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Original Article

Hypolipidemic Effect of Extracts from *Abelmoschus esculentus* L. (Malvaceae) on Tyloxapol-Induced Hyperlipidemia in Mice

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Abstract *Background: Abelmoschus esculentus* L. (Malvaceae) has been used for a long time as a daily food in many countries because of its nourishing components. Extracts from *A. esculentus* are known to ameliorate not only hyperglycemia but also hyperlipidemia in diabetic mice induced by alloxan and streptozocin. However, its hypolipidemic activity has not yet been clearly studied. *Objective:* Hypolipidemic activity of the extracts from total plant by dichloromethan (AE1) and methanol (AE2), and from fruit by dichloromethane (AE3) and methanol (AE4) was studied and compared to that of simvastatin (Zocor®). *Methods:* Hyperlipidemia in mice was induced by single intra-peritoneal injection of 300 mg/kg of tyloxapol. Studied extracts were orally administered at dose equivalent to 30 g of dry extract/kg immediately after tyloxapol injection. *Results:* Cholesterol levels decreased 56.45%, 55.65%, 41.13%, 40.50% and 53.63%, respectively in groups orally administered AE1, AE2, AE3, AE4 and simvastatin as compared to the tyloxapol injected group. Triglyceride levels in treated groups had no significant difference as compared to simvastatin group except the AE4 treated group. *Conclusion: A. esculentus* could lower cholesterol and triglyceride levels in hyperlipidemic mice.

Keywords: *Abelmoschus esculentus,* hyperlipidemia, simvastatin, tyloxapol

INTRODUCTION

Hyperlipidemia has been implicated in atherosclerosis, which is the primary cause of heart disease and stroke. Many hypolipidemic drugs have already been proved to be useful in lowering serum lipid levels in patients. However, its side effects in long-term treatment have been frequently reported and its prices are still expensive. Thus, efforts to develop effective and better hypolipidemic drugs had led to the discovery of natural agents.

Abelmoschus esculentus L. (or *Hibiscus esculentus* or Okra) – Malvaceae is used for a long time as an edible vegetable in many countries, and commonly eaten in Vietnam because of its nourishing components. Traditionally, it is believed that the plant is useful in the treatment of inflammatory disorders, constipation, retention of urine, and etc. On the other hand, a number of previous studies have reported that *Abelmoschus* sp. possessed hypoglycemic effect.[1] However, there is a little study regarding its hypolipidemic effect.

The aim of this present study is to investigate and evaluate the hypolipidemic effect of *A. esculentus* extracts on tyloxapol-induced hyperlipidemia in mice to provide scientific evidence for development of *A. esculentus* as a potential natural oral hypolipidemic agent or functional food.

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MATERIALS AND METHODS

Plant Materials and Chemicals/Reagents

Fresh fruits and total plants of *Abelmoschus esculentus* L. including trunk, root, leaves were collected from a local market in Long An province, Vietnam during June 2007. These plant materials were washed well, cut into small spieces, air-dried and ground to crude powder. It was exhaustedly extracted in a Soxhlet using respective two solvents, dichloromethane and methanol.

Tyloxapol (Triton WR-1339) was purchased from Sigma-Aldrich Chemie GMBH, simvastatin (Zocor®) 20 mg from Merck Sharp & Dohme, cholesterol reagent and triglyceride reagent from Biolabo, France.

Animals

ddY mice weighing 20 ± 2 g of either sex were obtained from Pasteur Institute, HCM city and kept on a standard environmental condition, fed on a pellet diet and tap water *ad libitum*. The mice were deprived of food in the night before experiment.

Hypolipidemic Activity

Animals were randomly divided into seven groups of eight mice each. The mice in group I received saline as control group (0.1 ml/10 g body weight) while those of other groups were injected intra-peritoneally 3% tyloxapol solution with a single dose of 300 mg/kg to induce hyperlipidemia. Treatment was conducted (per os) immediately after tyloxapol injection. The mice in Group II were untreated while in Group III were administered orally 80 mg/kg of simvastatin, once per day. Animals in Group IV and V were administered orally dichloromethane (AE1) and methanol (AE2) extracts from total plant, respectively at doses of 30 g of dried extract/kg, once per day. Animals in Group VI and VII were administered orally dichloromethane (AE3) and methanol (AE4) extracts from fruits, respectively at doses of 30 g of dried extract/kg once per day.

Blood samples were taken from tail vein. Serum was analyzed for total cholesterol and triglyceride levels after 24-hour tyloxapol injection by using the enzymatic-colorimetric method.

Statistical Analysis

All data were expressed as mean ± S.E.M. The data was evaluated by Kruskal-Wallis test and MannWithney test using Minitab 14. Differences between groups were considered significant when $p < 0.05$.

RESULTS

Lipid profiles of serum total cholesterol and triglycerides of experimental mice from various groups are summarized in Table 1. The results show that total cholesterol and triglyceride levels were significantly increased in tyloxapol injected mice as compared to those of saline control mice after 24-hour injection of tyloxapol or saline solution. Cholesterol and triglyceride levels of tyloxapol injected mice were more than two folds higher compared to those of control mice. Treatment with any extracts caused a considerable reduction in the total cholesterol and triglyceride levels, comparable to simvastatin.

Table 1. Total cholesterol and triglyceride levels in experimental groups

Groups	n	Cholesterol (mg/dl) (mean ± S.E.M.)	Triglyceride (mg/dl) (mean ± S.E.M.)
Control	8	107.96 ± 11.35	187.23 ± 30.23
Tyloxapol	8	243.54 ± 67.13	511.34 ± 122.42
Simvastatin	8	115.31 ± 15.17	208.40 ± 73.84
AE1 extract	8	108.84 ± 21.87	198.72 ± 37.39
AE2 extract	8	110.81 ± 11.65	209.36 ± 19.73
AE3 extract	8	146.25 ± 40.00	214.58 ± 70.56
AE4 extract	8	147.88 ± 48.20	297.69 ± 65.96

Hypolipidemic Effect of Extracts from Total Plant

Our data indicated that the two extracts from total plant reduced remarkably cholesterol and triglyceride levels in experimental mice (Figure 1). No difference was found between cholesterol and triglyceride levels of AE1 and AE2 treated groups and those of simvastatin treated group, suggesting the equivalence of hypolipidemic effect of AE1, AE2 and simvastatin. Thus, these three experimental treatments had action in diminution of high-cholesterol and high-triglyceride levels induced by tyloxapol**.**

Hypolipidemic Effect of Extracts from Fruit

Results show that cholesterol and triglyceride levels of treated groups either with simvastatin or with the extracts from fruit, AE3 and AE4, reduced significantly as compared to tyloxapol-induced hyper-lipidemic untreated group (Figure 2). There was no notable difference among cholesterol levels of AE3 and AE4 treated mice and that of simvastatin treated mice. However, hypotriglyceridemic activity of AE4 was less than that of simvastatin.

Comparison of Hypolipidemic Effect of Extracts from Total Plant and Fruit

A notable reduction in cholesterol levels of mice treated with the extracts was observed 24 hours after tyloxapol injection. Cholesterol levels decreased 56.45%, 55.65%, 41.13% and 40.50%, respectively, in groups orally administered AE1, AE2, AE3, and AE4 as compared to the tyloxapol injected group (Table 2). Furthermore, there

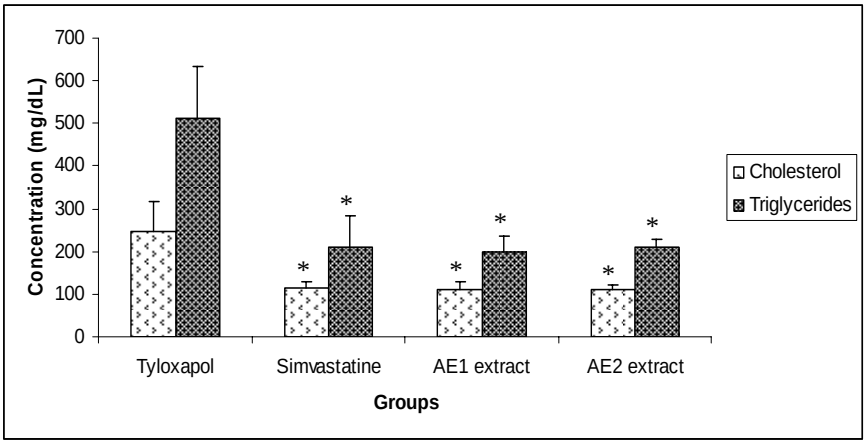

Figure 1. Hypolipidemic effect of total plant extracts. $^{*}p < 0.05$ as compared to tyloxapol injected group.

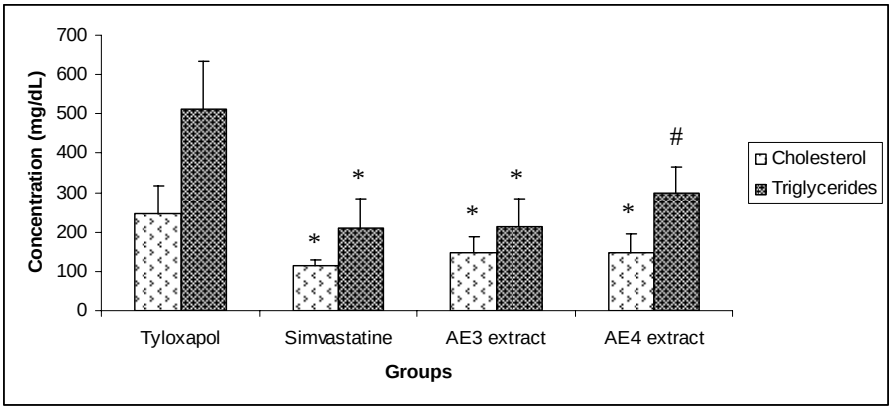

Figure 2. Hypolipidemic effect of fruit extracts. $^{*}p < 0.05$ as compared to tyloxapol injected group. $^{\#}p < 0.05$ as compared to simvastatin treated group.

Table 2. Reduction in cholesterol and triglyceride levels in treated mice as compared to tyloxapol injected mice

Groups	Reduction in cholesterol level (%)	Reduction in triglycerides level (%)
Simvastatin	53.63	59.29
AE1 extract	56.45	61.25
AE2 extract	55.65	59.10
AE3 extract	41.13	58.12
AE4 extract	40.50	41.88

was no significant difference among groups. With regards to triglyceride levels, the statistic results show that AE4 treated group was significantly different in comparison with the other three extract treated groups ($p < 0.05$). Hence, hypotriglyceridemic activity of AE4 was the least among all extracts.

DISCUSSION

This study was performed to investigate the hypolipidemic effect of extracts from *A. esculentus* on tyloxapol-induced hyperlipidemia in mice. Preliminary experiments demonstrated that 24 hours after administration, all four extracts from total plant by dichloromethane (AE1) and by methanol (AE2), also from fruit by dichloromethane (AE3) and by methanol (AE4) remarkably reduced the cholesterol and triglyceride levels in the plasma of hyperlipidemic mice. The hypocholesterolemic effect of all extracts was comparable to the effect of simvastatin. The hypotriglyceridemic effect of AE1, AE2, and AE3 was also comparable to the effect of simvastatin, while that of AE4 was lower than simvastatin.

On the other hand, according to experimental data, cholesterol and triglyceride levels in AE1 and AE2 treated groups were lower than those of other treated groups including simvastatin treated group. This suggests an orientation about the extraction and the purification of principal ingredients possessing good hypocholesterolemic and hypotriglyceridemic activity from total plant of *A. esculentus.*

Until now, there are few studies on the therapeutic effects or chemical components of this plant. The *in vitro* binding of bile acids by *A. esculentus* fruit was mentioned in the recent study.[2] Considering cholestyramine (bile acid-binding, cholesterol-lowering drug) as 100% bound, the relative *in vitro* bile acid binding on dry matter and total dietary fiber basis was 16% and 54%, respectively. Bile acid binding for *A. esculentus* was significantly higher than for all the other vegetables (such as asparagus, green beans, carrots, cauliflower). The results suggests its hypolipidemic effect by decreasing absorption of cholesterol from diet.

In the present study, tyloxapol–a detergent agent was used to produce hyperlipidemia in mice. Its mechanism of action is known to accelerate the hepatic cholesterol synthesis in phase I (after 24-hour tyloxapol injection).[3,4] Hence antihyperlipidemic effect of extracts from total plant and fruit of *A. esculentus* could be due to interfering with cholesterol biosynthesis. More studies are needed to elucidate hypolipidemic activity of these extracts for the purpose of application of *A. esculentus* in hyperlipidemia treatment and prevention.

CONCLUSION

In conclusion, extracts from total plant of *A. esculentus* by dichloromethane (AE1) or methanol (AE2) and extracts from fruit by dichloromethane (AE3) or methanol (AE4) possessed hypolipidemic activity in tyloxapol-induced hyperlipidemia in mice. The extracts may be useful in lowering cholesterol and triglyceride levels in hyperlipidemia. Further studies in other animal species and in other hyperlipidemic models are required to elucidate its hypolipidemic activity.

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