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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 is used for a long time as a daily food in many countries because of its nourishing components. Extracts from *Abelmoschus esculentus* have been 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 studied clearly.
Objective: Hypolipidemic activity of the extracts from total plant by dichloromethan (AE1), methanol (AE2) and from fruit by dichloromethan (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 30g 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. Triglycerids levels in treated groups had no significant difference as compared to simvastatine group except methanolic extract from fruit (AE4) administered group.
Conclusion: *Abelmoschus esculentus* is also useful in diminishing cholesterol and triglycerids levels in hyperlipidemic mice.
Keyword: *Abelmoschus esculentus* L., 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 were more reported and its prices were 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,…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 *Abelmoschus esculentus* L. extracts on tyloxapol-induced hyperlipidemia in mice and provide scientific evidence for development of *Abelmoschus esculentus* L.as a potential natural oral hypolipidemic agent or functional food.

MATERIALS AND METHODS

Materials

Plant materials and chemicals/reagents

Fresh fruits and total plants of *Abelmoschus esculentus* L. included trunk, root, leaves were collected in a local market in Long An province, Vietnam which were collected during the month of 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 respectively 2 solvants: dichloromethan and methanol.

Tyloxapol – Triton WR 1339 were purchased from Sigma-Aldrich Chemie GMBH; Simvastatin ZOCOR 20mg from Merck Sharp & Dohme; Cholesterol reagent from Biolabo, France; Triglycerides reagent from Biolabo, France.

Animals

ddY mice weighing 20 ± 2g of either sex were obtained from Pasteur Institute, HCM city and kept on a standard environmental conditions, 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 (0.1ml/10g B.W.) while the mice 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 belonging to Group II were untreated, Group III were administered orally 80 mg/kg of simvastatine, once per day. Group IV and V were administered orally dichloromethan (AE1) and methanol (AE2) extracts from total plant, respectively in the doses of 30 g of dried extract/kg, once per day. Group VI and VII were administered orally dichloromethan (AE3) and methanol (AE4) extracts from fruits, respectively in the 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 triglycerides levels after 24-hour tyloxapol injection. The total cholesterol and triglycerides were performed by using enzymatic-colorimetric method.

Statistical analysis

All data were expressed as mean ± SEM. 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 triglycerids 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 triglycerides levels in tyloxapol injected mice were more two folds higher than those of control mice. Treatment with one of four extracts caused a considerable reduction in total cholesterol level, similarly to that of reference mice using Simvastatine (Zocor).

Table 1. Total cholesterol and triglyceride levels in experimental groups

Groups	Number	Cholesterol (mg/dL) m± SEM	Triglyceride (mg/dL) M± SEM
Control	8	107.96±11.35	187.23±30.23
Tyloxapol	8	243.54 ± 67.13	511.34 ± 122.42
Simvastatine	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 two extracts from total plant reduced remarkably cholesterol and triglycerides levels in experimental mice. No difference was found between cholesterol and triglycerides levels of AE1 and AE2 extracts treated groups and those of simvastatine treated group. It suggested the equivalence of hypolipidemic effect of AE1, AE2 extracts and simvastatine.

Thus, these 3 experimental treatments had action in diminution of high-cholesterol and high-triglycerides levels induced by tyloxapol.

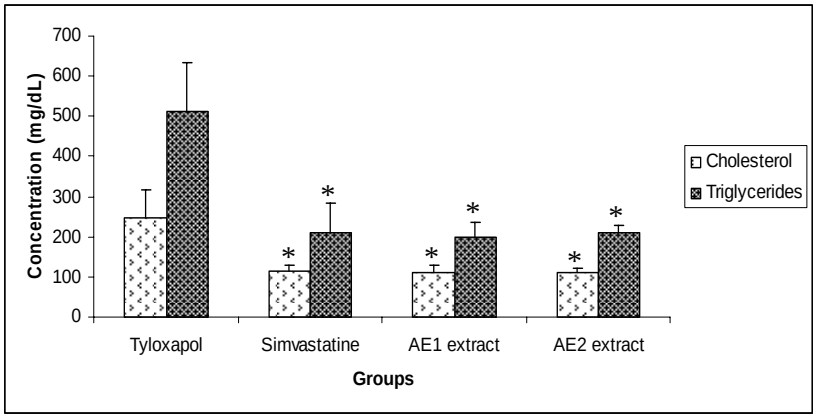

Figure 1. Hypolipidemic effect of total plant extracts. *P<0.05 as compared to tyloxapol injected group.

Hypolipidemic effect of extracts from fruit

Results show that cholesterol and triglycerides levels of treated groups either with simvastatine or with AE3, AE4 extracts reduced significantly as compared to tyloxapol – induced hyperlipidemic untreated group. There was no notable different among cholesterol level of AE3 and AE4 treated mice and that of simvastatine treated mice. However, hypotriglyceridemic activity of AE4 was less than that of simvastatine.

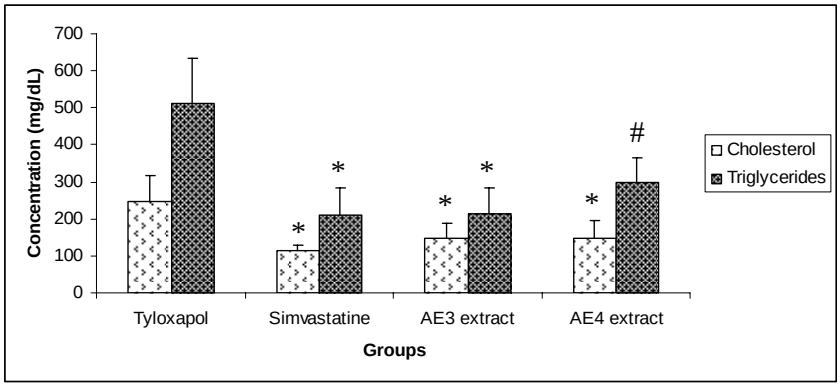

Figure 2. Hypolipidemic effect of fruit extracts. *P<0.05 as compared to tyloxapol injected group. #P<0.05 as compared to simvastatine treated group.

Comparison of hypolipidemic effect of extracts from total plant and fruit

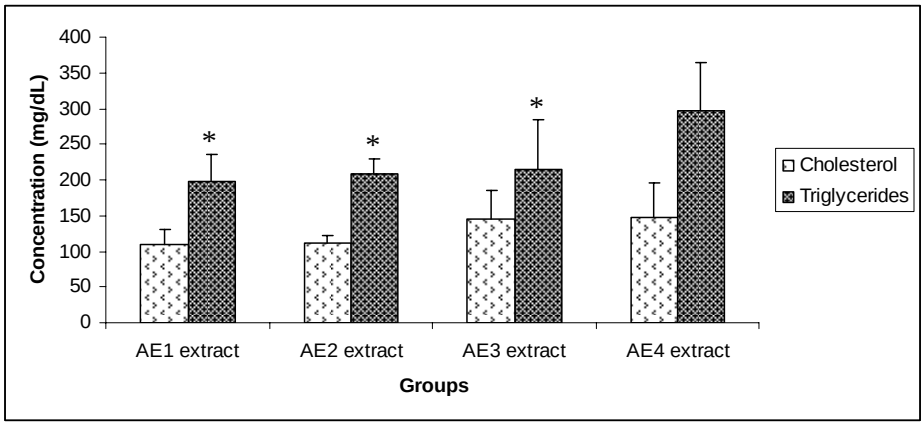

Figure 3. Hypolipidemic effect of studied extracts. *P<0.05 as compared to AE4 extract treated group.

A notable reduction in cholesterol levels of 4 extracts treated groups was observed 24 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 had no significant different among groups. With regards to triglycerides levels, the statistic results showed that AE3 extract treated group was significantly different in

comparison with other 3 extract treated groups ($P<0.05$). Hence, hypotriglyceridemic activity of AE3 extract was least than other extracts.

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

Groups	Reduction in cholesterol level (%)	Reduction in triglycerides level (%)
Simvastatine	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

DISCUSSION

This study was brought out to investigate the hypolipidemic effect of extracts from *Abelmoschus esculentus* L. on tyloxapol – induced hyperlipidemia in mice. Preliminary experiments demonstrated that 24h after administration, all 4 extracts from total plant by dichloromethan (AE1) and by methanol (AE2), also from fruit by dichloromethan (AE3) and by methanol (AE4) remarkably reduced the cholesterol and triglycerides levels in the plasma of hyperlipidemic mice. The hypocholesterolemic effect of 4 extracts was equivalent with this effect of simvastatine. The hypotriglyceridemic effect of AE1, AE2, and AE3 was similar to simvastatine effect but AE4 extract effect reduced triglycerides levels less than simvastatine.

On the other hand, according to experimental data, cholesterol and triglycerides levels in AE1 and AE2 extracts treated groups (extracts from total plant by methanol and dichloramethan, respectively) were lower than those of other treated groups included simvastatine treated group. It suggested an orientation about the extraction and the purification of principal ingredients possessing good hypocholesterolemic and hypotriglyceridemic activity from total plant of *Abelmoschus esculentus* L.

Until now, there are little studies about the therapeutic effects or chemical components of this plant. The in-vitro binding of bile acids by *Abelmoschus esculentus* L. 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 *Abelmoschus esculentus* L. was significantly higher than for all the other vegetables (asparagus, green beans, carrots, cauliflower...). These results suggested its hypolipidemic effect by decrease in absorption of cholesterol from diet.

In 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 *Abelmoschus esculentus* L. 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 *Abelmoschus esculentus* L. in hyperlipidemia treatment and prevention.

CONCLUSION

In conclusion, extracts from total plant of *Abelmoschus esculentus* L. by dichloromethane (AE1), methanol (AE2) and extracts from fruit by dichloromethane (AE3), methanol (AE4) have hypolipidemic activity in tyloxapol – induced hyperlipidemia in mice. Studied extracts may be useful in diminishing cholesterol and triglycerids levels in hyperlipidemia. Further studies in others animal species and in other hyperlipidemic model are required to elucidate its hypolipidemic activity.

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