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Ruta chalepensis L Considerable Action against Obesity or Hyperlipidemia in Body

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Abstract

Objective: This study was designed to estimate the efficacy of *Ruta chalepensis* L in falling the cholesterol levels in hypercholesterolemic rabbits.

Materials and methods: Hypercholesteremia was induced in normal rabbits by adding 0.75 g cholesterol and 1.5 g bile salt in normal diet that were used for the experiments. Dried leaves and fruits powder of Ruta chalepensis L was administered as feed supplement at 5 g and 10 g dose levels to the hypercholesterolemic rabbits. Plasma and liver lipid profiles, fecal bile acid, hepatic HMG-CoA reductase, malondialdehyde, cholesterol and neutral sterols were estimated by using standard methods.

Results: Feed supplementation with 5 g and 10 g of *Ruta chalepensis* L resulted in a significant decline in hepatic and plasma lipid profiles. The feed supplementation increased the HMG-CoA reductase activity and bile acid production in all groups of rabbits with immediate increase in fecal cholesterol excretion and in fecal bile acid. The activities of catalase, SOD and ascorbic acid content increased significantly in both the experimental groups (5 and 10 g supplemented groups). On the other hand, the concentration of malondialdehyde in these groups (5 and 10 g supplemented groups) decreased significantly, that was an indication of decreased lipid peroxidation.

Conclusion: The present study demonstrates that addition of *Ruta chalepensis* L fruits leaves powder at 5 g and 10 g level as feed supplement reduces the plasma and hepatic lipid (cholesterol) levels and also decreases lipid per-oxidation.

Keywords: Feed supplement; HMG-CoA reductase; Lipid profile

Introduction

Now a day, Coronary artery disease becomes a serious medical dilemma that pretends to have an influence on the majority of people every year. Progression of coronary artery disease (CAD) and atherosclerosis is happened due to the raised level of serum lipids primarily of cholesterol along with initiation of reactive oxygen species (ROS) (Lopez- Garcia et al., 2004).The most predisposing risk factors for the development of atherosclerosis and coronary artery diseases are stress, sedentary habits, alcohol intake, abuse of tobacco, genetic susceptibility and dietary habits e.t.c.(Visavadiya, 2005). It has been anticipated that adoption of various preventive measures (physical exercise, lifestyle modification, alteration in dietary habits, restraint from alcohol and tobacco use) can diminish the incidence of several cardiac problems through medical alliance all over the world. Reduction in lipid profiles can be efficiently achieved by consumption of phytosterols and natural antioxidants that have potential to alleviate the peroxidation alteration of lipoproteins and atherosclerosis (Ikeda and Sugano, 1998).

Ruta chalepensis L is one of the plants that belongs to Rutacease family and has shown to have remarkable medicinal properties and is considered as an important remedy for a number of ailments in many systems of medicine. This botanical family is best known for its extensive wide variety of tropical and subtropical plants including up to 1500 species having distribution in 150 genera (Figure 1) [1-10].

Instruments

The following instruments were used

Rotary Evaporator Laborota 4002 with Digital Heating bath (Heidolph Instruments, Schwabach, Germany), Incubator (Memmert, W. Germany), Hot Air oven Digital PID Control, (Memmert, W. Germany), UV-VIS Spectrophotometer. Pharma Spec. UV-1700 (Shimadzu, Germany), Analytical Balance, L 420 S, Sartorius (Germany), Refrigerated Centrifuge 2-16KC, (Sigma, Germany).

High fat diet

The experimental diet consists of high fat diet that include, butter and desi ghee. 100gm Butter contains, Total Fat 81.21gm in which saturated Fat 51.36 gm and Cholesterol 215 mg.1 tablespoon Desi ghee contains total 12.72 gm Fat, in which 7.92 gm saturated fat, 3.67 gm monounsaturated fat and 0.47 gm polyunsaturated fat.

Plant materials

To prepare extracts of crude parts include *Ruta chalepensis* L fruit, leaves and bark parts over a wide polarity range, dried fruit of Ruta chalepensis L plant was collected from local market, Papar Mandi, Lahore. For authentication, *Ruta chalepensis* L fruit was subjected to Herbarium, Botany Department, Government College University, Lahore. A voucher specimen was certified under Voucher No. GC. Herb. Bot. 2213 and has been preserved in the laboratory for future

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Figure 1: Terminal parts of *Ruta chalepensis* L (leaves, fruit, flower and seed).

reference. The material was dried under shad, pulverized by mechanical chopper into powder form and store in polythene bag for further analysis.

Animals grouping

- Experimental subject: Rabbits
- Total experimental subjects used: 15(5 groups of three)
- Experimental diet :

A. Normal diet (ND) standard pelleted chow.

B. High fat diet (ND) supplemented with butter and desi ghee.

- Preparation of the subjects before experimentation:

Before alienation in to 5 groups of 3, rabbits were accustomed to the experimental facility for about 2 weeks and accommodated in standard metallic wire gauge cages in a room maintained at temperature of 23 ± 1°C and nearly 55 ± 5% of relative humidity. Three rabbits were randomly selected as one the five groups and categorized as a normal group which had free access to fodder (normal diet) for approximately 5 weeks. And the remaining 12 rabbits were fed with a high-fat diet and normal diet (HIGH FAT DIET + ND) and were divided into following 4 groups on the random basis.

Normal Group	**High Fat diet Groups**			
Group 1	Group 2	Group 3	Group 4	Group 5
normal diet	High Fat diet	High fat diet + Orlistat	High fat diet + Methanloic extract of *Ruta chalepensis L*	High fat diet Chloroform extract of *Ruta chalepensis L*

Body weight (gained/ loss) were calculated every week. In order to ascertain the serum lipid profile, blood samples were collected every week. Serum biochemical enzymatic analysis was made following a 12-h fast. By the use of centrifugation, the serum was isolated at speed of 4,000 rpm for 10-12 min and kept at the temperature of 86°C for determination of the serum lipid profile. The study was approved by the Ethical Committee of the University College of Pharmacy, and was carried out in accordance with the standard guidelines for maintenance and use of experimental animals (Rabbits).

Estimation of biochemical parameters

After the conclusion of the experiment, the animals were subjected to overnight fasting and killed under mild anesthesia.

Plasma

Blood samples were drawn by retro-orbital puncture using a fine sterile capillary tube and the plasma was used for the estimation of total lipids, total cholesterol, (Mc Gowan et al. [5]) triglycerides,(Folch et al. [1]) HDL-cholesterol,(Mc Gowan et al. [5]), LDL-cholesterol, VLDL-cholesterol. The atherogenic index was calculated as described. The base line plasma lipid profiles, the fecal bile acid, cholesterol and neutral sterol profiles were determined prior to the treatment regime.

Liver

Hepatic lipids (Total hepatic triglycerides and cholesterol) were extracted (Folch et al. [1]) and estimated gravimetrically (Mc Gowan et al. [5]). HMG-CoA reductase activity was assayed by the method and expressed as the ratio of absorbance of HMG-CoA to mevalonate. This was taken as the index of HMG-CoA reductase (Yamamoto et al. [9]) activity. Hepatic bile acid was estimated by the method of Snell and Snell. (Snell and Snell [7]) Malonaldehyde, catalase, superoxide dismutase and total ascorbic acid content were assayed using standard methods (Niehaus and Samuelsson [6]).

Fecal matter

Fecal bile acid, cholesterol and neutral sterols were extracted (Kaiek et al. [3]) and estimated (Snell and Snell [7]).

Statistical Analysis

The results for body weights and lipid profile were expressed as mean ± Standard error of mean. All statistical analysis was performed using SPSS 12.0 software. Significant differences of means among the groups treated with different extracts of crude plant part, standard drug (orlistat) and high fat diet group were determined using analysis of variance (ANOVA) and Dunnett Test. Results were considered to be significant at P values less than 0.05.

Results

Extraction

The crude chloroform and methanolic extract were obtained from

plant, Ruta *chalepensis* L were examined for consistency and their percentage yield. The values obtained from the extraction are given in Table 1, while that of fractions are given in Table 2.

Preliminary phytochemical analysis

To identify the chemical nature of the extracts, preliminary qualitative chemical test were performed on methanolic and chloroform extracts of the crude plants parts. The results are given in Table 3.

Proximate analysis of plant powder

Proximate analysis was conducted on the plant powder. The results of moisture content, total ash, acid insoluble ash, sulphated ash, alcohol and water soluble extractive values are given in Table 4.

Determination of anti-obesity effect

Rabbit's weight reduction was calculated after treating with plant extracts and standard drug. The results were then compared across all groups. The results are shown in Tables 5 and 6.

Determination of lipid profile

The measure of cholesterol, LDL level, HDL level and triglyceride in all 4 groups was calculated. The results are tabulated in Tables 7-9.

Discussion

Different solvents were utilized to do the extraction of distinct phytochemical groups and particular compounds were often extracted by special extractants. Temperature, solvent, light, humidity and pH are the different physical parameters that considerably play an important role to enhance the quality of extraction and effectiveness of crude extract. In addition to water, methanol; ethanol; chloroforml; benzene; n-hexane; and ethyl acetate are the other solvents that were utilized in extraction process. From the fruit of *Ruta chalepensis* L, two extracts of distinct nature were made. Crude plant extracts are usually a combination of active and non-active compounds. For the reason of possessing different polarities, the methanol and chloroform extracts of *Ruta chalepensis* L plant were taken into account in this study.

By the use of methanol and chloroform, 4.15% w/w (83 gm) and 3.9% w/w (78 gm) were the calculated % age yields of powdered fruit of *Ruta chalepensis* L respectively (Table 1). Furthermore, for the estimation of the entire flavonoids and phenolic contents in the respective fraction, an additional various fractionation of Methanolic exract of *Ruta chalepensis* L was prepared. The percentage yield of different factions were 16% (4.0 gm), 14% (3.5 gm), 11.6% (2.9 gm), 15.2% (3.8 gm), 36% (9.0 gm) for n-Hexane, Chloroform, Ethyl Acetate, n-Butanol and Aqueous respectively (Table 2). It has been documented from some previous phytochemical analysis that Ruta chalepensis L also contain various other constituents such as alkaloids, coumarins, volatile substances, terpeniods, flavonoids, and furoquinolines. So from this study on *Ruta chalepensis* L, it is inferred that steroids, flavonoids, tannins, saponin, cardio glycosides, carbohydrates, alkaloids are present in different plant extract.

Conclusion

It is inferred from the above study that direct analysis on the powdered fruit of *Ruta chalepensis* L have showed the moisture content of approx. 5.25% which is an indication of the fact that stability of the crude plant extracts is enhanced by low moisture content. Reduced moisture content can also protect the microbial growth. The total ash value of powdered fruit of *Ruta chalepensis* L was 9.4% which lies within the standard limit. Estimation of ash value is an important indicator of the quantitative standards that is helpful in the determination of purity and authenticity of crude plant parts. The total ash value is represented by physiological and non-physiological ash values that give an indication of biochemical and environmental contaminants. The study on *Ruta chalepensis* L has confirmed the presence of various important chemical compounds through the phytochemical investigation, which play a considerable role in the treatment of obesity by possessing medicinal properties. This fact has been confirmed by administrating plant extracts of *Ruta chalepensis* L to five groups of albino rabbits in contrast to standard medication such as "orilstat" having anti-obesity property. The two types of diets were given to the rabbits (3 rabbits were on normal diet and the remaining 12 rabbits were on high fat diet). The

Name of Plant	Part Used	Weight of crude powder	Extracts	Nature of Extracts	Extracts Weight	Percentage Yield of Extracts
Ruta chalepensis L	**Fruit**	**1.5 Kg**	Methanol	Semisolid	83 gm	4.15%
			Chloroform	**Semisolid**	**78 gm**	**3.90%**

Table 1: Extractive values of crude plant materials.

Name of Plant	Weight of Methanolic Extract	Fractions	Nature of Fraction	Fraction weight	Percentage Yield of Fraction
Ruta chalepensis L	**25 gm**	n-Hexane	Semisolid	4.0 gm	16.00%
		Chloroform	Semisolid	3.5 gm	14%
		Ethyl Acetate	Semisolid	2.9 gm	11.60%
		n-Butanol	Semisolid	3.8 gm	15.20%
		Aqueous	solid	9.0 gm	36%

Table 2: % yield of different fraction of extracts of crude plant materials.

Phytoconstituents	*Ruta chalepensis L*	
	Methanol	Chloroform
Alkaloids	+ + +	+ + +
Carbohydrates	+ +	+
Glycosides	+ +	– – –
Flavonoids	+ + +	+
Fixed oil	+ + +	+ + +

Table 3: Phytochemical constituents of the plant extracts.

Parameter	*Ruta chalepensis L*
	Values in (%) w/w
Total ash	9.5
Acid Insoluble ash	4.6
Water soluble ash	7.2
Sulphated ash	10.8
Moisture Content	5.23
Alcohol soluble extractive	15.5
Water soluble extractive	9.7

Table 4: Physicochemical Parameter of powdered Plants parts.

Treatment	Ordinary light	Short UV	Long UV
		(254 nm)	(365 nm)
Powder as such	Brown	Light Brown	Brown with fluorescence
Powder + Aniline	Reddish Brown	Light Brown	Dark Brown
Powder + Barium Chloride	Yellow	Yellow	Light Violet fluorescence

Table 5: Behavior and Fluorescent analysis of powdered fruit of Ruta *chalepensis* L on treatment with different chemical reagents.

No. of rabbits	Body weight before high fat diet (gm)	Body weight after high fat diet (gm)
1	1300	1360
2	1420	1460
3	1410	1500
4	960	1000
5	1420	1420
6	1160	1240
7	1400	1560
8	1300	1420
9	1300	1370
10	1400	1420
11	1300	1360
12	1500	1600

Table 6: Measurement of change in body weight before and after administrating high fat diet (monitored for *Ruta chalepensis L* plant study).

Groups	Baseline	After 7 days	After 14 days	After 21 days
high fat diet	17.53 ± 0.56	16.35 ± 0.34	15.53 ± 0.65	14.61 ± 0.73
orlistat treatment + high fat diet	17.61 ± 1.20	15.07 ± 0.56	14.35 ± 0.98	15.37 ± 1.28
methanolic extract of ruta chalepensis l + high fat diet	15.89 ± 0.99	15.07 ± 1.51	17.35 ± 0.87	16.27 ± 0.48
chloroform extract of ruta chalepensis l + high fat diet	16.26 ± 1.42	16.17 ± 0.82	15.89 ± 1.51	15.62 ± 1.35

Table 7: Effect of crude extracts of the Ruta chalepensis L on serum High Density Lipoprotein level (mg/dl) of obese rabbits.

Groups	Baseline	After 7 days	After 14 days	After 21 days
high fat diet	83.42 ± 12.29	86.91 ± 9.62	86.98 ± 8.41	86.39 ± 7.33
orlistat treatment + high fat diet	78.85 ± 10.25	74.98 ± 9.41	69.97 ± 3.72	62.88 ± 2.99*
methanolic extract of ruta chalepensis L + HFD	73.19 ± 6.86	75.37 ±6.21	78.59 ± 7.79	71.95 ± 7.11
chloroform extract of ruta chalepensis L + HFD	74.84 ± 0.34	84.30 ± 3.65	81.22 ± 4.21	79.66 ± 4.04

Table 8: Effect of crude extracts of the Ruta chalepensis L on serum Low Density Lipoprotein level (mg/dl) of obese rabbit.

Treatment	Ordinary light	Short UV (255 nm)	Long UV (364 nm)
Powder as such	Brown	Light Brown	Brown with fluorescence
Powder + Aniline	Reddish Brown	Light Brown	Dark Brown
Powder + Barium Chloride	Yellow	Yellow	Light Violet fluorescence
Powder + Chloroform	Light Green	Yellow	Light Purple fluorescence
Powder + 5% Ferric chloride solution	Dark Brown	Creamy Brown	Dark brown
Powder + Formaldehyde	Light Yellow	Light Violet	Blue fluorescence
Powder + Formic Acid	Dark Yellow	Brown	Violet fluorescence
Powder + Glacial Acetic Acid	Light Green	Violet	Violet fluorescence
Powder + Dilute HCl	Yellow	Orange	Dark Violet
Powder + Iodine Solution	Dark Red	Red	Black
Powder + NH_4OH	Brown	Pink	Blue fluorescence
Powder + 50 % Nitric acid	Dark Yellow	Light Brown	Green fluorescence
Powder + 1 M Potassium hydroxide solution	Brownish Yellow	Orange	Green
Powder + Sodium Carbonate soln.	Yellow	Brown	Blue fluorescence
Powder + 5% sodium hydroxide	Orange	Orange	Light Green
Powder + 50 % H_2SO_4	Brownish Green	Light Brown	Blue
Powder + 60 % H_2SO_4	Dark Brownish Green	Brown	Green fluorescence
Powder + Silver Nitrate	Yellow	Yellow	Blue fluorescence
Powder + water	Yellow	Yellow	Blue fluorescence

Table 9: Behavior and Fluorescent analysis of powdered fruit of Ruta chalepensis L on treatment with different chemical reagents.

blood serum samples from every rabbit were collected and analyzed with the help of Enzymatic Kits. At the end, we have concluded from the obtained serum samples that plant extracts of *Ruta chalepensis* L has pronounced effect on LDL, TG and HDL cholesterol levels.

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