decreases from 3.7% to 9.5 %.

Lot 5 used Metformin the body weight of mice decreased 11.7 % (Drug Metformin has effect on decreasing body weight and blood lipid, FDA).

Lot 1 did not treat with *Docynia indica* (W.) Decne fruit extracts were expressed the increase of body weight 11.9%.

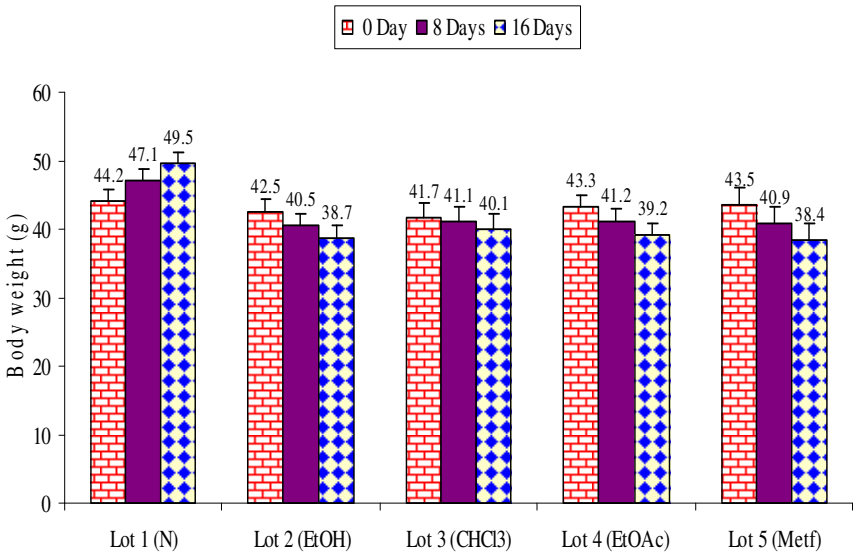


Fig. 4. Effect of repeated oral administration (14 days) of *Docynia indica* (W.) Decne fruit extract fractions (650 mg concentrate /kg) on body weight.

In addition, hypoglycemia, total cholesterol, triaglycerol, LDL reducing the effect of *Docynia indica* (W.) Decne fruit extracts were demonstrated clearly (fig.5, fig.6, fig.7 and Fig.8). Where as, blood HDL-c was increased. This results proved hypolipidemic effect of D. indica extracts in obese mice.

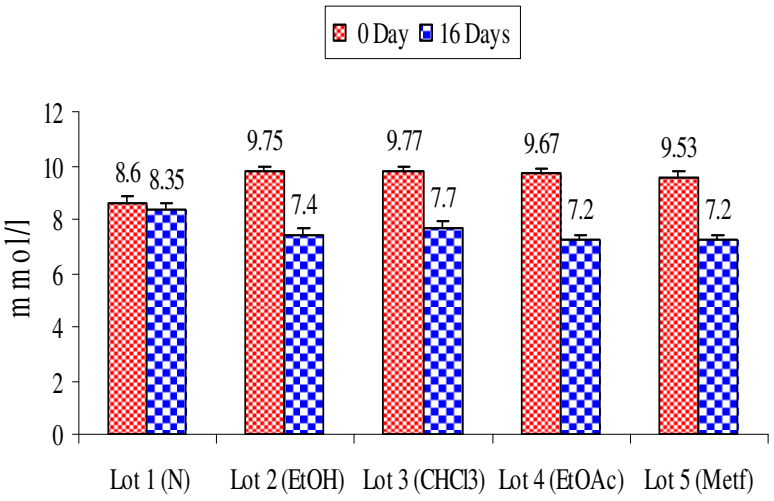


Fig. 5. Effect of repeated oral administration (14 days) of *Docynia indica* (W.) Decne fruit extract fractions (650 mg concentrate/kg) on blood glucose concentration.

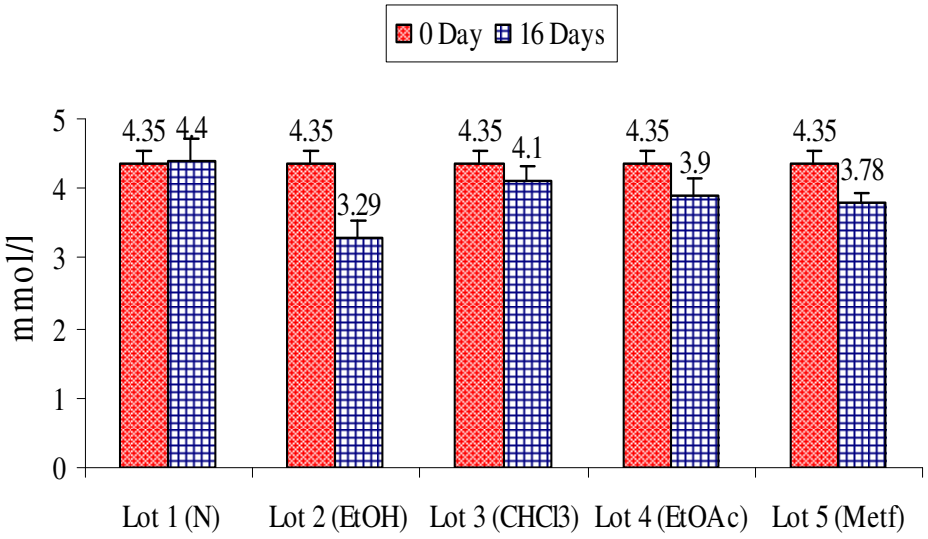


Fig. 6. Effect of repeated oral administration (14 days) of *Docynia indica* (W.) Decne fruit extract fractions (650 mg concentrate/kg) on blood cholesterol concentration.

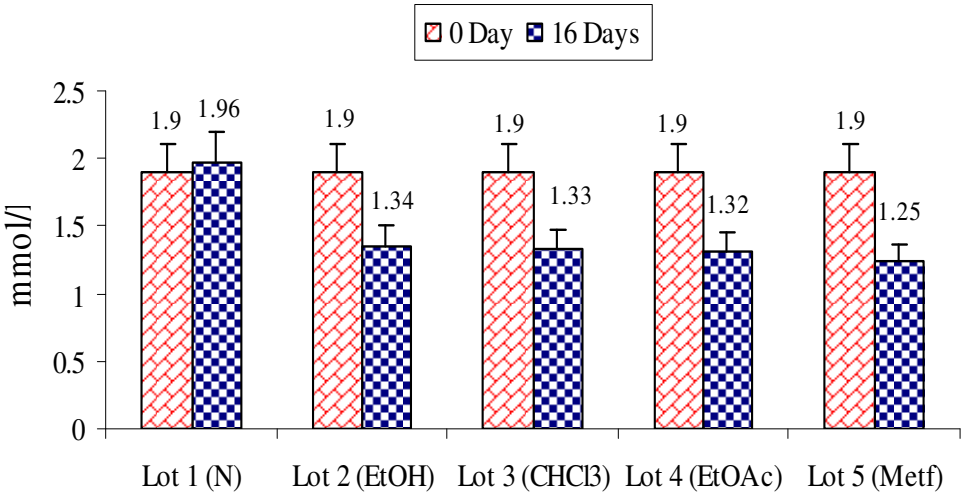


Fig. 7. Effect of repeated oral administration (14 days) of *Docynia indica* (W.) Decne fruit extract fractions (650 mg concentrate/kg) on blood triacylglycerole concentration.

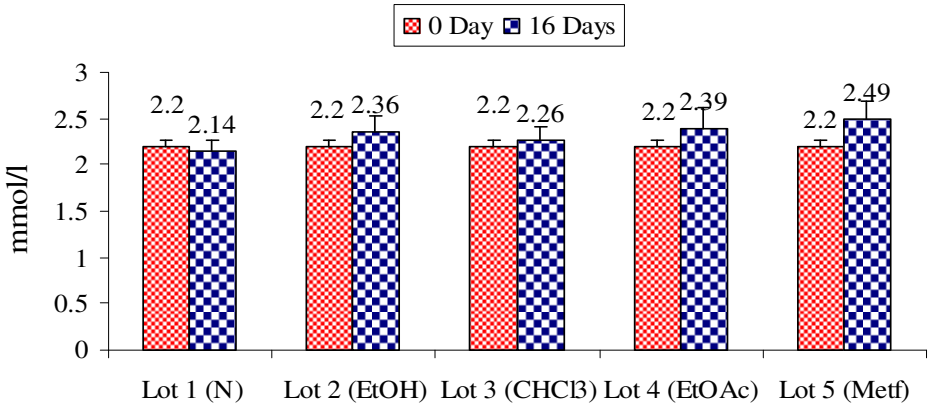


Fig. 8. Effect of repeated oral administration (14 days) of *Docynia indica* (W.) Decne fruit extract fractions (650 mg concentrate/kg) on blood HDL-c concentration.

Especially, the effect of daily repeated oral administration (16 days) of *Docynia indica* (W.) Decne fruit extracts on blood glucose concentration was proved clearly: Lot 2 decreases 11.9% (total ethanol fraction), Lot 4 decreases 14.3% (ethyl acetate fraction)

## 4. Conclusions

Natural products from *Docynia indica* (W.) Decne fruit extracts consist of polyphenol compounds, especially flavonoids and alkaloids.

The model of experimentally obese mouse (*Mus musculus* Swiss strain) was established in mice fed high lipid diet for 28 days. The results show that body weight, blood lipid concentration such as total cholesterol, triacylglycerole and LDL-c of experimentally obese mice increase clearly compared to control mice. Hypolipidemic, Hypoglycemic, body weight reducing effects in obese mice treated with *Docynia indica* (W.) Decne fruit extracts (650mg/kg) were demonstrated clearly.

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# Tác dụng chống béo phì và giảm trọng lượng của dịch chiết quả Táo mèo *Docynia indica* (Wall.) Decne trên mô hình chuột béo phì thực nghiệm

Nguyễn Thị Thanh Loan[1], Hoàng Thị Minh Tân[2], Vũ Thị Hạnh Tâm[3], Chử Lương Luân[3], Lê Mai Hương[4], Đỗ Ngọc Liên[3]

[1]*Đại học Sư phạm Hà Nội, 136 Xuân Thủy, Hà Nội, Việt Nam*

[2]*Đại học Sư Phạm Hà Nội 2, Xuân Hòa, Vĩnh Phúc, Việt Nam*

[3]*Trường Đại học Khoa học Tự nhiên, ĐHQGHN, 334 Nguyễn Trãi, Hà Nội, Việt Nam*

[4]*Viện Hóa học Các hợp chất Thiên nhiên, Viện Khoa học và Công nghệ Việt Nam, 18 Hoàng Quốc Việt, Hà Nội, Việt Nam*

Mục tiêu của nghiên cứu là đánh giá tác dụng giảm trọng lượng, chống béo phì từ các phân đoạn dịch chiết quả Táo mèo *Docynia indica* (Wall.) Decen trên mô hình chuột béo phì thực nghiệm.

Chuột đực chủng Swiss 4 tuần tuổi cân nặng 14-15 gram mua từ Viện Vệ sinh Dịch tễ Trung ương được nuôi ở trong phòng có nhiệt độ (25 ± $2^0$C) và độ ẩm (80% ± 10 %) dưới điều kiện 12h sáng và tối. Chuột được ăn và uống nước tự do sau đó được chia thành nhóm, nhóm đầu tiên được nuôi bằng thức ăn chuẩn của Viện Vệ sinh Dịch tễ TW (nhóm đối chúng) các nhóm còn lại được ăn bằng thức ăn có hàm lượng chất béo cao trong thời gian 28 ngày.

Nhóm chuột ăn thức ăn béo đã tăng trọng lượng lên 44,4% trong cùng thời gian so với nhóm chuột ăn thường. Ngoài ra, hàm lượng các thành phần trong máu chuột ăn thức ăn béo như nồng độ cholesterol tổng số, tryglycerid, LDL-c, và glucose tăng lên tương ứng là 67,3%, 46,2%, 28,6% và 18,3%, so với chuột ăn thức ăn thường. Khả năng chống béo phì giảm trọng lượng ở những lô chuột được điều trị bằng các phân đoạn dịch chiết từ quả *Docinia indica* (W.) đã được chứng minh. Kết quả giảm trọng lượng ở chuột béo phì ở mỗi phân đoạn khác nhau là khác nhau 9.5% (phân đoạn EtOAc), 3,8% (phân đoạn Chloroform) và 8,9% (ở phân đoạn ethanol tổng số) so với chuột béo phì không điều trị ($p<0,05$).

Ngoài ra, chuột béo phì được điều trị trong 14 ngày bằng phân đoạn dịch chiết ethylacetate của quả Táo mèo đã biểu hiện giảm các chỉ số lipid máu như : TC 10,3%, TG 31,16 %; đặc biệt là nồng độ glucose máu ở chuột béo phì giảm 14,3% so với lô đối chứng ($p\leq 0,05$).