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RESEARCH ARTICLE

Study of the Effects of *Prunus Domestica* L. Seeds on Some Blood Biochemical Parameters in Hypercholesterolemic Female Rats

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Abstract

Background: The present study investigate the effect extracts of *Prunus Domestica* red (PDR) and *Prunus Domestica* yellow (PDY) seeds on some biochemical parameters in hypercholesterolemic female rats.

Materials and methods: Twenty four healthy female rats (*Rattus norvegicus*) (mean weight, 220-250g) were divided into following four groups with 6 animals in each group, all rats in bio-chemical parameters are divided into normal control group (I), hypercholesterolemic positive control group (II), group (III) of hypercholesterolemic experimental rats received the PDR extract at 50 mg/kg of body weight for 20 days on daily basis. While group (IV) rats is received the PDY extract at 50 mg/kg of body weight for 20 days on daily basis. Whilst blood samples collects after 20days.

Results: The results lipid profile showed a significant increase ($P<0.05$) in serum TC, TG, LDL,VLDL and Atherogenic Index levels between group (II) rats compared with normal control group (I). Serum HDL levels are a significant decreases in group (II) ($P < 0.05$) compared with normal control group (I). PDR and PDY extracts are significantly decreased in serum TC, TG, LDL,VLDL and Atherogenic Index levels between groups (III & IV) compared with group (II). PDR and PDY extracts are significantly increased in serum HDL between groups (III & IV) compared with group (II).

Conclusion: The PDR extract reduces the lipid levels more effective than PDY extract.

Introduction

Many studies have highlighted several pharmacological properties in medicinal plants or their isolated constituents including antihyperlipidemic antioxidant, anti-diabetes, antibacterial, antiviral and anti-ulcer activities (Nergard *et al.*, 2005; Meléndez and Capriles, 2006). Plants have formed the basis of traditional medicine systems that have been in existence for thousands of years. Even in modern times, plant-based systems continue to play an essential role in health care. It has been estimated by the WHO that approximately 80% of the world's population from developing countries rely mainly on traditional medicines for their primary health care. Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants. They also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes (Sajad *et al.*, 2013). *Prunus domestica* commonly known as Plum Alu-Bukhara, Alucha found commonly in Pakistan, India, Afghanistan and Persia Persia (Gupta, 2003; Narayan and

Kumar ,2003). *P.domestica* are fruits rich in phenolic compounds, characterized by relatively high antioxidant activity, higher than oranges, apples or strawberries (Kayano *et al.,* 2002; Leong and Shui, 2002).

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to prevalence and severity of coronary heart diseases (Ananthi *et al.,* 2014). Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death (Davey,1993). Hyperlipidemia characterized by elevated serum total cholesterol and low density and very low density lipoprotein cholesterol and decrease high density lipoprotein are the risk factor for coronary heart diseases. Hyperlipidemia associated lipid disorders are considered to cause the atherosclerotic cardiovascular disease (Saravanan *et al.,* 2003). Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease (Kaesancini and Krauss, 1994) . The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease (Davey and Pekkanen,1992). Currently available hypolipidemic drugs have been associated with number of side effects (Ananthi *et al.,* 2014).

MATERIALS AND METHODS

Collection of Plant:

Prunus domestica L. seeds were collected in July 2012 from local markets in *nasiriyah* city at Iraq, then it was authenticated and specimen of plant was classified in biological department-college of science at university of Thi qar in Iraq by Asst. prof. Hayder Radhi . The seeds were cleaned , washed by distilled water, dried at room temperature for four weeks, ground as powder and kept in Dark glass containers for further use.

Preparation of Solvent extracts:

After shade drying the dried seeds were powdered in mixture grinder. The powdered seeds was macerated with distilled water for 72 hrs at room temperature with occasional stirring. It was then filtered through Whatman filter paper. The filtrate was air dried and stored in refrigerator for further use as PDR and PDY aqueous extract. The yield of the extract was 10%(w/w). During experiment the crude extract was diluted with distilled water just before administration to animals.

Experimental Animals:

Twenty four healthy female rats *(Rattus norvegicus*) weighing (220-250 g) used in the present study. Animals were housed in the animal house of Biology Dept. Science College, Thi-Qar University. Experiments were achieved between May-2013 & June-2013. Animals were housed in iron boxes bedded with wooden chips. During the experimental period six animals were kept in each box and they were housed under standard laboratory conditions (12h light: 12h dark photoperiod (LD) at 22 ± 2 C^{o} and relative humidity 45-55%. Animals were fed on standard rat pellet and tap water *ad libitum*

Effects of PDR and PDY Extracts on Lipid Profile:

- Method of Food Preparing (High Cholesterol Diet)

5% of high cholesterol diet prepared from 50 g of cholesterol dissolved in 200 g of olive oil and heated in a water bath, and after soluble cholesterol in the oil were added to 1 kg of feed, then was cut into small pieces fit with the size of the holes in the lid iron to boxes, to facilitate the process taken up by rats (Cook et al., 1950).

-Laboratory Animals:

Experimental animals were divided into four groups (6 rats in each group) upon the following designed: **Group I**: Control (normal) that were treated daily with (0.2 mL DMSO). **Group II**: Rats were treated with daily high cholesterol diet for 20 days (Cook *et al.,* 1950). **Group III**: Rats were daily treated with (50 mg/kg) of PDR extract besides high cholesterol diet for 20 days. **Group IV**: Rats were daily treated with (50 mg/kg) of PYR extract besides high cholesterol diet for 20 days.

Blood Samples:

5mL of blood were drawn from each animal of experimental groups, by heart puncture method after 12 hours fast. Using 60 gauge syringes, the sample was transferred into clean tube, left at room temperature for 15 minutes for clotting, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in a clean tube in the refrigerator at 2-8ºC until the time of assay.

Biochemical analysis:

The used reagents were supplied by Biolabo (France), and Serum total cholesterol was measured according to (Allan and Dawson, 1979) and Serum TG was measured according to (Tietz *et al.*, 1999). while serum HDL was measured according to (Lopes-Virella , 1977) and measurement of LDL and VLDL according to (Friedwald *et al.*, 1972). LDL , VLDL and atherogenic index concentration was measured as follows:

LDL (mg/dL) = Total cholesterol – (HDL + VLDL)

VLDL(mg/dL) = serum TG /5

Atherogenic Index = LDL / HDL

Statistical Analysis:

Statistical analysis was done using the software SPSS version 17.0; the results were expressed as mean ± standard deviations (Mean ± SD). One way ANOVA-test was used to compare parameters in different studied groups. P-values ($P < 0.05$) were considered statistically significant.

RESULTS AND DISCUSSION

Serum TC, TG, LDL, VDL and Atherogenic Index concentration was changed as shown in table (1). During 20 days, there was a significant increase in the serum concentration of TC, TG, LDL, VDL and Atherogenic Index in group (II) as compared with normal control group (I) ($P < 0.05$). At these times, there was a significant reduction in the serum concentration of TC in groups (III & IV) as compared with group (II) ($P < 0.05$), with greater decrement in TC, TG, LDL, VDL and Atherogenic Index concentration in PDR extract treated group (III) than this with PDY extract treated group (IV) ($p < 0.05$). On the other hand, non significant differences can be observed between (III & IV) groups compared to control group (I). Serum HDL concentration was changed as shown in table (1). During 20 days, there was a significant decrease in the serum concentration of HDL in group (II) as compared with normal control group (I) ($P < 0.05$). At these times, there was a significant increase in the serum concentration of HDL in groups (III & IV) as compared with group (II) ($P < 0.05$), with greater increment in HDL concentration in PDR extract treated group (III) than this with PDY extract treated group (IV) ($p < 0.05$). On the other hand, non significant can be observed between(III & IV) groups compared to control group (I).

Cholesterol is a waxy substance produced by the liver and supplied in the diet. It is vital for the body to function properly (i.e., for hormone and bile acid production). Most of the body's cholesterol is madein the liver, with excess production occurring when the diet is rich in saturated (mainly animal) fats. Triglycerides are composed of fatty acids and glycerol. Like cholesterol, they circulate in the blood but are stored in body fat. When a fatty meal is eaten, triglyceride (and glucose) levels increase significantly. Gradually as the body processes the fat efficiently the level of triglycerides will decrease (Ananthi *et al.*, 2014). About 65 percent of TC is carried by low density lipoproteins. LDL cholesterol (known as "bad" cholesterol) is potentially harmful, becoming dangerous when it is oxidized. It is deposited onto the walls of arteries (eg.coronary arteries) to form atheromatous plaques. Particle size is important because largeparticles are less dangerous than small ones, which readily penetrate the arterial wall and are more easily oxidized (leading to endothelial dysfunction and atheromatous plaque formation) (Caselli *et al.*, 1977). VLDL cholesterol is composed mostly of cholesterol,with little protein. VLDL cholesterol is also often called "bad cholesterol" because it, too deposits cholesterol on artery walls. Increased levels of VLDL cholesterol are associated with atherosclerosis andcoronary heart disease. However, the risk of CVD cannot be judged on TC alone. Other factors make a significant difference. Cholesterol carried by HDL has been removed from the walls of blood vessels. HDL cholesterol is known as "good" cholesterol because it helps to clear excesslipids from the arteries (Ginsberg, 1998).

PDR & PDY provides an excellent source of polyphenolic compounds, fiber, potassium, and vitamin K, and dietary supplementation has been reported to have both antiresorptive and proanabolic effects (Johnson *et al.*,2008). This result is similar to the result of Nishi, *et al*. (2013) who reported that a more pronounced effect was found with low-dose chlorogenic acid than with high dose in elevated plasma cholesterol.

Phenolic components in prunes such as rutin and caffeic acid have also been reported to be active inhibitors of LDL (Teissedre *et al.*, 1996; Rice-Evans *et al.*, 1996; Nardini *et al.*, 1995). In other study, Phenolic components indirectly interferes in cholesterol synthesis pathway and inhibit HMGcoA reductase enzyme, reduce cholesterol production, also increase its bile secretions (Namasivayam, 2002). In the previous study, we have reported that chlorogenic acid showed the most potent anti-LDL oxidative activity than other compounds (Kim et al 2000). HDL reduced due to consumption of antioxidants (as phenolic compounds), acts to prevent thedevelopment of atherosclerosis (Décordé *et al.*, 2008). The phenolic compounds have beneficial effects on human health through their prevention of degenerative pathologies, such as cardiovascular disease and cancer (Kadoma and Fujisawa, 2008). Polyphenolic extract exhibited a significant hypolipidemic effect through the reduction of VLDL

levels (Chidambaram *et al.*,2007). In a previous study, more pronounced effect was found with low-dose of ferulic acid than with high dose in serum concentration of TG (Sri Balasubashini *et al*., 2003).

Table(1): Effects of PDR and PDY extracts on serum lipid profile.

Animal groups	n	Cholesterol mg/dL Mean ± S.D	T.G mg/dL Mean ± S.D	HDL mg/dL Mean ± S.D	LDL mg/dL Mean ± S.D	VLDL mg/dL Mean ± S.D	Atherogenic Index Mean ± S.D
I	6	90.21 ± 1.49 [b]	55.41 ± 2.12 [b]	40.60± 1.50 [a]	38.53 ± 1.01 [b]	11.08± 0.85[b]	0. 94 ± 0.19 [b]
II	6	199.25 ± 1.59 [a]	71.16 ± 2.11 [a]	27.22 ± 1.01 [b]	157.80 ± 2.09 [a]	14.23 ± 0.65 [a]	5.79 ± 0.38 [a]
III	6	109.65 ± 1.28 [b]	59.22 ± 1.99 [b]	38.23 ± 1.12 [a]	59.58± 2.12 [b]	11.84 ± 0.57 [b]	1.55 ± 0.15 [b]
IV	6	115.12 ± 1.60 [b]	61.33 ± 1.85 [b]	37.55 ± 1.13 [a]	65.31 ± 2.02 [b]	12.26 ± 0.74 [b]	1.73 ± 0.17 [b]

Note: Each value represents (Mean ± SD) values with non identical superscript (a, b …etc.) were considered significantly different ($P \leq 0.05$).
n=no. of animals.

CONCLUSIONS

The PDR extract reduces the lipid levels more effective than PDY extract.

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