VNU Journal of Science, Natural Sciences and Technology 25 (2009) 179-187

Anti-obesity and body weight reducing effect of *Fortunella japonica* peel extract fractions in experimentally obese mice

Do Ngoc Lien*, Nguyen Thuy Quynh, Nguyen Hoang Quang, Nguyen Thi Thanh Ngan

Faculty of Biology, College of Science, VNU, 334 Nguyen Trai, Hanoi, Vietnam

Received 15 April 2009

Abstract. The objective of the present study was to develop an experimentally obese mice model and to investigate antiobesity and body weight reducing effect of *Fortunella japonica* peel extract fractions.

Male mice (*Mus musculus*, Swiss race weighed at 14-15g) purchased from National Institute of Hygiene and Epidemiology (NIHE) were divided into 5 groups in which, the first group was fed with normal pellet diet (NPD) (Standard chow from NIHE), and other groups (2-5 groups) were fed high fat diet (HFD) containing 58% calories as fat for a period of 15 days.

The HFD mice exhibited significant increase in body weight about 44% as compared to NPD group at that time ($p< 0.05$). Besides, the HFD mice showed significant increase in blood total cholesterol, blood triglyceride LDL and glucose concentrations to be 32.20%, 44.0%, 57.50% and 24.60% respectively as compared to NPD mice. In addition, the effects of daily repeated oral administration for 8 days of 800 mg/kg of ethylacetate, chloroform and ethanol extract fractions from *Fortunella japonica* peels were studied. The obtained results showed that the body weights of HFD mice treated by different extract fractions were reduced by 9.2% (for ethylacetate extract), 8.2% (for chloroform extract) and 4.7% (for ethanol extract) as compared to vehicle (control, obese mice were treated by water replaced the extracts ($p<0.05$).

Besides, HFD mice treated by different extract fractions exhibited significant decrease in blood total cholesterol to 13.2% (for ethanol extract), 1.4% (for chloroform extract), 0.7% (for ethylacetate), in triglycerid 13% (for ethanol extract), 15.2% (for chloroform extract), 16.2% (for ethylacetate extract), in glucose 0.9% (for ethanol extract), 23% (for chloroform), 18.5% (for ethylacetate extract) as compared to control ($p<0.05$).

Keywords: Anti-obesity, *Fortunella japonica*, experimentally obese mice.

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 life-threatening diseases, including type II diabetes, hypertension, coronary heart diseases stroke and certain cancers [1,2]. The worldwide prevalence of obesity in adults is currently estimated to exceed 300 million [3]. Although altering eating and activity behavior, lifestyle is the cornerstone of anti-obesity the pharmacotherapy for obesity is necessary.

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

However, most of the medications listed by United States Food and Drug Administration (FDA) were only approved for short-term use because patient's complications to drug therapy usually were exhibited [1,3]. One of the novel strategies for anti-obesity is to exploit the natural products from traditional medicinal plants in form of plant extracts or functional food. However, pharmacological and action mechanisms investigations of natural compounds oriental remedies were limited [2,4-6].

For a long time, the mandarin (Fortunella japonica) is used to prepare traditional remedies for treatment of respiratory and digestive diseases, but its anti-obesity effect is not studied yet. In this report the anti-obesity effect of *Fornunella japonica* peel extract was studied on experimentally obese mice.

2. Materials and methods

- Mandarine (*Fortunella japonica* (Thunb) Swingle Rutaceae) peels were collected from plants grown in Hanoi.
- Characterization of some compounds was carried out by specialized techniques as specific colour reactions: shinoda, vanilin/HCL, etc. and thin layer chromatography on silicagel sheets (Merck Flufolien 60 F254) with solvent system including Toluen/ethylacetate/acetone/formic acid (5/3/1/1) ratio.
- Quantification of total phenolic compounds was carried out by Folin-ciocalteau technique (Orthofer et al, 1999).
- Determination of some blood biochemical data such as: glucose, cholesterol, triglycerid, HDL and LDL concentrations was carried by Olympus Analyzer, Japan
- The obtained data were calculated by biological statistics with MS-excel.

3. Results and discussion

Extraction process of natural products from F.indica peels

Using the solvents such as ethanol (EtOH), n-hexan, chloroform (CHCl3), ethlacetate (EtOAc) and water, we designed the process of natural product extraction from *Fortunella japonica* peels [7]. The scheme of extraction process was reprented in the Fig.1.

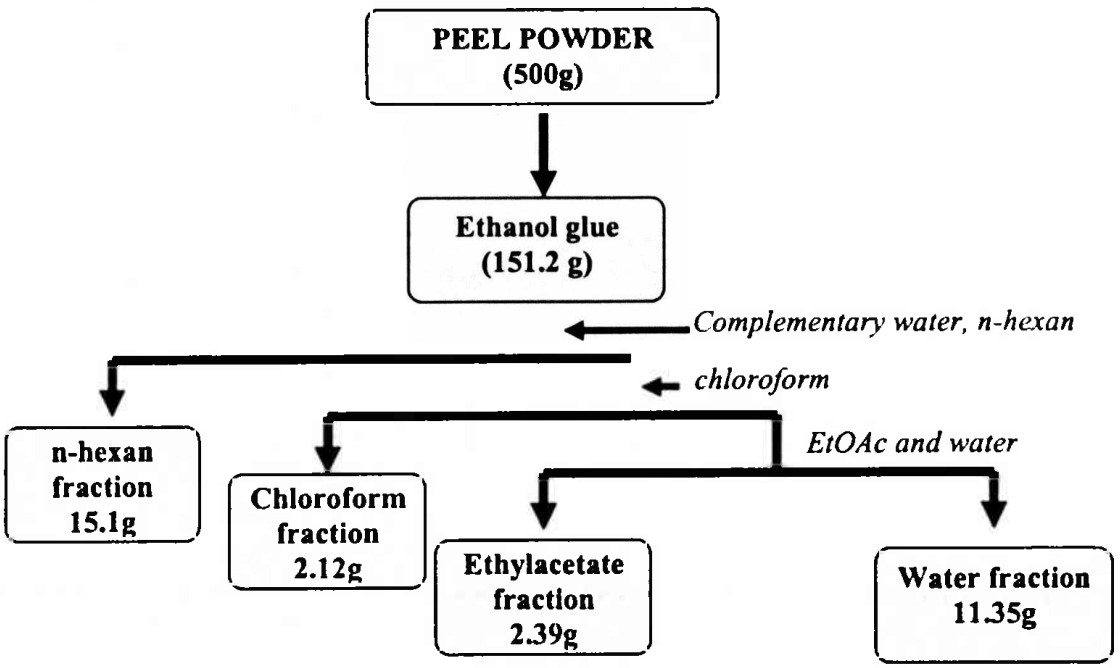


Fig.1. Process of isolation of natural products from Mandarine peels.

The ethanol extract fraction obtained from 500g of dry peel powder were concentrated in vacuum (151.2g) to preprare the different fraction concentrates such as n-hexan, chloroform, ethylcetate and water. The resuls show that natural products of two extraction fractions of n-hexan and water were recovered highly 15.1g (3.02% of dry peel powder), 11.35g (2.27% of dry peel powder) respectively (Fig. 1). In order to analyse the natural product composition from extraction fractions we used the specific reactions. The results were represented in the table 1.

Table 1. Characterization of natural products from *Fortunella japonica* peel extract fractions by specific reactions

Compounds		Specific reaction	Compound			
			Ethanol fraction	n-hexan fraction	Cloroform fraction	Ethylacetate fraction
Phenolic	Flavonoid	Shinoda	+	-	+	+
		H_2SO_4	+	+	+	+++
	Catechin	Vanilin/HCl	+	+	+	+
	Tannin	Vanilin/H_2SO_4	+	+	+	+
		Lead Acetate	++	+	++	++
		Gelatin/NaCl	+	-	+	+
	Other Polyphenol	10% NaOH	+	+	+	++
		5% $FeCl_3$	++	+	++	+++
Glycoside		Keller-killian	+	-	-	-
Alkaloid		Vans-Mayer	+	+	+	-
		Bouchardat	+	+	+	-
		Dragendroff	++	+	++	-

(+) positive level

The results listed in the table 1 show that the compositions of natural products from *Fortunella japonica* peel extract fractions consist of phenolic, glycoside and alkaloid compounds. However, phenolic compounds as flavonoids, tannin, catechin and alkaloid were found principally in ethanol, n-hexan and chloroform fractions. Especially, there is no alkaloid in the ethylacetate fraction.

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 including toluen / ethylacetate / acetone / formic acid (5/3/1/1 ratio). The results show that mandarine peel extract fractions were composed from12 to 16 bands of natural products (Fig.2).

Using the technique of Orthofer et al (1999) for quantification of phenolic compounds, we have determined polyphenols content of mandarin peel extract fractions. (Table 2)

(1) Ethylacetate fraction
(2) n-hexan fraction
(3) Chloroform fraction
(4) Total ethanol fraction

Fig. 2. Thin layer chromatography on silica gel sheet by solvent system: Toluen-ethylacetate-acetone-formic acid (5:3:1:1).

Table 2. Total polyphenol content of the concentrated fractions by Folin-Ciocalteau technique [8]

Fractions	Polyphenol content, dry weight (mg)	% of dry polyphenols compounds
EtOH	1,426.6	100
n-hexan	195.2	13.68
$CHCl_3$	577.2	40.4
EtOAc	580.2	40.6
Water	73.6	5.1

The results listed in the Table 2 show that ethylacetate and chloroform fractions expressed the highest concentrations of phenolic compounds, from 5.802 to 5.772 respectively (% dry polyphenol compounds).

Designing the model of experimentally obese mice

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

+ Lot 1: control, mice were fed with standard diet (National Institute of Hygiene and Epidemiology, Hanoi)

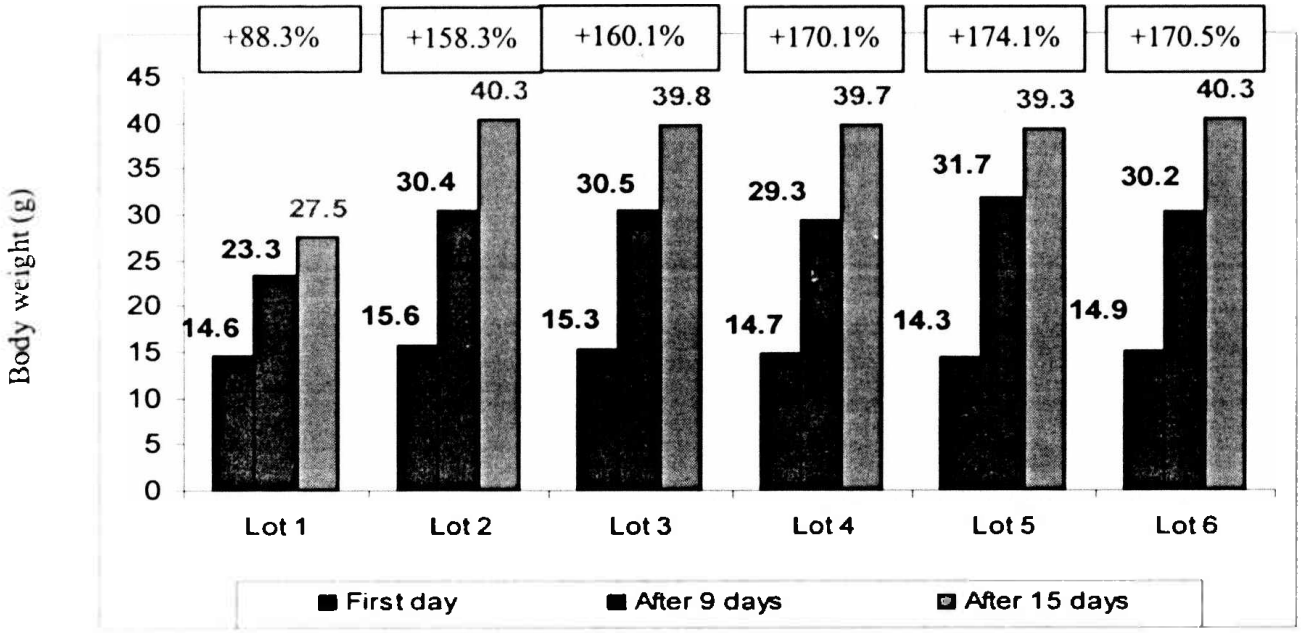
+ Lot 2 – 6: mice were fed with high lipid diet (HLD).

The composition of high lipid diet 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

Fistly, the body weight of experimental obese mice was determined in conparision with control mice (fed standard diet). The obtained result was represented in the Fig.4



Lot 1: fed standard diet
Lot 2: fed HLD
Lot 3: fed HLD
Lot 4: fed HLD
Lot 5: fed HLD
Lot 6: fed HLD

Fig. 3. A diagrammatic representation of weight increase of experimentally obese mice fed with high lipid diet after 15 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 15 days increase clearly from 158.3% to 174.8% compared with the first day. While, the body weight of control mice fed standard diet only increases 88.3% (Fig. 3)

In order to demonstrate the disorder of lipid metabolism of experimentally obese mice we have proceeded to analyze blood lipid compositions of experimentally obese mice compared to control mice. The results represented in the fig. 4 show that:

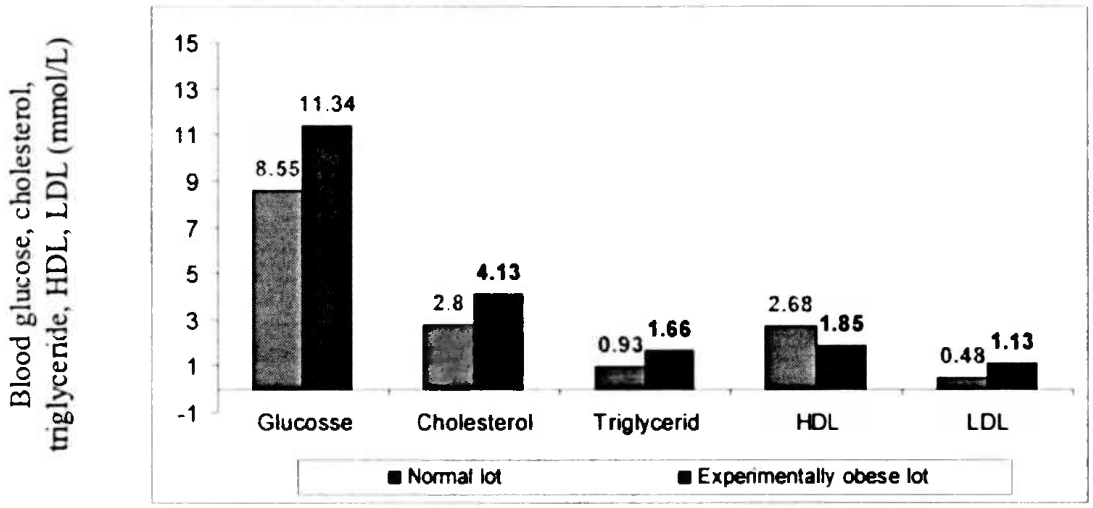


Fig. 4. Diagrammatic representation of glucose and lipid concentration of normal and obese mouse blood.

▪ The blood glucose concentration of obese mouse increases 24.6% compared with normal mouse

▪ The blood total cholesterol concentration of obese mouse increases 32.2% compared with normal mouse

▪ Blood HDL of obese mouse decreases 30.1% compared with normal mouse

▪ Blood LDL of obese mouse increases especially 57.5% compared with normal mouse

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

In order to treat the obese mice, we have designed the experimental schema for daily repeated oral administration (8 days) of *Fortunella japonica* peel extract fractions (800mg extract concentrace/kg).

The experimental schema was presented in the following:

+ Lot 1: Control mice fed with standard diet and drinking water ad libitum

+ Lot 2: Obese mice treated with ethanol fraction and drinking water ad libitum

+ Lot 3: Obese mice treated with chloroform fraction and drinking water ad libitum

+ Lot 4: Obese mice treated with ethylacetate fraction and drinking water ad libitum

+ Lot 5: Obese mice treated with metformin (500mg/kg) and drinking water ad libitum

+ Lot 6: Obese mice fed normal diet and drink water (no treatment) and drinking water ad libitum

The results obtained show that the effect of daily repeated oral administration of mandarin peel extracts was prove clearly on decrease of body weight of obese mice.

- The body weight of all the obese mice treated mandarine peel extracts decreases from 4.7% to 9.2%.

- Lot 5 used Metformin decreased 9.1% (Drug Metformin has effect on decreasing body weight and blood lipid, FDA).

- Lot 1 and lot 6 did not treated with mandarin peel extracts were expressed the increase of body weight from 4% to 9.5%.

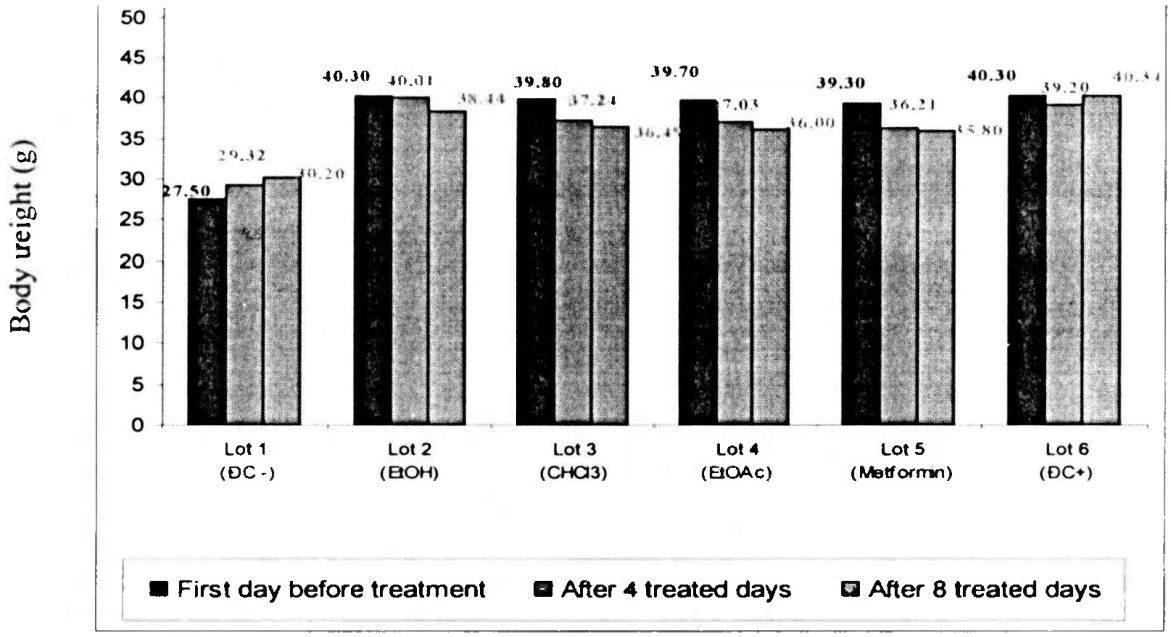


Fig. 5. Effect of repeated oral administration (8 days) of *Fortunella japonica* peel extract fractions (800 mg extract concentrace /kg) on body weight of mice.

In addition, the effect of mandarin peel extracts on blood glucose, total cholesterol, triglyceride concentrations was demonstrated clearly (fig.6, fig.7 and fig.8).

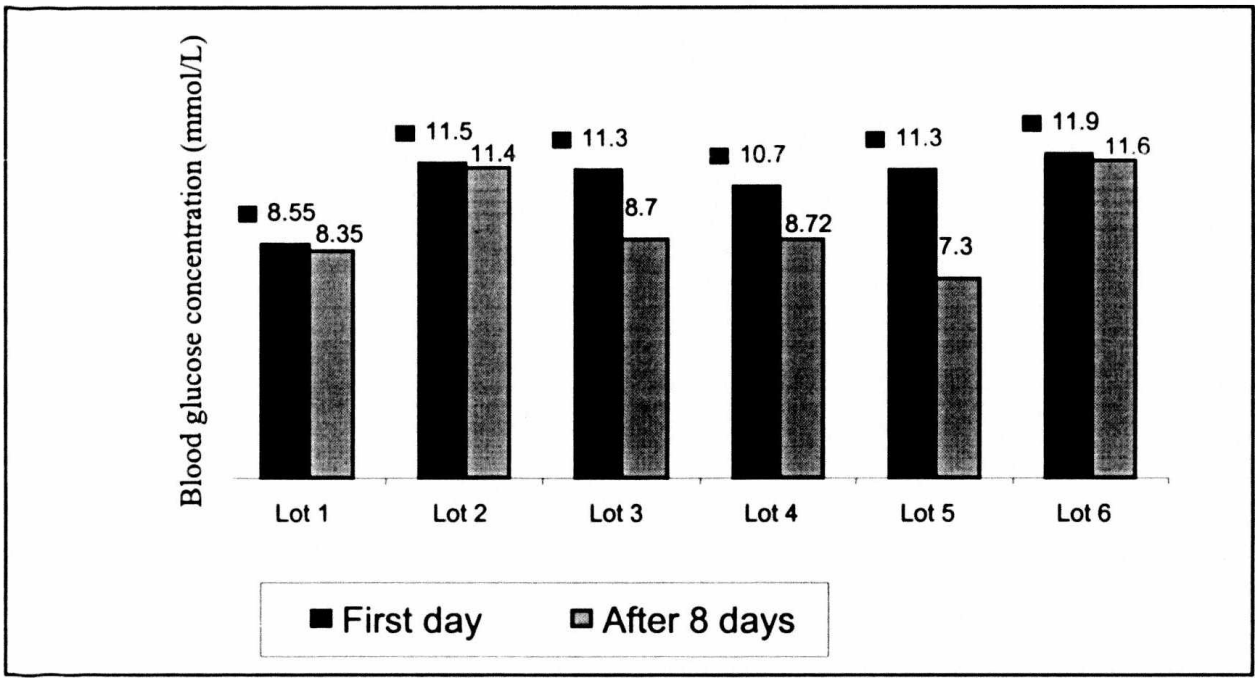


Fig. 6. Effect of repeated oral administration (8 days) of *Fortunella japonica* peel extract fractions (800 mg concentrate/kg) on blood glucose concentration.

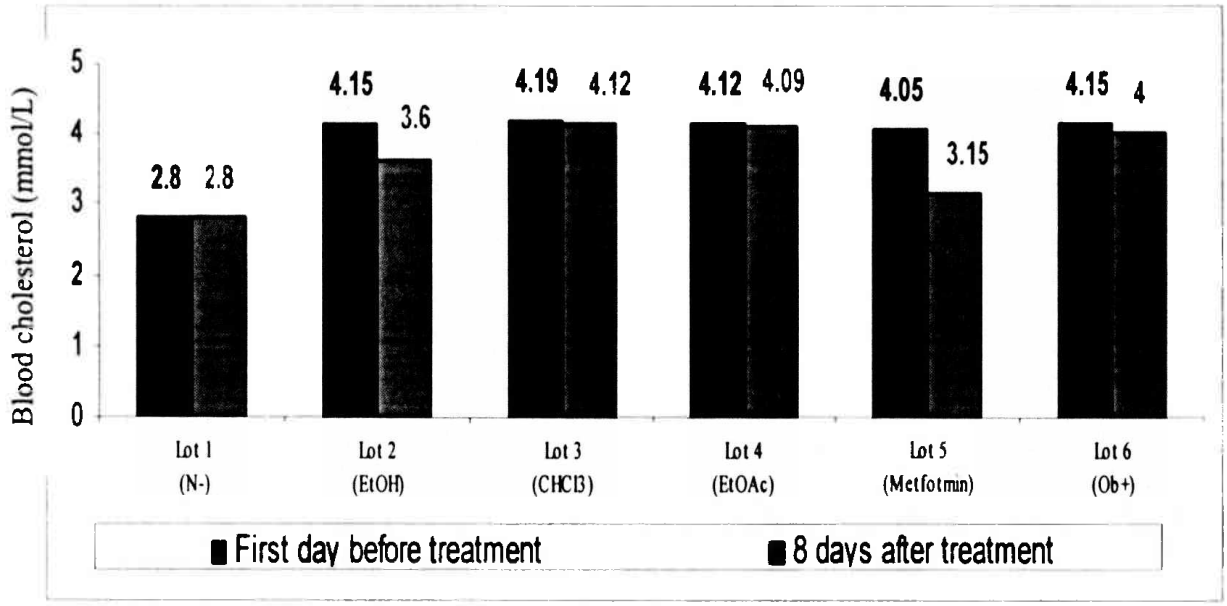


Fig. 7. Effect of repeated oral administration (8 days) of *Fortunella japonica* peel extract fractions (800 mg concentrate/kg) on blood cholesterol concentration.

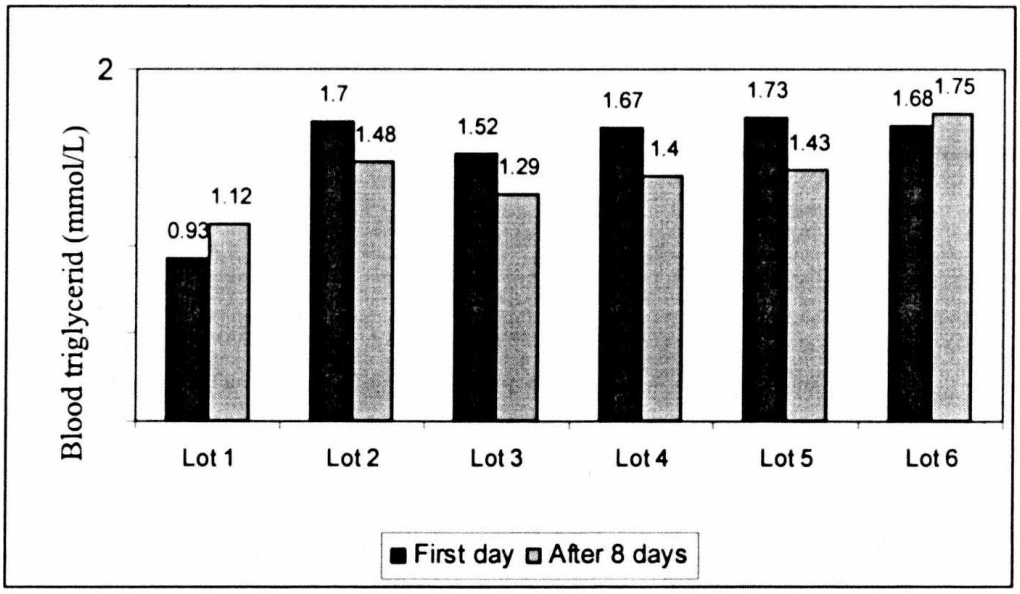


Fig. 8. Effect of repeated oral administration (8 days) of *Fortunella japonica* peel extract fractions (800 mg concentrate/kg) on blood triglyceride concentration.

Especially, the effect of daily repeated oral administration (8days) of mandarin peel extracts on blood glucose concentration was proved clearly: Lot 3 decreases 23% (Chloroform fraction), Lot 4 decreases 18.5% (ethylacetate fraction) (fig 6)

4. Conclusions

Natural products from *Fortunella japonica* peel extracts consist of essentially polyphenol compounds, especially flavonoid.

The model of experimentally obese mouse (*Mus musculus* Swiss strain) was established based on high lipid diet for 15 days. The results show that body weight, blood lipid concentration such as total cholesterol, triglyceride and LDL of experimentally obese mice increase clearly compared to control mice

Effect of repeated oral administration in 8 days of *Fortunella japonica* peel extract refractions (800mg concentrate/kg) on decreases of body weight, blood glucose and lipid concentration was proved in experimentally obese mice.

Acknowledgements

The authors wish to thank the Vietnam National University, Hanoi for the research grant of QDTD 0806 project and Professor Dr. Sc. Pham Thi Tran Chau for her valuable comments.

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Tác dụng chống béo phì và giảm khối lượng cơ thể của các phân đoạn dịch chiết vỏ quả quất cảnh (*fortunella japonica*) trên chuột béo phì thực nghiệm

Đỗ Ngọc Liên, Nguyễn Thúy Quỳnh,
Nguyễn Hoàng Quang, Nguyễn Thị Thanh Ngân

Khoa Sinh học, Trường Đại học Khoa học Tự nhiên, ĐHQGHN, 334 Nguyễn Trãi, Hà Nội, Việt Nam

Mục đích của nghiên cứu này là phát triển một mô hình chuột béo phì thực nghiệm để nghiên cứu tác dụng chống béo phì và giảm khối lượng cơ thể của các phân đoạn dịch chiết từ vỏ quả quất (*fortunella japonica*)

Chuột đực (Muss musculus, chủng Swiss)khối lượng 14-15g được mua của viện Vệ sinh Dịch tễ Trung ương được chia làm 5 nhóm trong đó nhóm 1 ăn thức ăn tiêu chuẩn bình thường (NPD) của viện Vệ sinh Dịch tễ Trung ương. Các nhóm từ 2 đến 5 được ăn bằng thức ăn hàm lượng chất béo cao (HFD) chứa 58% calorie là chất béo được nuôi trong thời gian 15 ngày. Chuột ăn HFD biểu hiện tăng trọng lượng cơ thể (44%) có ý nghĩa thống kê so với chuột ăn NPD, với $p < 0,05$. Ngoài ra chuột ăn béo đã được chứng minh là có nồng độ các chỉ số lipid máu tăng lên như cholesterol tổng số, triglycerid, LDL tương ứng là: 32,2%, 44%, 57,5%, đồng thời có rối loạn trao đổi gluxit được biểu hiện nồng độ glucose máu tăng 24,6% so với chuột dùng thức ăn chuẩn. Tiếp theo chuột béo phì được điều trị lặp lại hằng ngày trong 8 ngày bằng 85mg/kg thể trọng các phân đoạn diết ethylaxetat, chloroform và ethanol. Những kết quả chứng minh rằng: khối lượng của chuột béo phì được điều trị bằng các phân đoạn dịch chiết ở trên đều giảm xuống tương ứng là 9,2% (đối với phân đoạn ethylaxetat), 8,2%(đối với phân đoạn chloroform) và 4,7% (đối với phân đoạn ethanol) so với nhóm chuột kiểm tra (ăn thức ăn chuẩn) chỉ uống nước cất với độ khác biệt $p < 0,05$. Ngoài ra các chỉ số mỡ máu của chuột béo phì được điều trị bằng các phân đoạn dịch chiết khác nhau đều giảm xuống như cholesterol tổng số giảm 13,2% (đối với dịch chiết ethanol), giảm 1,4%, (đối với dịch chiết chloroform), 0,7% (đối với ethylaxetat) và triglycerid giảm 13% (đối với dịch chiết ethanol), giảm 15,2%(đối với dịch chiết chloroform), giảm 16,2% (đối với ethylaxetat). Đặc biệt là ở các chuột béo phì hàm lượng glucose máu giảm xuống ở hai phân đoạn dịch chiết ethylaxetat và chloroform tương ứng là 18,5% và 23%.