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COMPARATIVE ANTIHYPERLIPIDAEMIC EFFICACY OF *TRACHYSPERMUM AMMI* EXTRACTS IN ALBINO RABBITS

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ABSTRACT

Comparative antihyperlipidaemic efficacy of *Trachyspermum ammi* (L) Sprague (Ajowain*)* extracts in chloroform, methanol, petroleum ether and water was investigated in albino rabbits. Hyperlipidaemia was induced with butter fed *ad libitum* and oral intubation of cholesterol 400 mg/kg body weight. Simvastatin was used as a synthetic cholesterol lowering drug. The results suggested that chloroform and water extracts of *T. ammi* seed had no hypolipidaemic activity. However, methanol and petroleum ether extracts equivalent to its 2 g/kg body weight powder and Simvastatin (0.6 mg/kg body weight) were equally effective in treating hyperlipidaemia in albino rabbits. Moreover, petroleum ether extract appeared to be more potent than methanol extract on the basis of increasing the level of HDL-cholesterol and lowering the LDL-cholesterol more effectively than methanol extract. Petroleum ether extract reduced atherogenic index (total cholesterol/HDL-cholesterol) more effectively than methanol extract.

Key words: Lipid profile, antihyperlipidaemia, *Trachyspermum ammi*, Simvastatin.

INTRODUCTION

Mortality rate due to cardiovascular diseases has increased several folds in most developed and underdeveloped countries of the world. These cardiac aliments are directly related to hyperlipidaemia (Koh *et al*., 2002). Any abrupt change in blood lipid parameters such as total lipids, triglycerides and cholesterol and their deposition in the arterial wall of circulatory system may lead to atherosclerosis and arteriosclerosis. During the last two decades, both retrospective and prospective studies have shown strong correlation between levels of circulating lipids and mortality rates from coronary atherosclerotic heart disease (Anwar *et al*., 1999).

Several synthetic drugs have been reported having serious side effects (Javed *et al.*, 1994; Anwar *et al*., 1999). Therefore, like other ailments, attention is also being directed to the medicines of herbal origin to find out safer and cheaper drugs for hypolipidaemic activity (Shaila *et al.*, 1997; Wei *et al*., 2003; Suber, 2005; Visavadiya and Narasimhacharya, 2005).

Trachyspermum ammi (L.) Sprague, locally known as Ajowain, is reported to have platelet aggregation inhibitory action (Srivastava, 1988), antifungal potency (Dwivedi and Dubey, 1993) and blood pressure lowering action (Aftab *et al*., 1995*).* Recently, antihyperlipidaemic effect of *T.ammi* seed has been evaluated in albino rabbits. It was assessed that *T. ammi* powder at dose rate of 2 g/kg body weight and its equivalent methanol extract were effective lipid lowering agents (Javed *et al*., 2002). In continuation of this study, the present project was carried out to determine the comparative antihyperlipidaemic efficacy of *T.ammi* seed extracts equivalent to its 2 g/kg body weight powder in chloroform, methanol, petroleum ether and water.

MATERIALS AND METHODS

Forty two albino rabbits of either sex, ranging from 1 to 1.5 kg body weight, were used in the present study. After acclimatization for seven days, these rabbits were randomly divided into seven equal groups. All the animals were housed in individual iron cages. The rabbits were provided alfalfa (lucern) as normal routine feed twice a day, usually in the morning and evening. However, drinking water was available throughout 24 hours. Except one control group kept only on normal routine feed, rest of the groups were also fed with butter *ad libitum* and cholesterol (Cholesterol 90% E. Merck, Darmstadt, Germany) at a dose rate of 400 mg/kg body weight by oral intubation for 90 days to induce hyperlipidaemia.

Seeds of *T. ammi*, obtained from the Department of Botany, University of Agriculture, Faisalabad, were finely powdered with an electric grinder. The powdered seeds were extracted in chloroform (95%), methanol (99%), petroleum ether (99%) and distilled water, using soxhlet apparatus. Each extract thus obtained was evaporated under vacuum and the resultant dry material was stored for administration in rabbits. Tablet survive (Simvastatin, 20 mg, Warrick Pharmaceuticals, Islamabad, Pakistan) was used as cholesterol lowering synthetic drug.

The drugs were intubated orally with ball tipped intubation tube to individual experimental rabbits, in the morning at least one hour before the normal routine feeding. The feeding and drug administration schedule have been presented in Table 1.

Table 1: Feeding and drugs administration schedule in rabbits during the experimental period

Experimental groups	Treatments
Untreated control on normal routine feed	Normal routine feed 0 to 135 days + 5 ml distilled water
Untreated control on butter and cholesterol	Normal routine feed + butter *ad libitum* + cholesterol 400 mg/kg body weight in 5 ml coconut oil 0 to 90 days, normal routine feed 91 to 135 days
Treated control on Tablet Survive	Normal routine feed + butter *ad libitum* + cholesterol 400 mg/kg body weight in 5 ml coconut oil 0 to 90 days, normal routine feed + Tablets Survive 91 to 135 days
Chloroform extract of *Trachyspermum ammi*	Normal routine feed + butter *ad libitum* + cholesterol 400 mg/kg body weight in 5 ml coconut oil 0 to 90 days, normal routine feed + chloroform extract equivalent to 2g/kg seed powder 91 to 135 days
Methanol extract of *Trachyspermum ammi*	Normal routine feed + butter *ad libitum* + cholesterol 400 mg/kg body weight in 5 ml coconut oil 0 to 90 days, normal routine feed + methanol extract equivalent to 2g/kg seed powder 91 to 135 days
Petroleum ether extract of *Trachyspermum ammi*	Normal routine feed + butter *ad libitum* + cholesterol 400 mg/kg body weight in 5 ml coconut oil 0 to 90 days, normal routine feed + petroleum ether extract equivalent to 2g/kg seed powder 91 to 135 days
Water extract of *Trachyspermum ammi*	Normal routine feed + butter *ad libitum* + cholesterol 400 mg/kg body weight in 5 ml coconut oil 0 to 90 days, normal routine feed + water extract equivalent to 2g/kg seed powder 91 to 135 days

From 0-135 days, in individual animals of each group, jugular blood samples were taken at. 0, 30, 60, 90, 105, 120 and 135 days. The serum was separated and stored at -4ºC till analysis. Serum lipid profile parameters of individual animals were determined with reagent kits (Randox, Randox laboratories, UK). Lipid profile parameters included total lipids, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol) and low-density lipoprotein cholesterol (LDL-cholesterol). Student's t-test was used to compare mean values of individual groups with the control group.

RESULTS AND DISCUSSION

Hyperlipidaemia produced as a result of 90 days feeding butter *ad libtum* and cholesterol 400 mg/kg body weight along with the normal routine feed is given in Fig. 1. It shows that there were about 2.5 to 7 times increase in lipid profile parameters at day 90 than their respective values at day 0, except HDL-cholesterol which showed 1 to 1.5 fold decrease. However, 3.5 to 9 times increase in these parameters have been reported in albino rabbits fed with atherogenic diet and cholesterol (400 mg/kg) for 120 days (Purohit and Daradka, 2001).

Antihyperlipidaemic efficacy of the chloroform, methanol, petroleum ether and water extracts of *T. ammi* seed equivalent to 2 g/kg body weight of its powder against lipid profile parameters has been shown in Tables 2-6. It can be seen that in case of chloroform and water extracts, lipid profile parameter values seen at post cholesterol feeding day 90 did not significantly change ($P>0.05$) at post treatment days 105, 120 and 135. However, the reductions in lipid profile parameters (mg/dl) induced by methanol and petroleum ether extracts were significant ($P<0.05$). Thus, methanol and petroleum ether extracts of *T. ammi* seed significantly ($P<0.05$) lowered the lipid profile parameters including total lipids, triglycerides, total cholesterol and LDL-cholesterol. Moreover, methanol extract and Simvastatin produced similar effects in terms of percentage reduction of lipid profile parameters at post treatment day 135. Further, the methanol extract more effectively lowered total cholesterol (76%), followed by triglycerides (70%), LDL-cholesterol (65%) and total lipids (51%) on post treatment day 135. However, *T. ammi* extract in methanol did not show any increase in the value of HDL-cholesterol after its medication in hyperlipidaemic albino rabbits. Similar observations have been reported by Jahromi *et al*. (1993) after oral administration of extract of *Pterocarpus marsupium* (120 mg/kg) for 14 days in triton induced hyperlipidaemic rats. These studies showed that ethanolic extract of *P. marsupium* reduced the levels of serum triglycerides, total cholesterol and LDL-cholesterol without any significant effect on the level of HDL-cholesterol. Similar effects in lowering lipid profile parameters were observed in hyperlipidaemic rabbits after administration of alcoholic extract of *Lyceum europeium* (Kalhoro *et al*., 1996). However, earlier studies showed that ethanol extract of *Brassica oleracea* administrated in hyperlipidaemic rats increased HDL-cholesterol level (Jahodar *et al*., 1995

Petroleum ether extract of *T. ammi* seed administrated to hyperlipidaemic albino rabbits

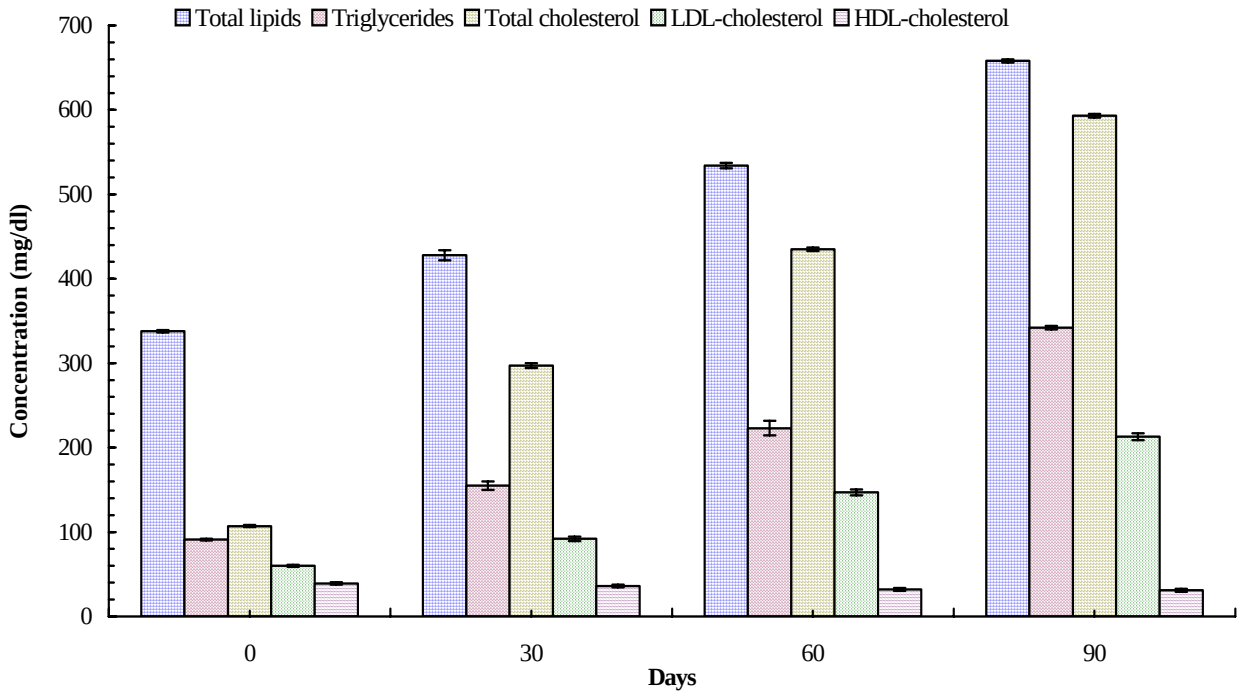

Fig. 1: Mean (± SE) concentrations of serum lipid profile parameters in rabbits fed with butter *ad libitum* and cholesterol 400 mg/kg body weight.

significantly ($P<0.05$) decreased serum levels of total lipids, triglycerides, total cholesterol and LDL-cholesterol. At the same time, it significantly increased serum HDL-cholesterol level. On post treatment day 135, the percentage reduction of the lipid profile induced by petroleum ether extract and Simvastatin was similar ($P>0.05$). It can also be seen that amongst the lipid profile parameters, petroleum ether reduced more effectively the level of cholesterol (76%), followed by triglycerides and LDL-cholesterol (70% each) and total lipid (52%). These effects are almost similar to those produced by the methanol extract. Moreover, it is evident that the petroleum ether increased the level of HDL-Cholesterol (42%), while this effect was non-significant ($P>0.05$) in case of methanol extract. However, petroleum ether extract of *Lyceum europium* when administered in hyperlipidaemic rabbits did not produce any significant change in plasma lipid profile parameters (Kalhoro *et al*., 1996). On the other hand, administration of petroleum ether extract of *Allium sativum* and *Allium cepa* in albino rats significantly prevented rise in serum cholesterol and serum triglyceride levels, caused by atherogenic diet (Lata *et al*., 1991).

Methanol and petroleum ether extracts and Simvastatin induced reductions in lipid profile parameters as 51, 52 and 60% in total lipids, 70, 70 and 73% in triglycerides, 76, 78 and 83% in total cholesterol and 65, 70 and 74% in LDL-cholesterol (Fig. 2). HDL-cholesterol did not increase after the methanol extract administration. However, petroleum ether extract and Simvastatin increased HDL-cholesterol levels by 42 and 57%, respectively.

These results suggest that methanol and petroleum ether extracts of *T. ammi* seed equivalent to its 2 g/kg body weight powder and Simvastatin 0.6 mg/kg body weight were equally effective in treating hyperlipidaemia in albino rabbits. Petroleum ether appears to be more potent on the basis of increasing the level of HDL-cholesterol and decreasing the LDL-cholesterol more effectively (70%) than methanol extract (65%). There is an inverse relationship between plasma HDL-cholesterol level and coronary heart disease (Jahromi *et al*., 1993). Moreover, apo-B-containing lipoprotein fractions are thought to be responsible for cholesterol deposition in atherosclerotic plaques (Choi *et al*., 1991). So, an increase in HDL-cholesterol and a reduction in LDL-cholesterol would be advantageous clinically. The potency of petroleum ether lies also in its ability to reduce atherogenic index (total cholesterol/HDL-cholesterol) more effectively (23.2 to 2.5) than that reduced by methanol extract (22.9 to 4.6) from post cholesterol feeding day 90 to after treatment day 135. However, during this period, Simvastatin reduced this ratio from 23.6 to 2.5. Further comprehensive chemical and pharmacological investigations are suggested to elucidate the exact mechanism of these effects and to isolate the active principles responsible for these effects.

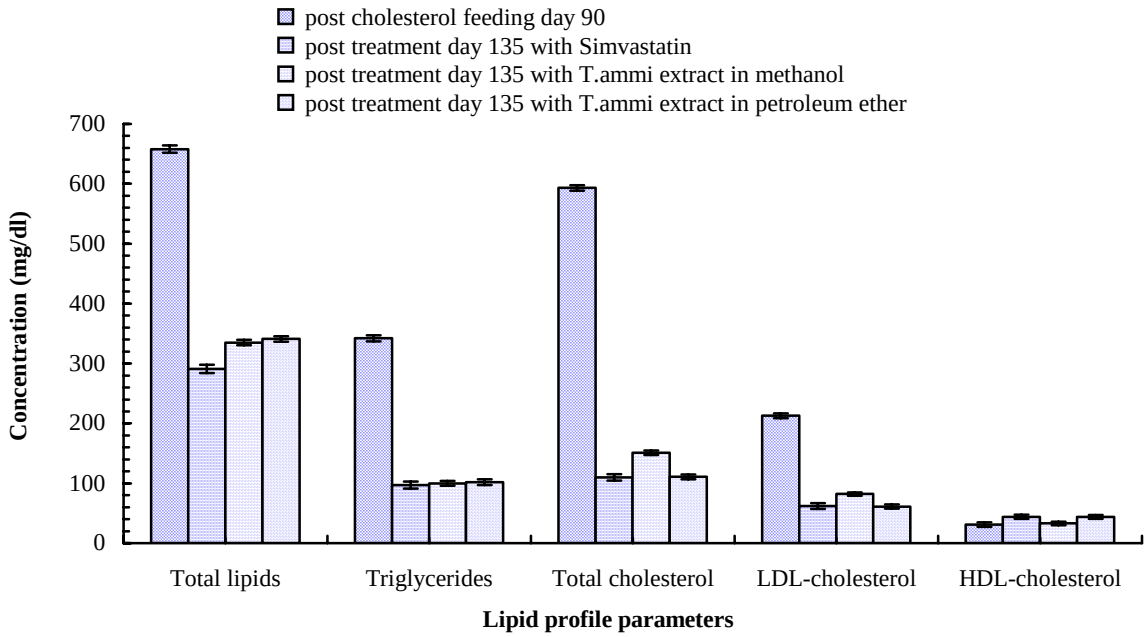

Fig. 2: Comparative antihyperlipidaemic efficacy of *T. ammi* extracts in methanol and petroleum ether equivalent to 2 g/kg body weight of its powder, and Simvastatin 0.6 mg/kg body weight in albino rabbits.

Table 2: Mean (± SE) values of total lipids (mg/dl) and their percentage reductions in the serum of hyperlipidaemic rabbits (n=6) after treatment with *ammi* extracts in chloroform, methanol, petroleum ether and water and Simvastatin

Medication	Dosage (oral)	Post cholesterol feeding day 90	Post treatment days			Percentage reduction on post treatment days		
			105	120	135	105	120	135
Untreated control on normal routine feed	-	338 ± 2.01	342 ± 1.43	342 ± 1.34	334 ± 1.25	-	-	-
Untreated control on butter and cholesterol	400 mg/kg	704 ± 1.93	652 ± 1.47	633 ± 1.38	615 ± 1.24	-	-	-
Treated control on Simvastatin	0.6 mg/kg	713 ± 1.99	302 ± 1.33*	292 ± 1.23*	288 ± 1.00*	58 ± 4.61	59 ± 5.36	60 ± 5.97
Trachyspermum ammi extract in chloroform	Equivalent to 2.0 g/kg of powder	698 ±1.39	621 ± 1.21	551 ± 1.16	502 ± 1.10	11 ± 3.01	21 ± 4.11	28 ± 4.91
T. ammi extract in methanol	Equivalent to 2.0 g/kg of powder	732 ± 1.96	534 ± 1.67*	401 ± 1.34*	360 ± 1.07*	27 ± 5.14	45 ± 6.09	51 ± 7.12^{NS}
T. ammi extract in petroleum ether	Equivalent to 2.0 g/kg of powder	703 ± 1.48	503 ± 1.26*	393 ± 1.14*	335 ± 1.01*	28 ± 2.58	44 ± 3.41	52 ± 4.67^{NS}
T. ammi extract in water	Equivalent to 2.0 g/kg of powder	721 ± 2.01	679 ± 1.74	630 ± 1.51	591 ± 1.30	6 ± 3.64	13 ± 4.70	18 ± 5.31

n = Number of animals in each group,

* = significantly less (P≤0.05) than the pretreatment value at 90 days,

NS = Non-significantly (P>0.05) different from respective value obtained with Tablet Survive (Simvastatin).

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