Action of cholecystectomy and the alcoholic extract of *Taraxacum officinale* leaves on plasma lipid profile in rabbits

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Received 31/8/2005, Accepted 7/3/2006

Abstract

Two experiments were carried out in this study. The first experiment was conducted to investigate the effect of cholecystectomy on lipid profile [total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), very-low density lipoprotein (VLDL), and high-density lipoprotein (HDL)]. The second experiment was aimed to investigate the effect of *Taraxacum officinale* leaves on plasma lipid profile of cholecysectomic rabbits. Accordingly, in Experiment one, 12 rabbits were used which allocated randomly into two groups, group one subjected to removal of gall bladder and group two was normal rabbits served as control group. In experiment two, (12) chlecysectomized rabbits were also divided randomly into two groups, first group received (250 mg/kg) B.W. of alcoholic extract of *T. Officinale* leaves for three weeks and second group received 2ml of 0.9% NaCl. Plasma concentrations of TC, TG, HDL, LDL and VLDL in all groups in both experiments were measured every two weeks in experiment one for one month and weekly for experiment two.

The results showed that the removal of gall bladder (cholecystectomy) caused marked increase ($P<0.01$) in plasma TC, TG, VLDL, and LDL levels with significant reduction ($P<0.01$) in HDL level compared with control normal rabbits. While the administration of *T. officinale* to cholecystectomized rabbits caused highly significant ($P<0.01$) decreased in TC, TG, VLDL, & LDL concentrations and marked increase ($P<0.01$) in HDL concentration. It can be concluded that, cholecystectomy leads to hyperlipidemia and *T. officinale* can relieve it.

Key words: *Taraxacum officinale*, Dandelion, Cholecystectomy, Plasma lipid, Lipoprotein.

Introduction

The surgical removal of gall bladder (cholecystectomy) in human being is increased recently. However, the cholecystectomized patients maintain a good health and nutrition with constant slow discharge of bile into the duodenum **[1].** Although eventually the bile duct becomes somewhat dilated, and more bile tends to enter the duodenum after eating than normal person **[1]**. It has been known for several decades that elevated blood cholesterol and triglycerides are associated with an increase risk of cardiovascular diseases **[2]**.Many medical herbs have been used in treatment of hyperlipidemia and atherosclerosis such as *Cassia auriculata* **[3]**, *Nigella sativa* **[4],** *Pueraiae radix* **[5]** and *Zingiberis officinale* **[6].**

Cholesterol is an essential component in animal cell membranes and a precursor in biosynthesis of all steroid hormones, vitamin D and bile salt **[7]**. Lipoprotein is a complex of protein and lipid with a characteristic density, size and chemical composition. Generally, lipoproteins transport lipids in blood between the sites of their absorption, liver, and various tissues that utilize lipids. The major lipoprotein classes in the body are chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL) and these are important in clinical diagnosis **[1]**.

One of many important functions of the liver is to secret bile; the hepatic bile enters the gall bladder for storage until need in duodenum **[8].** Bile plays an important role in fat digestion and absorption, moreover, bile serves as a means for excretion of waste products from blood (like bilirubin, end product of hemoglobin destruction, and excesses of cholesterol synthesized by the liver cells **[9]**. Accordingly, this study was aimed to investigate; firstly the effect of cholecytectomy on plasma lipid profile (TC, TG, HDL,VLDL& LDL) and secondly effect of alcohol extract of *T. officinale* on the parameters of plasma lipid in cholecystectomic rabbits.

Materials & Methods

Plant Materials: Leaves of *Taraxacum officinal*e were purchased from local markets in Basrah Province / Iraq. The plant was identified at Collage of Science / University of Basrah.

Preparation of Alcohol Extract of *Taraxacum officinale:* After cleaning, the leaves were dried in the shadow at room temperature and then grounded into powder form by using electrical mill for 3 minutes. Twenty-five grams of ground leaves powder were refluxed with (250 ml) (70% EtOH-water) (Iraq medical alcohol 96%) for (12 hr.), then cooled and filtered. The solvent was dried and concentrated by using Rotary evaporator (PUCHI Rotavpor-RE, Switzerland) at (50ºC). The dryness of the extract was completed by using oven at 50ºC) yielding black green powder (1gm) **[6]**.

Animals housing: Twenty four male rabbits (1-1.5 kg) weight and (6-7) months) of age were brought from local market /Basrah. The rabbits (6 rabbits/cage) housed in wire silk cages measuring (1x0.5x0.5 m) under controlled conditions of temperature (25 ±3ºC) and relative humidity (50±5%). Upon arrival, animals were acclimated for four weeks and were maintained on a regular feed consist from alfalfa and concentrate pullet (crude protein 15%, ground Soya bean 6%,wheat flour 50%, wheat barn 25%,saturated fat 2%,milk powder 2%and minerals &vitamins 1g/kg.). Water and food were given *ad labitum*. The animals were fasted 12hr. before collection of blood samples.

Experimental Design: two experiments have been done.

Experiment one: Effect of cholecystectomy on plasma lipid profile. Twelve rabbits were divided equally and randomly into two groups: **Group I:** the animals in this group were subjected to cholecystectomy. **Group II:** In this group, normal animals were placed as control group.

Experiment two: Effect of alcohol extract of *T. officinale* on plasma lipid profile in cholecystectomic rabbats: Twelve rabbits were subjected to cholecystectomy operation. These animals were allocated randomly into two groups: ***Group I:*** The cholecystectomized animals of this group were administrated orally and daily the alcoholic extract of the leaves of *T. officinale* (250 mg/kg B.W.) for 3 weeks. The dose was depending on that used by Alwan **[10]**. ***Group II:*** In this group, the animals were gall bladder removed surgically and received 2ml of normal saline and served as cholecystectomy control group.

Cholecystectomy: Preparations of animals to operation include clapping and shaving of the abdominal region, then the site of operation were cleaned and disinfected.

The animals were anaesthetized by intramuscular injection of Ketamine hydrochloride (10mg/kg B.W.) and Zylazine (5mg/kg B.W.) **[11].** The surgical operation of cholecystectomy was done according to that described by Archibald **[12]**.

Blood Sampling and plasma preparation: For both experiments, fasting blood samples were collected from ear margin vein by using syringe (2ml) and transferred into EDTA tubes immediately. Blood was then centrifuged at (4,000 rpm) for (10 minutes) to remove red blood cells and recover plasma, this plasma sample was used in biochemical measurements **[13]**.

Total Cholesterol Determination: Plasma total cholesterol (TC) was enzymatically measured by using a linear chemical kit (BIOLABO S.A., France) **[14, 15]**

***Plasma Triglyceride Determination*:**Plasma triglyceride (TG.) concentration was measured by using special chemical kit (SYRBIO/GPO-PAP, Syria) **[9]**.

***Plasma High- density lipoprotein (HDL)*:** Plasma HDL concentration was measured enzymatically as described by Alwan **[9]**
***Plasma Very low- density lipoprotein (VLDL)*:** Plasma VLDL concentration was calculated by divided plasma TG by five **[16].**
Plasma Low- density lipoprotein (LDL): Plasma LDL concentration was calculated by applying the equation: LDL = TC – (HDL +TG/5 **[17].**
Statistical analysis: The results were analyzed by one-way ANOVA and independent t-test by using SPSS version 9. The least significant difference (LSD} test was used to determine the differences between group.

Results

Experiment One: Effect of cholecystectomy on plasma lipid profile:

The plasma lipid profile after 2 and 4 weeks of the removal of gall bladder (cholecystectomy) is illustrated in Table (1). There were a marked increases ($P<0.01$) in plasma TC, TG, VLDL and LDL levels in cholecystectomic rabbits as compared with normal control group, after 4 weeks post operation. On the contrary, the mean value of plasma HDL-C concentration decreased significantly ($P<0.01$) from (21.6 ± 2.3) in the control group to (17.4±1.52) in cholecystectomic group after 4 weeks post operation.

Table (1).Effect of cholecystectomy on plasma lipid profile in rabbits

Groups	TC (mg/dl)		TG (mg/dl		HDL (mg/dl)		VLDL (mg/dl)		LDL (mg/dl)	
	2wk	4wk	2wk	4wk	2wk	4wk	2wk	4wk	2wk	4wk
Cholecystectomic rabbits	A 86.7* ± 5.55	B 118.8* ± 17.0	A 170.0* ± 14.4	B 188.6* ± 6.35	A 19.6* ± 1.2	B 17.4* ± 1.52	A 34.0* ± 2.9	B 38.3*± 1.54	A 32.6* ± 7.27	B 62.8* ± 16.92
Normal control rabbits	A 78.4 ± 7.49	A 78.6 ± 6.99	A 146.0 ± 18.52	A 146.5 ± 16.73	A 21.5 ± 1.86	A 21.6 ± 2.30	A 29.9 ± 3.78	A 30.1 ± 3.26	A 25.4 ± 8.22	A 26.8 ± 10.38

Values are expressed as mean ± SD. n=6/group.
***Denote differences between groups, P<0.01 vs. control.**
Capital letters denote differences within groups, P<0.01 vs. control.

Experiment Two: Effect of alcoholic extract of *T. officinale* leaves on plasma lipid profile in cholecystectomic rabbits:

***Plasma total cholesterol*:** In the post treatment period, there was a highly significant depression ($P<0.01$) in plasma total cholesterol in the group which received 250mg/kg B.W. of alcoholic extract of *T. officinale* with time noticed in Table (2).

The lowest reduction ($P<0.01$) in total cholesterol was noticed after 3weeks of alcoholic extract administration compared with untreated cholecystectomic group. ***Plasma triglycerides* concentration:** Table (3) shows that there is reduction, but failed to reach significant level, in plasma TG concentration in cholecystectomic rabbits after one week compared with control cholecystectomic group**.** Plasma TG concentration significantly decreased ($P<0.01$) after two and three weeks of treatment with alcoholic extract of *T. officinale* leaves as compared to cholecystectomic rabbits received (2ml of 0.9% NaCl).
***Plasma HDL concentration*:** Table (4) shows level of plasma HDL in cholecystectomic treated with *T. officinale.* The treated group showed non-significant elevation ($P>0.05$) in plasma HDL concentration in comparison with untreated group after one week of

treatment. However, these treated animals produced a significant elevation (P<0.01) in plasma HDL after second and third week compared with untreated control group.

***Plasma VLDL concentration*:** The results of plasma VLDL concentration in the treated and control cholecystectomic rabbits were presented in Table (5). The results showed that the oral administration of *T. officinale* for one week caused significant decrease (P< 0.01) in plasma VLDL compared with pretreatment and untreated control group. After second and third week of oral administration of plant extract, the results showed there were further decrements in plasma VLDL level compared with control group.

***Plasma LDL concentration*:** Depending on the results clarified in Table (6), there was a significant difference (P<0.01) in plasma LDL level between both groups. A maximal reduction in plasma LDL concentration was obtained post three weeks of treatment compared with control cholecystectomic group that received physiological saline.

Table (2). Effect of alcoholic extract of *T. officinale* on plasma total cholesterol (TC) concentration in cholecystectomic rabbits.

Groups	Total cholesterol(mg/dl)			
	0wk	1wk	2wk	3wk
Alcoholic extract to cholecystectomic rabbits	Aa 118.74 ±18.43	Ab 98.84 ±16.72	Bc 80.84 ±9.15	Bd 64.87 ±8.71
Control cholecystectomic rabbits	Aa 118.81 ±16.99	Aa 112.42 ±14.60	Aa 106.67 ±11.93	Ab 103.93 ±13.97

Values are expressed as mean SD. n=6/group.
Capital letters denote differences between groups, P<0.01 vs. control.
Small letters denote differences within groups, P<0.01 vs. control.

Table (3). Effect of alcoholic extract of *T. officinale* on plasma triglyceride (TG) concentration in cholecystectomic rabbits.

Groups	Triglyceride (mg/dl)			
	0wk	1wk	2wk	3wk
Alcoholic extract to cholecystectomic rabbits	Aa 186.21 ±12.20	Bb 150.39 ±14.71	Bc 119.91 ±13.93	Bd 98.95 ±11.40
Control cholecystectomic rabbits	Aa 188.65 ±6.35	Aa 184.15 ±7.69	Aa 183.97 ±7.76	Aa 183.58 ±9.00

Values are expressed as mean SD. n=6/group.
Capital letters denote differences between groups, P<0.01 vs. control.
Small letters denote differences within groups, P<0.01 vs. control.

Table(4). Effect of alcoholic extract of *T. officinale* on plasma HDL concentration in cholecystectomic rabbits.

Groups	HDL (mg/dl)			
	0wk	1wk	2wk	3wk
Alcoholic extract to cholecystectomic rabbits	Aa 17.38 ±1.28	Ab 19.08 ±1.25	Bb 20.88 ±0.87	Bc 21.95 ±2.10
Control cholecystectomic rabbits	Aa 17.37 ±1.52	Aa 17.88 ±1.63	Aa 18.58 ±1.54	Aa 18.63 ±1.72

Values are expressed as mean SD. n=6/group.
Capital letters denote differences between groups, P<0.01 vs. control.
Small letters denote differences within groups, P<0.01 vs. control.

Table(5) . Effect of alcoholic extract of *T. officinale* on plasma VLDL concentration in cholecystectomic rabbits.

Groups	VLDL (mg/dl)			
	0wk	1wk	2wk	3wk
Alcoholic extract to cholecystectomic rabbits	Aa 37.24 ±2.44	Bb 30.07 ±2.94	Bc 23.48 ±2.23	Bd 19.19 ±2.28
Control cholecystectomic rabbits	Aa 38.63 ±1.54	Aa 36.82 ±1.53	Aa 36.79 ±1.55	Aa 36.71 ±1.80

Values are expressed as mean SD. n=6/group.
Capital letters denote differences between groups, P<0.01 vs. control.
Small letters denote differences within groups, P<0.01 vs. control.

Table (6).Effect of alcoholic extract of *T. officinale* on plasma LDL concentration in cholecystectomic rabbits.

Groups	(mg/dl)			
	0wk	1wk	2wk	3wk
Alcoholic extract to cholecystectomic rabbits	Aa 64.12 ±19.92	Ab 49.68 ±17.17	Bc 36.47 ±8.63	Bd 23.13 ±8.00
Control cholecystectomic rabbits	Aa 62.84 ±16.92	Aa 57.55 ±14.51	Ab 51.29 ±12.38	Ac 48.58 ±14.45

Values are expressed as mean ±SD. n=6/group.
Capital letters denote differences between groups, P<0.01 vs. control.

Small letters denote differences within groups, P<0.01 vs. control.

Discussion

Experiment one, effect of cholecystectomy on plasma lipid profile

The result of the present study showed that after four weeks cholecystectomic operation in rabbits leads to significant changes in lipid profile system. These changes were manifested by increase in plasma TC, TG, LDL, VLDL and decrease in HDL. However, the pathogenesis of cholecystectomy operation in rabbit has not elucidated. These result in accord with the results of Xu *et al.* **[18]** who have been described marked inhibition of cholesterol 7α-hydroxylase after 2 weeks of cholesterol feeding to cholecystectomic rabbits. This inhibition caused elevation of plasma TC. The events which lead to the observed changes in plasma TC concentration might be due to changes in steroid absorption and excretion, intestinal bile acid pool size and bile acid binding in the intestine **[19]**. These events that lead to a greater reabsorption of bile acids by the small intestine into circulation lead to increased uptake by the liver **[19]**. The consequence was an inhibition in the conversion of the cholesterol to bile acids by the liver. The resultant elevation in liver cholesterol content leads to an increase in VLDL production, decreased in lipoprotein receptor activity, and an accumulation of VLDL and LDL in the plasma **[20].**

The plasma TG concentration increased in cholecystectomic rabbits in the present study. This result may be due to an increase in plasma VLDL level which acts as a carrier for the TG in the plasma. Partial deficiency of lipoprotein lipase associated with increased output of lipoprotein from the liver due to high cholesterol level that may contribute to the elevation of plasma TG level **[21]**.

When plasma TC was increased a marked increase in the production of VLDL by the liver and intestine was resulted. Moreover, a subsequent clearance of VLDL by the liver is reduced to a down regulation of cell-surface lipoprotein receptors **[19].**

Experiment two: Effect of alcoholic extract of *T. officinale* on plasma lipid profile in *cholecystectomic rabbits:*

The results revealed that the oral administration of (250 mg/kg B.W.) alcoholic extract of *T. officinale* to cholecystectomized rabbit caused significant reduction of TC, TG, VLDL and LDL while HDL increased after three weeks of treatment. The hypolipidemic effect of Dandelion may be explained by the presence of significantly large amount of sterol bitter like sitosterol, stimasterol, taraxasterol, homotaraxasterol and inulin in *T officinale* leaves **[22].**

The inhibitory effect of this plant to TC may be due to its effect on cholesterol synthesis **[23, 24].** Otherwise, it may be due to its binding affinity of its ingredients with bile acid and enhance the faecal bile acid excreation **[25]**. The effect of this plant on TG may be also due to inhibition of TG synthesis by inhibiting fatty acid production **(24)** or on the enzymatic system of triglyceride synthesis **[4].** The increase in HDL was in commtant with decrease in plasma TC level which can be explained due to the cholesterol in HDL being transported back to liver for excreation out of the body **[26].**

The reasons for the decrease in VLDL level in treated cholecystemized animals may be due to suppression of hepatic cholesterol synthesis and decreasing in production of short chain fatty acids with decrease in TG formation **[27, 28, 29].**On the other hand, the active ingredients in this plant may have an effect on the key enzymatic activities involved in synthesis of VLDL **(30).** Dandelion (*T. officinale*) in this study was effective in reducing plasma LDL; this reduction may be due to up regulation of LDL-receptors in the liver cells **[31].** Khudiar **[25]** emphasized the effect of Parsey seeds on lowing plasma LDL levels by increase LDL-receptors, thus stimulating the hepatic uptake of LDL.

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تأثير استئصال المرارة والمستخلص الكحولي لنبات الهندباء على صورة الدهون في الأرانب

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الخلاصة

لقد أجريت في هذه الدراسة تجربتان: ألأولى لمعرفة تأثير إجراء عملية استئصال المرارة على صورة الدهون التي تشمل نسبة الكولسترول الكلي (TC) والكليسيريدات الثلاثية(TG) والشحوم البروتينية ذات الكثافة العالية(HDL) والشحوم البروتينية ذات الكثافة الواطئة(LDL) والشحوم البروتينية ذات الكثافة الواطئة جدا(VLDL) في بلازما دم الأرانب. أما التجربة الثانية تهدف دراسة تأثير المستخلص الكحولي لأوراق نبات الهندباء على صورة الدهون في بلازما دم الأرانب المستأصلة المرارة، ولأجل ذلك أستخدم في التجربة الأولى (12) أرنبا قسموا عشوائيا إلى مجموعتين؛ المجموعة الأولى أجريت لهم عملية استئصال ألمرارة والمجموعة الثانية حيوانات طبيعية كمجموعة سيطرة. أما التجربة الثانية فأستخدم لها أيضا (12) أرنبا أجريت لجميعهم عملية استئصال المرارة وقسموا عشوائيا إلى مجموعتين:المجموعة الأولى أعطوا عن طريق الفم(250ملغم/كغم) من وزن الجسم من المستخلص الكحولي لأوراق نبات الهندباء والمجموعة الثانية أعطوا (2مل) من المحلول الفسيولوجي (NaCl 0.9%). جمعت عينات الدم في المجموعة ألأولى كل أسبوعين ولمدة شهر بعد أجراء العملية، أما في التجربة الثانية فجمعت عينات الدم أسبوعيا ولمدة ثلاثة أسابيع.

أظهرت النتائج بأن عملية استئصال كيس المرارة سببت زيادة معنوية عالية ($P<0.01$) في مستويات بلازما الدم من (LDL, VLDL, TG, TC) مع انخفاض معنوي عال ($P< 0.01$) في مستوى HDL مقارنة مع مجموعة أرانب السيطرة. بينما أدى أعطاء مستخلص نبات الهندباء إلى ألأرانب ألمستأصلة المرارة إلى انخفاض معنوي عال ($P<0.01$) في LDL, VLDL, TG, TCمقرونا بزيادة معنوية في تركيز HDL في بلازما الدم.

من النتائج يمكن الاستنتاج بأن عملية استئصال المرارة تؤدى إلى رفع مستوى الكولسترول والبروتينات الدهنية في بلازما الدم. ويمكن استخدام أوراق نبات الهندباء كدواء بديل لتخفيضة.