HYPOLIPIDEMIC AND ANTIOXIDANT ACTIVITY OF PHOENIX DACTYLIFERA L. IN ALBINO WISTAR RATS

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ABSTRACT

Objective: To investigate the hypolipidemic and antioxidant activity of phoenix dactylifera L. in albino wistar rats. Methods: Rats were divided into four groups, groups 1 received water and 5% carboxymethyl cellulose (CMC); groups 2 received control and ethanolic extract phoenix dactylifera groups 3 received triton WR1339 with 5% carboxymethyl cellulose (CMC) and group 4 received triton WR1339 with 5% carboxymethyl cellulose (CMC) and ethanolic extract phoenix dactylifera for the total experimental period of 8 days.

Results: The results showed significantly elevated levels of serum thiobarbituric acid reactive substances (TBARS), total cholesterol, triglycerides, VLDL, LDL and significantly lowered enzymic antioxidant activity of superoxide dismutase (SOD) and HDL in triton WR1339 treated rats compared with the control. Ethanolic extract phoenix dactylifera administration to rats with triton WR1339 induced hyper lipidemia significantly decreased the levels of thiobarbituric acid reactive substances, total cholesterol, triglycerides, VLDL, LDL and significantly elevated the activity of superoxide dismutase and HDL in the serum compared with those of the unsupplemented triton WR1339 treated rats. The different biochemical parameters registered a significant rise in serum of triton WR 1339 treated rats as compared to the normal control. Conclusion: These findings suggest that the hypolipidemic and antioxidant activity of phoenix dactylifera L. in albino wistar rats.

KEYWORDS: Phoenix dactylifera (L); hypolipidemic and antioxidant activity; triton WR 1339.
INTRODUCTION
Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart disease. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death. Hyperlipidemia is characterized by elevated serum total cholesterol, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease.\[1\] Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease.\[2\] Currently available drugs have been associated with number of side effects.\[3\]

Pathophysiology of hyperlipidemia: Decreased clearance of triglyceride-rich lipoproteins due to inhibition of lipoprotein lipase and triglyceride lipase, other factors such as peripheral insulin resistance, carnitine deficiency, and hyperthyroidism may contribute to lipid abnormalities and in nephrotic syndrome, decreased effective plasma albumin circulation results in increased lipoprotein synthesis to maintain plasma oncotic pressure.\[1\] The dates contain at least 6 vitamins. The contents of B6, B3, B2 and B9 vitamins in 100g of dates flesh provide 9% of an adult’s daily RDA (Recommended Dietary allowance).\[4\]

Dates fruits pulp contains phytochemicals like phenolics, sterols, carotenoids, anthocyanins, procyanidins and flavonoids. The ratio and concentrations of these constituents depend on the type of the fruit, stage of fruit picking, location and soil conditions. These phytochemicals also contribute to the nutritional and organoleptic properties of the fruits.\[5\]

Medicinal uses: Leprosy, asthma, bronchitis, tuberculosis, fever, sore eyes or stye, sexual depility, constipation and indigestion. General weakness, cough and throat disorder. Antifungal activity, antioxidant properties, hepatoprotective action, nephroprotective action, gastrointestinal protective activity, anticancerous activity, anti-inflammatory activity, anti-hyperlipidemic activity, immunostimulatory activity, gonadotropic activity and anti-diarrhoeal activity.\[6\]

MATERIALS AND METHODS

Plant Material
Phoenix dactylifera were obtained from a local market in mayiladuthurai. Fresh dates are dried under shade. The coarsely powdered dates was stored in polythene bags at room temperature.
Preparation of ethanolic extract

About 20g of the powdered *Phoenix dactylifera* were exhaustively extracted in 200ml of 70% ethanol using soxhlet apparatus. The residue was filtered and concentrated in vacuo to a syrupy consistency.

Animals

A healthy Swiss albino rats were housed in well ventilated hygienic atmosphere. Animals with 120-150g were used for our study. Animals were fed with commercial rate feed (Saidurga feeds & foods, Bangalore) and tap water ad libitum. After randomization in to various groups, the rats were acclimatized for a period of 2-3 days in the new environment before initiation of experiment.

Chemicals

All the chemicals used in the experiment were of analytical grade.

Experimental Design

In the experiment, a total of 24 rats were used. The rats were divided in to 4 group of 6 rat in each group.

Group I → Control rats received water and orally administered with 5% CMC.

Group II → Control + Ethanolic extract of *Phoenix dactylifera* (200 mg/Kg BW).

Group III → Triton WR 1339 (400 mg/Kg BW), With 5% CMC.

Group IV → Triton WR 1339 (400 mg/Kg BW) + Ethanolic extract of *Phoenix dactylifera* (200 mg/Kg BW).

Sample collection

After 8 days of herbal treatment, the blood sample were collected from the anaesthetized rats by puncturing the orbital sinus. After the collection of blood, it was allowed to stand for 10 mts.

Biochemical measurements

Serum total cholesterol Zlatkis *et al.*\(^7\) triglycerides Foster and Dunn\(^8\) VLDL, LDL and HDL Nerurkar and Tarkar\(^9\), TBARS Yagi\(^10\) SOD Kakkar *et al.*\(^11\)

Statistical analysis

Data were analysed by one way analysis of variance followed by Duncan’s multiple range test using SPSS for Windows (v. 11.0; SPSS Inc., Chicago, IL, USA). Results are presented...
as means ± SD of six rats in each group. Values of P < 0.05 were regarded as statistically significant and the data are represented as mean ± SD for the absolute values or percent of controls as indicated in the vertical axis legends of figures. The statistical significance of differential findings between the experimental groups and control was determined.

RESULTS

Table 1: Level of total cholesterol, triglycerides, VLDL, HDL, LDL, TBARS and SOD in serum of normal and experimental groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>VLDL (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>TBARS (nmoles/mL)</th>
<th>SOD (U* (mg protein)-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82.11±7.90a</td>
<td>55.50±5.34a</td>
<td>15.97±1.53a</td>
<td>24.70±2.37b</td>
<td>26.18±2.52a</td>
<td>3.37±0.48a</td>
<td>10.60±1.04</td>
</tr>
<tr>
<td>Control + Phoenix dactylifera</td>
<td>87.31±8.40a</td>
<td>60.07±5.78a</td>
<td>16.11±1.55a</td>
<td>25.61±2.46b</td>
<td>30.70±2.95a</td>
<td>12±0.47a</td>
<td>10.69±1.05</td>
</tr>
<tr>
<td>Triton</td>
<td>204.51±19.68b</td>
<td>479.40±6.14b</td>
<td>24.48±2.35b</td>
<td>19.08±1.83a</td>
<td>158.59±15.26b</td>
<td>5.26±0.60b</td>
<td>6.39±0.61</td>
</tr>
<tr>
<td>Triton + Phoenix dactylifera</td>
<td>92.41±8.89a</td>
<td>71.40±6.87a</td>
<td>14.68±1.41a</td>
<td>24.78±2.38b</td>
<td>31.93±3.07a</td>
<td>3.45±0.49a</td>
<td>9.68±0.98</td>
</tr>
</tbody>
</table>

Values are means ± S.D. of six rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (Duncan's multiple range test).

U*-Enzyme required for 50% inhibition of NBT reduction / min/mg Hb for serum

Table 1 Shows the activities of total cholesterol and triglycerides VLDL, LDL, TBARS, HDL and SOD. The level of total cholesterol and triglyceride, VLDL, LDL and TBARS were significantly increased in high fat diet fed rats as compared to the control rats. Supplementation with Phoenix dactylifera extract and high fat diet treated rats (group 4) significantly decreased the level of serum total cholesterol and triglyceride as compared to the unsupplemented high fat diet fed rats (group 3; p<0.05). Supplementation of Phoenix dactylifera extract alone did not produce any significant changes in the levels of total cholesterol and triglyceride, VLDL, LDL and TBARS. In contrast, the level of serum HDL and SOD were significantly decreased in high fat diet fed rats (group 3) as compared to the control rats (group 1). Supplementation with Phoenix dactylifera extract to high fat diet fed rats (group 4) significantly (p<0.05) increased the level of HDL and SOD as compared to the unsupplemented high fat diet fed rats (group 3).
DISCUSSION
Triton WR1339 is a non ionic detergent with surface tension reducing properties \[12\]. Systemic administration of the Triton WR1339 is reported to inhibit lipoprotein lipase and there by elevates serum cholesterol and a triglyceride level in rats \[13\]. Cholesterol is essential for human life. It builds and repairs cells and it is used to produce sex hormones like estrogen and testosterone. It is converted to bile acids to help digestion and formation of vitamin D on the skin’s surface and it is found in large amount in brain and nerve tissue. Blood cholesterol elevation is due to some external disturbance of lipid metabolism \[14\]. The excess of triton WR-1339 increases the TG level which is one of the causes of hardening of the arteries \[13\]. Elevated levels of TG and blood cholesterol are the major risk factors for heart diseases. The high concentration of plasma cholesterol observed in triton WR-1339 rats as compared to the control rats in the present study agree with previous findings \[15\]. Increase in serum levels of cholesterol and triglycerides due to triton WR-1339 injection result mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism \[16\]. Triton WR-1339 induced hyperlipidemia is biphasic. Triton WR-1339 causes hyperlipidemia via inhibition of lipolysis of triglyceride (TG) rich lipoprotein and increase in hepatic cholesterol synthesis by enhancing the activity of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG CoA) reductase as the rate-limiting enzyme in cholesterol synthesis. A rise in LDL may cause deposition of cholesterol in the arteries and aorta and hence it is a direct risk factor for coronary heart disease. \[17\]

We observed significantly reduced levels of TC and TG in the serum of *Phoenix dactylifera* extract treated rats, thus showing the beneficial effect of *Phoenix dactylifera* extract. In this context, Prasanna, \[18\] have reported decreased in cholesterol and TG levels on *Phoenix dactylifera* extract treated rats. Regarding the mechanism of action these plant extracts may have caused decrease in serum cholesterol and triglycerides. \[19\]

Decreased level of HDL and increased LDL and VLDL levels were observed in triton WR-1339 treated rats compared to control. These lipoproteins are chemically modified by oxidation or glycation in the initial stages of atheroma formation. The oxidized or modified lipoproteins do not react with LDL receptors leading to esterification of cholesterol and conversion of macrophages to foam cells in atherosclerosis and obesity-associated disorders. Increased level of serum LDL-cholesterol results in increased risk for the development of atherosclerosis. \[20\] It is well known that HDL-cholesterol levels have a protective role in
coronary artery disease. HDL-cholesterol is reported to have a preventive function against atherogenesis since an independent inverse relationship between blood HDL-C levels and cardiovascular risk incidence has been reported [21]. HMG CoA reductase is the rate-limiting enzyme in the cholesterol biosynthesis pathway. It converts HMG CoA to mevalonate. HMG CoA reductase activity was indirectly measured in terms of the ratio between HMG CoA and mevalonate. The ratio was found to be inversely proportional to HMG CoA reductase activity, indicating that an increase in the ratio inferred a decrease in the enzyme activity. [22] Elevated LDL levels promote atherosclerosis and other cardiovascular disease. Low level of HDL is associated with high risk of coronary artery disease. [23]

We observed significantly reduced levels of VLDL and LDL in the serum of Phoenix dactylifera extract treated rats, thus showing the beneficial effect of Phoenix dactylifera extract. These results are in agreement with the observations of previous researchers and this could be due to decreased in absorption of cholesterol or an increase in HDL cholesterol [18]. The lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformability, reduced erythrocyte survival and lipid fluidity [24]. The thiobarbituric acid assay is the most popular method of estimation of malondialdelyde level, which is an indication of lipid peroxidation and free radical activity. The increase in lipid peroxidation, a degradative process of membraneous polyunsaturated fatty acid has been suggested by the increase in malondialdelyde in triton WR-1339 induced hyperlipidemic in circulation. In our study, there was significantly elevated level of TBARS in circulation of rats on triton WR-1339 treatment (Group 3). These results are in agreement with the observations of previous researcher [25]. The elevated levels of LPO products in the plasma in our study may be due to diffusion of LPO products from the site of inflammation into circulation [26]. Furthermore, RBCs are rich in polyunsaturated fatty acids, molecular oxygen, and ferrous ion and thus erythrocytes are easily susceptible to lipid peroxidative damage. [27]

Administration of Phoenix dactylifera extract to triton WR-1339 treated rats significantly decreased the level of TBARS, which may be due to the antioxidant property. In this context, Naskar et al [28] have also reported that the Phoenix dactylifera has antioxidant activity in invivo and invitro studies. Decreased lipid peroxidation on Phoenix dactylifera administration suggests the decreased impact of reactive oxygen species (ROS) on lipid membrane, thus increased protection against hyperlipidemia.
Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells prevent damage to lipids, proteins, enzymes, carbohydrates DNA. There are some synthetic antioxidant compounds such as butylatedhydroxytoluene, butylatedhydroxyanisole tertiary butyl hydroquinone which are commonly used in processed foods.\(^{[29]}\) SOD acts as scavengers of free radicals and reduce the toxicity of oxygen. Oxygen toxicity may be caused by the superoxide free radical tissues are protected from superoxide by the specific enzyme superoxide dismutase. SOD is a ubiquitous chain-breaking antioxidant found in all aerobic organisms. It is a metalloprotein widely distributed in all cells and plays an important protective role against oxidative damage induced by reactive oxygen species. SOD converts superoxide ion (O\(_2^-\)) to hydrogen peroxide (H\(_2\)O\(_2\)) and the hydrogen peroxide thus formed is degraded by CAT and GPx. The decrease in the SOD activity may be associated with the elevation of the intracellular concentrations of H\(_2\)O\(_2\).\(^{[30]}\) The decrease in SOD activity could be due to oxidative inactivation of the enzyme due to excessive reactive oxygen species generation.\(^{[31]}\)

Administration of *Phoenix dactylifera* extract to triton WR-1339 treated rats increased the levels of SOD. This may be due to their increased utilization to scavenge the significantly elevated levels of ROS that are formed on triton WR-1339 treatment. This suggests that the maintenance of SOD by *Phoenix dactylifera* extract was mainly due to inactivation of ROS via its radical scavenging effects, sparing antioxidant enzymes such as SOD.\(^{[32]}\)

In the present bring out the hypolipidemic activity on phoenix dactylifera against triton WR 1339 induced hyperlipidemic in rats. The use of dates as iron tonic seems to be effective. To rationalise use of the plant however, more work needs to be carried out at molecular level.

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