

Comparative studies on rabbit plasma lipid profile fed with *Silybum marianum* oil, sunflower oil and vegetable ghee

Ghosia Lutfullah¹, Aziz-Ur-Rahman^{1,2*}, Aftab Ahmad², Taufiq Ahmad³, Amjad Ali⁴ and Jan Alam⁵

¹Centre of Biotechnology & Microbiology, University of Peshawar, Pakistan

²State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing, PR China

³Food Chemistry, NIFA, Peshawar, Pakistan

⁴Department of Statistics, Islamia College University, Peshawar, Pakistan

⁵Pak International Medical College, Peshawar, Pakistan

Abstract: Present work is aimed to compare the physicochemical characterization and biochemical effects of oil extracted from *Silybum Marianum* and Sunflower oil, collected from Peshawar (Pakistan). To investigate the comparative effects on the body weight, organ weight and lipid profile, the crude oil of *Silybum marianum*, edible sunflower oil and vegetable ghee were given to three groups of rabbits under study. Percent proximate composition and food consumption of all rabbits were determined which showed no significant statistical variation. There is no data available about *Silybum marianum* oil on animal model in literature. This study clearly revealed that oil from *Silybum marianum* significantly reduces plasma cholesterol level in rabbits. A threefold higher Triglyceride levels was observed in vegetable ghee feeding groups compared with the sunflower and *Silybum marianum* oil feeding groups. The crude oil of *Silybum marianum* was found to be safe in rabbits compared with sunflower oil and vegetable ghee. The results of these studies revealed most valuable information and also support the refining and purification to convert this non-edible oil to edible oil.

Keyword: Lipid profile, vegetable ghee, sunflower oil, *Silybum marianum* oil.

INTRODUCTION

Silybum marianum (Milk thistle) is an annual or biennial plant originated from Europe, United States, India and Pakistan. It grows to a height of 3-10 feet with an erect stem that bears large, alternating and prickly-edged leaves following season from May to August and each stem bears a single, large purple flower ending in sharp spines. The fruit portion of plant is glossy brown or grey with spots. On average the fruit is about 6.7 mm long, 3 mm wide and 1.5 mm thick. This plant belongs to family composite (Omidbaigi and Nobakht 2001; Luper 1998). The seeds of different localities of *Silybum marianum* yield good oil by expression, which contains 26-39% fixed oil, composed of unsaturated and saturated fatty acids. The other constituents of oil are free fatty acids, campesterol, stigmasterole, mono, di and triglycerides. The defatted seed contains an excellent quality and quantity of proteins, ranging from 20-24% containing amino acids such as aspartic acid, glycine, cystine and glutamic acid (Subhan *et al.*, 1995) *Silybum marianum* contains silymarin, which is composed of flavonolignans; a unique group of carbohydrates consist of silydianin, isosilybin, dihydrosilybin, silychristine and silybin, which is the most active biological component. Silymarin is found in the highest concentration in the fruit portion of the plant but is also present in the leaves and seeds (Szentmihalyi *et al.*, 1998) Total 23 element (Al, As, B,

Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, P, Pb, S, Ti, V and Zn) have been identified in the seed of this plant (Fiebrich and Koch 1979) *Silybum marianum* has been known since ancient times and recommended in traditional European and Asiatic medicines mainly for treatment of liver disorders and as an antioxidant (Dehmlow *et al.*, 1996; Stephen *et al.*; 2010). Seeds of *Silybum marianum* (milk thistle) have been used for more than 2000 years to treat liver and gall bladder disorders, including hepatitis, cirrhosis, jaundice, and to protect the liver against poisoning from chemical and environmental toxins, including snake bites, insect stings, mushroom poisoning, and alcohols. Silymarin is hepatoprotective, antioxidant, and enhance liver detoxification (Vladimir *et al.*, 2005; DeLa Puerta *et al.*, 1996; Santosh and katiyar, 2005; Nin skottova *et al.*, 2000). It also helps in the protection of glutathione depletion and has anti-inflammatory effect (12 Skottova and krecman, 1998; Deak *et al.*, 1990; Lang *et al.*, 1990). In addition silymarin also have been shown to increase hepatocyte protein synthesis thereby promoting hepatic tissue regeneration (15 Hem Lata *et al.*, 2004). Clinical study also shows that silymarin acts as an anti-cancer and anti-inflammatory agent (Laurence *et al.*, 1996). Animal study has demonstrated that silybinin reduces the conversion of hepatic cells into myofibroblasts, slowing or even reversing fibrosis (Fidelis and Achuba 2005). Clinical studies show that silymarin has immunomodulatory effects (Lang 1990). Rats were fed on diet containing

*Corresponding author: e-mail: aziz_biotech@yahoo.com

silymarin and silibinin a decrease level of cholesterol and lipoprotein were found in the plasma. This can be suppressed by improvement of bioavailability of silibinin. The administration of silymarin reduces plasma levels of cholesterol and low-density lipoprotein (LDL) cholesterol in hyperlipidaemic rats, whereas silibinin does not reduce plasma levels of cholesterol in normal rats; however, it does reduce phospholipids levels, especially those transported in LDL. In the experimental model of hepatic injury produced by thioacetamide, silymarin did not appear to be able to normalize the reduction in triglycerides in serum (Hem Lata *et al.*, 2004; Laurence *et al.*, 1996). In human, the flavinoid containing oil contributes greatly to the dietary source of antioxidant and decrease the risk of coronary heart diseases (Laurence *et al.*, 1996; Fidelis and Achuba, 2005). Therefore, a comprehensive study is needed to convert this neglected, unwanted and abundantly available weed to a valuable source of oil and protein.

Silymarin and silybinin extracted from the *Silybum marianum* seed are reported for their hypocholesterolemic effects in rats (Hem Lata *et al.*, 2004; Laurence *et al.*, 1996). While no literature is reported on the *Silybum marianum* seed oil fed to rabbit's model. In the present work the rabbits were fed with nonconventional *Silybum marianum* oil (SMO) in-order to compare the effects of conventional sunflower oil (SFO) and vegetable ghee (VG) on total cholesterol, triglyceride and high density lipoprotein, organs weight, dress weight and food consumption.

MATERIALS AND METHODS

Rabbits of local strain were obtained from Pakistan Council of Scientific & Industrial Research, Peshawar (PCSIR) as well as from local market and their colony was raised at Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan.

Study Design

Some seeds were obtained from Pakistan council of scientific & industrial research, Peshawar, Pakistan. These seeds were cultivated at nuclear institute of food & agriculture (NIFA), Peshawar. The oil was extracted from those seeds, which were grown for cultivation in NIFA. The grown seeds were collected, cleaned, dried & stored in cold dark conditions. Oil was extracted chemically by using petroleum ether & mechanically by conventional oil expeller. The crude oil was filtered & store in bottles in dark conditions to prevent photo-oxidation. Fully ripened and healthy seeds of *Silybum marianum* were collected from ring road side, Peshawar, Pakistan. Their crude oil was extracted with same procedure, which is used in this study.

An independent experiment with 30% fat energy was conducted at NIFA, Peshawar using male rabbits. Young

male rabbits, weighing between 1000-1200g were selected for these experiments from a locally bred strains stock. Rabbits were divided into four groups and housed under uniform environmental conditions and maintained on test diets containing *Silybum marianum* oil extracted from seeds collected from ring road side and seeds grown in NIFA, commercial sunflower oil, and commercial vegetable ghee at 30% fat energy levels. The control group was fed on mixed green forage and carrot. Each treatment was replicated thrice with three animals in each case. Feed and water were provided regularly during the entire experimental period. Animals were housed in steel cages with wire tops and wooden bottoms and maintained at ambient conditions (20-30°C) for a period of 90 days with an approximate 12 hours light/dark photo-period.

Animals were weighed weekly and slaughtered at the end of the experiment for blood collection. Blood sample from each animal was collected in a test tube containing ethylene di amine tetra acetate (EDTA) and stored at 20°C. Then the blood was centrifuged for 10 min. at 3000 rpm to obtain plasma and analyzed for lipid profiles spectrophotometrically by using commercial clonitol kits (Spain), available in the market.

Formulation of experimental diets

Experimental diets were prepared on the basis of 30% fat energy levels for each oil or fat and the control was maintained on mixed forage only. The rabbits in the control lot were fed mixed green forage only in order to simulate prevailing husbandry practices in Pakistan. The test diets were almost isocaloric, isonitrogenous and isofibrous containing Wheat bran, Chickpea, Salt and Vitamin/mineral mixture. The proximate composition of experimental diet and *Silybum marianum* seeds oil were determined. Total 30 rabbits were taken and randomized in three replicated groups as R1 (fed on 30% vegetable ghee), R2 (fed on 30% sunflower oil) and R3 (fed on 30 % *Silybum marianum* oil). Food consumption of each rabbits /day, Weight gain and % weight gain of entire group was calculated at the end of experiments.

RESULTS

Physiochemical properties including; peroxide value, free fatty acid, optical density, beta-carotene, anisidin and refractive index of selected seeds oil were observed in this study and are reported in table 1. The mean peroxide value analyzed for *Silybum marianum* seeds oil (Ring Road & NIFA) and sunflower oil were 7.00 meq/kg, 6.59 meq/kg and 3.00 meq/kg, respectively. The results revealed a slight variation in the mean free fatty acid value (1.147%, 1.144% and 1.091%) for *Silybum marianum* (Ring Road & NIFA) and sunflower oil respectively. The mean optical density value of 0.831, 0.681429 and 0.253 were observed for *Silybum marianum* (ring road seeds oil, NIFA seeds oil) and sunflower oil respectively. The oil obtained from Ring Road seeds

Table 1: Mean value of oil parameter

Sample	POV	FFA	OD	β . caroten	R. index	IV	AV
Ring road seed	7.00	1.15	0.831	4.43	1.468	109.8	1.899
NIFA seed	6.595	1.144	0.68	4.56	1.468	109.89	1.853
R2	3	1.091	0.253	27	1.473	107	2.4

Table 2: Mean value of proximate composition of diet

Sample	Protein%	Fats %	Carbohydrates %	Moisture %	Ash %	Fiber %	Total
Ring road seed	23	27	32.88	3.94	5.9	7.28	100
NIFA seed	23.5	27.5	33.88	3.86	3.98	7.28	100
R3 diet	12	22.44	49.88	5.76	2.62	7.3	100
R2 diet	12.25	22.33	49.89	5.13	3.1	7.3	100
R1 diet	12.86	22.4	49.54	5.18	2.72	7.3	100

Table 3: Mena values of organs and

Group	Liver (g)	Kidney (g)	Heart (g)	Spleen (g)	TC mg/dl	HDL mg/dl	TG mg/dl	Glucose mg/dl
Control	29.83	7.600	3.333	0.3667	20.68	22.55	60.11	119.6
R1	29.50	5.833	3.000	0.2833	148.2	42.53	626.4	126.2
R2	33.00	5.667	2.167	0.2333	128.6	37.54	150.6	105.9
R3	34.50	7.667	3.167	0.3500	61.93 ^B	23.97	134.8	104.2
LSD Value	8.198	2.112	1.246	0.1786	29.92	17.08	60.84	12.98

Table 4: Analysis of variance results

One way analysis of variance	Liver	Kidney	Heart	Spleen	Total cholesterol	HDL	Triglycerides	Glucose
F-Ratio	0.9481	2.833	1.841	1.302	60.38	2.137	213.4	7.223
P value	0.4646	0.1062	0.2179	0.3390	0.0001	0.1737	0.0001	0.0115
Are mean sig. different? (P<0.05)	No	No	No	No	Yes	No	Yes	yes
Number of group	4	4	4	4	4	4	4	4

revealed the highest peroxide, free fatty acid and optical density mean value. The mean refractive index value analyzed for ring road seeds oil, NIFA seeds oil, and sunflower oil were 1.468, 1.468 and 1.473, respectively. The Anisidin mean value of 1.899, 1.852 and 2.40, was exhibited by seeds oil from, ring road, NIFA and Sunflower respectively. The β -carotene content analyzed showing sunflower oil i.e. 27 parts per million (ppm), NIFA seeds and ring road seeds oil with mean values of 4.564 ppm and 4.43 ppm, respectively. Sunflower oil showed the highest Refractive Index, Anisidin and β -carotene mean value.

To study the weight of different organs all the rabbits were slaughtered, organs were removed, weighed individually and were physically examined. The mean weights for liver, kidney, heart and spleen in grams and mean values of total cholesterol, High Density Lipoprotein, Triglyceride and Glucose in mg/dl are reported in table 3 and fig. 1. We used One-Way Analysis of Variance (ANOVA) technique for the comparison of means and its results are given in table 4.

The results in table 4 show that no significant difference has been found in the mean weights of all organs. So, there is no significant effect of R1, R2 and R3 on the weight of organs. By looking into the absolute comparisons of means, R3 gave the maximum mean weights for liver (34.50g) and kidney (7.67g) and 2nd highest mean weights for heart (3.17 g) and spleen (0.35 g). In case of heart (3.33g) and spleen (0.37g) the control group gave us the maximum mean weights as compared to treatments R1, R2 and R3. Another notable feature of the data is that R2 group gave us minimum mean weights for kidney (5.67g), heart (2.17g) and spleen (0.23g).

DISCUSSION

Hyperliporoteinaemias with a significant increase of serum cholesterol and its carrier LDL are known to be associated with an increased risk of heart diseases (Orolin J *et al.*, 2007). In accordance with results obtained by Krecman *et al.* and Skottova *et al.*, the present study showed that polyphenolic fraction of silymarin can positively modify lipoprotein profiles and

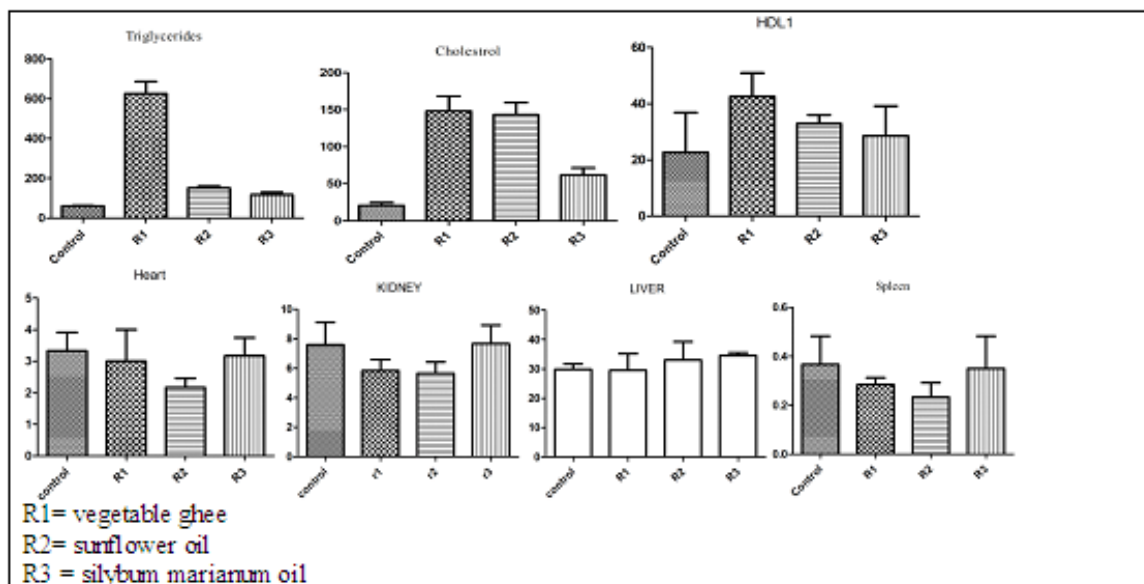


Fig. 1: Comparative studies on rabbit plasma lipid profile fed with *Silybum marianum* oil, sunflower oil and vegetable ghee

antihypercholesterolemic effects of this fraction were dose-dependent (Skottova N *et al.*, 2003). It has been shown recently that silymarin has beneficial effects on some risk factors of atherosclerosis owing to its hypolipidemic properties (Orolin J *et al.*, 2007). Recent data suggest that the inhibition of cholesterol absorption caused by silymarin could be a mechanism contributing to the positive change in plasma lipoprotein profile (Sobolova L *et al.*, 2006). Recent evidence suggests that silybinin is not as effective as silymarin and other constituents of silymarin may be responsible for its anti-hyperlipidemic effect or that the bioavailability of silybinin alone might be lower than that of silybinin as a compound of silymarin (Krecman V *et al.* 1998).

In our Study a significant difference has been found in the total cholesterol level. Compared to the control group total cholesterol has been increased in all the treatment groups. It can be seen that the maximum mean total cholesterol is given by vegetable ghee (R1) which seems to be natural followed by the sunflower oil (R2) and *silybum marianum* oil (R3) gave the minimum mean total cholesterol. It is important to mention that our results are consistent with the previous studies (Orolin J *et al.*, 2007; Skottova N *et al.*, 2000). No significant differences has been reported by the ANOVA results for high density lipoprotein (HDL) concentration among control and treatment groups but HDL increase has been observed for treatment groups R1 and R2 as compared to the control group. A slight decrease in HDL has been reported by our results. Significant variation has been observed in the mean triglycerides level of different groups with a maximum of 626.4mg/dl for R1. Further, LSD result also show that the mean value for R1 is significantly greater than the other two groups as well as the control group.

Significant differences have also been reported by ANOVA results for glucose among the groups with a maximum mean glucose level for R1 group. The glucose level for R2 and R3 has been decreased and increased in case of R1 compared to the control group (table 4). high density lipoprotein (HDL) concentration among control and treatment groups but HDL increase has been observed for treatment groups R1 and R2 as compared to the control group. A slight decrease in HDL has been reported by our results. Significant variation has been observed in the mean triglycerides level of different groups with a maximum of 626.4mg/dl for R1. Further, LSD result also show that the mean value for R1 is significantly greater than the other two groups as well as the control group. Significant differences have also been reported by ANOVA results for glucose among the groups with a maximum mean glucose level for R1 group. The glucose level for R2 and R3 has been decreased and increased in case of R1 compared to the control group.

CONCLUSION

This study suggests that oil from *Silybum marianum* significantly reduces plasma cholesterol level in rabbits. The crude oil of *Silybum marianum* was found to be safe in rabbits compared with sunflower oil and vegetable ghee. The results of these studies revealed most valuable information and also support the refining and purification to convert this non-edible oil to edible oil.

ACKNOWLEDGMENTS

The authors are indebted to the Director, Nuclear Institute for Food and Agriculture (NIFA) Peshawar, for providing research facilities.

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