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Research article

ANTIHYPERLIPIDIMIC EFFECT OF *SIMMONDSIA CHINENSIS* SEEDS EXTRACTS IN RABBITS

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ABSTRACT

Objective: The present study was designed to examine the effect of 70% ethanol extract of *Simmondsia chinensis* seeds on lipid profile in rabbits. **Material and methods**: The plant extract was orally administered to the atherogenic rabbits (atherogenic diet + cholesterol powder supplement at 400 mg/kg/body weight/ day dissolved in 5 mL coconut oil) at dose of 500mg/kg body weight/day. During the hall period of the experiment blood samples were collected and serum was analyzed for lipid profile. At the end of the experiment the animals were sacrificed; the heart and the liver were collected and stored at -20°C until assayed. Biochemical analysis of blood serum and tissue (liver and heart muscle) were performed for cholesterol, phospholipids and triglycerides. In addition blood serum was analyzed further for HDL-cholesterol. **Results**: All the results were by administration of *Simmondsia chinensis* seeds. Serum cholesterol levels dropped from 957.57 to 223.3 and further to 143.4 by the end of the experiment. Similarly, phospholipids statistically analyzed using student's t-test. Hypolipidaemic nature of *Simmondsia chinensis* extract was studied in hyperlipidaemic rabbits. The increased cholesterol levels were brought to normal and triglycerides levels were reduced. The tissues lipids profiles of liver and heart muscle showed similar changes in those noticed in serum lipids. Conclusion: We can conclude from these results that a *Simmondsia chinensis* seed possesses active hypolipidaemic constituents. The results suggest the validity of *Simmondsia chinensis* seeds clinical use in hypolipidaemic control, after their toxicological investigation.

Key words: *Simmondsia chinensis*, hypolipidemia, triglyceride, cholesterol

INTRODUCTION

Hyperlidaemia is the current medical as well social problem, specially associated with diabetes mellitus leading to increasing morbidity and mortality. The major risk factors of hyperlipidemia are associated with atherosclerosis which predisposes ischemic heart disease and cerebrovascular disease (Brown and Goldstein, 1990). In type 2 diabetic patients there is mild to moderate hypertriglyceridemia, low level of high density lipoprotein (HDL) and over production of very low density lipoprotein (VLDL) (Ginsberg, 1991). Serum total cholesterol is also increased (Florey et al, 1973). In the present century modern medicine draws its nourishment from the rich legacy of traditional medicine. Simmondsin, a 2-cyanomethylene)-3 hydroxy 4,5 dimethoxy cyclohexyl b-D-glucoside, is a dietary supplement that is extracted from the seed of jojoba plant (Simmondsia chinensis). Jojoba is native to the Sonora desert of the American South West and Mexico. Oil is extracted from the seed for commercial use in cosmetics and shampoos. The remaining meal is approximately 30% protein and has a markedly suppressive effect on food intake in a variety of species. Several early studies suggested that simmondsin is toxic, as rats fed jojoba meal at 5–10% of rations for 94 days decreased food intake to such an extent that the rats became emaciated and died (Booth et al, 1974). More recent studies suggested that *simmondsin* extracted from jojoba could inhibit food intake without direct toxicity. In 1980, Verbiscar et al. reported that five mice died when fed *simmondsin* at 750 mg/kg for 14 days and three surviving mice showed signs of hepatotoxicity and possible intestinal hemorrhage. Intraperitoneal administration of the same dose, however, did not decrease body weight of rats nor were there any other drug-induced effects (Verbiscar et al, 1980). Subsequent pair-feeding studies, using more moderate levels of simmondsin, suggested that its effects are primarily because of decreased voluntary food consumption. Rats fed 250 mg/kg for 5 days showed no toxicological influences on biochemical parameters of the liver, pancreas and kidneys and no pathological changes were found in kidney, liver, pancreas, stomach, intestine, testis and seminal vesicle (Cokelaere et al, 1992).

Teratological studies found lower birth weights of jojobatreated rat dams compared with a pairfed group, to be because of lower body weight gain during gestation; offspring showed no other abnormalities and subsequently reproduced normally (Cokelaere et al, 2001). Simmondsin may reduce food intake by increasing satiety (Cokelaere et al, 1992, Cokelaere et al, 2000, Flo et al, 2000) via an indirect interaction with the peripheral CCKA system. Cokelaere et al. (Cokelaere et al, 1995) found that simmondsin's effect on satiety was abolished by administration of a CCKA receptor-antagonist (devazepide), which had previously been shown to inhibit the anorexic effect of exogenous CCK in both rats and mice (Lotti et al, 2000, Silver et al, 1989). Vagotomy, in rats, also reduced the effects of both exogenously administered simmondsin and CCK, (Cokelaere et al, 1995) suggesting that simmondsin's augmentation of satiety, like that of CCK, is mediated, in part, by the vagus nerve. A study by York et al., (York et al, 2000) however, renewed concerns about simmondsin's toxicity. In this study, simmondsin had negative effects on hematopoiesis in rats even at doses that did not affect food intake or body weight. The authors concluded that simmondsin is 'toxic with profound effects on the hematopoietic system.' In this study, the aim is to determine the effect of oral administration of *Simmondsia chinensis* plant extract on the lipid profile fed albino Rabbits.

MATERIALS AND METHODS

Animals: Adult healthy albino rabbits weighing 1.6-1.7 kg were housed individually in metallic cages in an air-conditioned room (26 ± 2 °C) and were fed control diet (standard pellets). This diet was supplemented with green leafy vegetables and water *add labitum.* The average consumption of diet was calculated 200 g day. Atherogenic diet was prepared by mixing wheat flour, milk powder, dried egg yolk.Hydrogenated fat, butter, salt jaggery and vitamins as shown in Table 1.

Table 1: Atherogenic diet

Component	Control (g %)	Atherogenic diet (g %)
Protein	20	15
Carbohydrate 65 60	65	60
Sucrose	3	3
Fat	5	15
Salts	4	4
Vitamin	1	1
Fiber	2	2

In addition to the atherogenic diet, the rabbits were fed with cholesterol (400 mg/kg body weight/day) dissolved in 5 ml coconut oil.

Plant and treatment

Seeds of *Simmondsia chinensis* were collected from Jordan University of science and technology farms during September, 2011. The seeds were dried and grinded into powder. Powder was extracted by water–ethanol mixture (70|30 V\V) for 6 hrs. This step was repeated three times then the filtrate was pooled and concentrated under vacuum keeping a temperature less than 50oC. The concentrate was dissolved in a normal saline and used. The extract, 400 mg/kg, dissolved in 1ml normal saline was administered orally to rats using animal feeding intubation's needles (Popper and Sons, New York).

Determination of LD50 in mice

Determination of LD50 in mice was conducted to determine the dose to be given to rabbits. Graded doses of the aqueous extract of *Simmondsia chinensis* in 0.2 distilled water were administered intraperitonealy to six groups of six non fasted male albino mice (25-30 g each). They were housed in transparent plastic cages at 24 °C. Mortality was noted after 1 h (Litchfield and Wilcoxon, 1949).

Experimental design: Rabbits were divided in the following groups of eight animals each:

Group A: Vehicle (5 mL normal saline) treated control (120 days)
Group B: Atherodiet + cholesterol feeding (120 days, 400 mg cholesterol/kg body 3 weight/day in 5 mL coconut oil)

Group C: Atherodiet + cholesterol feeding (120 days, atherodiet withdrawn + *Simmondsia chinensis* (70% EtOH) 1.2 g kg-1 body weight/day (120-150 days)

Group D: Atherodiet + cholesterol feeding (120 days, Atherodiet withdrawn + *Simmondsia chinensis* (70% EtOH) extract 1.2 g kg-1 body weight/day (120-180 days)

At the end of the experiment all the animals were sacrificed and the heart, the aorta and the liver were removed, cleaned from the fat and adhering connective tissue and stored at -20 °C until assayed. Biochemical analysis of blood serum and tissue (liver and heart muscle) were made for cholesterol [Zlatkis et al, 1953], phospholipids [Zilversmit and Davis, 1950] and triglyceride (Gottfried and Rosenberg, 1973). In addition blood serum was analyzed further for HDL-cholesterol (Burnstein et al, 1970).

Statistical analysis: Data were expressed as Mean ± SD [statistical package for social sciences (SPSS, version 11.5)]. Differences between control and *Euphorbia prostrata* exposed groups were analyzed using either the Chi-square test, t-test or nonparametric (Sheskin, 2004), when applicable. A p-value of <0.05 was considered significant (Ipsen and Feigl, 1970).

RESULTS

A non-significant reduction in the body weights was noticed in rabbits fed with cholesterol diet and later treated with *Simmondsia chinensis* extract (Groups C and D) in comparison with the initial body weights. A non-significant change in hear weight of cholesterol fed rabbits. Liver weight was significantly increased in cholesterol fed rabbits (Table 2). *Simmondsia chinensis* (70% EtOH) extract feeding (Groups C and D) resulted in a significant lowering of total cholesterol, triglycerides and phospholipids of liver and ventricular heart muscles in comparison with cholesterol fed rabbits. In group D the reduction was on higher side (Table 3). A nine-fold increase was observed in serum cholesterol in treated rabbits fed with atherogenic diet ($p \leq 0.001$). In addition a significant reduction in the blood serum cholesterol was recorded in both *Simmondsia chinensis* treatment group (C and D). Serum triglyceride increased significantly ($p \leq 0.001$) after cholesterol feeding but was subsequently reduced after *Simmondsia chinensis* extract treatment. An increased in phospholipids and HDL cholesterol followed by cholesterol diet could be corrected by *Simmondsia chinensis* extract feeding (Table 4). The LD50 of the aqueous extract of *Simmondsia chinensis* was 4.14 g kg-1 body weight [Litchfield and Wilcoxon, 1949] method which represents 20.54 g of 15 crude powdered plant material 1 kg body weights.

Table 2: Change in body, liver and heart weight after cholesterol/ *Simmondsia chinensis* (70% EtOH) extract feeding in rabbits (8 animals per treatment)

Treatment	Body weight (kg)		% Body weight	
	Initial	Final	Liver	Heart
Group A	1.71±0.36	1.167±0.58	2.13± 0.45	0.21± 0.67
Group B	1.61±0.16	1.57±0.78	4.12± 0.15	0.24± 0.62
Group C	1.68±0.42	1.64±0.28	2.16± 0.58	0.22± 0.65
Group D	1.63±0.23	1.59±0.74	2.09± 0.81	0.19± 0.53

[a]$p \leq 0.05$ [b]$p \leq 0.01$, [c]$p \leq 0.001$ [d]NS=Non-significant

Table 3: Change in tissue lipids after cholesterol/ *Simmondsia chinensis* (70% EtOH) Extract feeding in rabbits (8 animals per treatment)

Treatment	Cholesterol(mg/g)		Triglycerides(mg/g)		Phospholipids (mg/g)	
	Liver	Heart	Liver	Heart	Heart	muscles
Group A	9.34± 0.06	6.71± 0.32	3.76± 0.20	4.52±0.22	7.68±0.14	9.09 ±0.81
Group B	17.44± 0.42 [c]	8.56± 0. 6 [c]	5.61±0.27 c	11.74±0.67 [c]	12.9±0.78 [c]	10.63±0.66 [c]
Group C	11.86± 0.18 [c]	7.14±0.3 [c]	5.28±0.52 [b]	6.28±0.25 [d]	9.55±0.61 [c]	10.18±0.33 [c]
Group D	10.20±0.17 [c]	6.93± 0.6 [c]	4.11±0.17 [c]	4.81±0.12 [c]	8.22±0.18 [c]	9.22±0.11 [c]

[a]$p \leq 0.05$ [b]$p \leq 0.01$ [c]$p \leq 0.001$ [d]NS=Non-significant

Table 4: Change in Serum analysis after cholesterol/ *Simmondsia chinensis* (70% EtOH) extract feeding in rabbits (8 animals per treatment)

Treatment	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)	HDL cholesterol (mg/dl)
Group A	108.3±3.7	71.7± 1.84	187.22± 4.8	29.7± 1.5
Group B	957.57±3.5 [c]	314.6± 4.12 [c]	296.52± 8.11 [c]	270.65± 7.35[c]
Group C	223.3±5.63 [c]	106.8± 5.33 [c]	218.71± 4.23 [b]	69.43± 5.58 [c]
Group D	143.4± 6.54 [c]	88.90± 8.22 [c]	207.63± 6.34 [c]	52.38± 2.23 [c]

[a]p≤0.05 [b]p≤ 0.01, [c]p≤ 0.001 [d]NS=Non-significant

DISCUSSION

The present study was designed to investigate the hypolipidemic effects of *Simmondsia chinensis* (70% EtOH) extract on lipid profile on rabbits. Results of this study demonstrated that hypolipidaemic nature of *Simmondsia chinensis*. The increased cholesterol levels were brought to normal by adding of *Simmondsia chinensis* Serum cholesterol levels dropped significantly by the end of the experiment. Similarly, phospholipids and triglycerides levels were observed to be also reduced. The tissues lipids profiles of liver and heart muscle showed similar changes in those noticed in serum lipids. A positive correlation between cholesterol plasma concentration and the risk of coronary heart disease has been widely demonstrated by the lipid research Clinics Primary Prevention Trails (Choi et al, 1991). In order to find good means to decrease plasma cholesterol level with minimal toxicity.

The level of cholesterol in lipoprotein fractions has been shown to be a good indicator of atherosclerosis risk in rabbits (Azzarito et al 1996). Significant lowering of cholesterol after *Simmondsia chinensis* feeding indicates a risk reduction action. Plasma triglycerides and cholesterol carry the highest risk for ischemic heart disease (McBride, 2008). HDL and LDL cholesterol are significant variables and indicator for 11 coronary heart disease (Miller and Miller, 1975). It is reported that HDL is inversely related to total body cholesterol. Treatment with *Simmondsia chinensis* extract reduces serum cholesterol and triglyceride by 8 and 3.5 times, respectively. HDL alters the balance of unesterified cholesterol between plasma and cell by increasing its utilization in the lecithin cholesterol acyl transferase (LCAT) system to form cholesterol ester which moves rapidly into the cells. Decreased total cholesterol and phospholipid after *Simmondsia chinensis* extract feeding indicate the anti-atherogenic or hypolipidaemic nature of the plant product. Further reduction in total cholesterol, triglyceride and phospholipids of liver and ventricular heart muscle may be suggestive of a beneficial role of *Simmondsia chinensis* L. in hyperlipidaemic subject. The possible mechanism of lipid alteration might be cholestatic effect of *Simmondsia chinensis* in liver enhanced removal or catabolism of lipoproteins [Brattsand, 1975] and/or inhibition of lysosomal lipid hydrolytic enzymes secreted by the liver [Sherlock, 1998]. In conclusion *Simmondsia chinensis* possesses active hypolipidaemic constituents. Further chemical and pharmacological investigations are in progress.

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