Hypolipidemic and antioxidant activity of aqueous extract of fruit of *Withania coagulans* (Stocks) Dunal in cholesterol-fed hyperlipidemic rabbit model

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*Withania coagulans* (family: *Solanaceae*, English: *Indian Cheese Maker*, Hindi: *Doda Paneer*) fruit is known for its ethanopharmacological significance in health care system of India. Diet rich in high-fat is an important risk factor for diabetes, atherosclerosis and macro and microvascular complications. Treatment with aqueous extract of fruit of *W. coagulans* (aqWC; 250 mg/kg body weight) in cholesterol-fed animals resulted in significant decrease in the levels of total cholesterol, triacylglycerol, low density lipoprotein, tissue lipid content and acetyl CoA carboxylase activity whereas, the level of high density lipoprotein and activity of HMGCoA reductase also recovered partially. Treatment with aqWC also significantly decreased plasma lipid peroxide levels and increased reduced glutathione and superoxide dismutase activities. These results suggest that the aqueous extract of *W. coagulans* has potent lipid lowering and antioxidant activities.

**Keywords:** Hypercholesterolemia, Lipid lowering activities, Lipid metabolism, Oxidative stress, *Withania coagulans*

Daily meals rich in high fat and cholesterol may lead to hyperlipidemia with increased oxidative stress, which are major risk factors for various metabolic disorders i.e. diabetes, cardiovascular diseases (CVD) and atherosclerosis\(^1\). Various synthetic drugs and statins in particular, are used widely for their potent cholesterol lowering effect in patients, however these drugs are not free from side effects. The drugs from medicinal plants offer an attractive alternative, due to their negligible side effects and cost effectiveness\(^2\).

Fruit of *Withania coagulans* (Stocks) Dunal (family: *Solanaceae*; English: Indian Cheese Maker, Hindi: Doda Paneer) is known to possess a variety of biological activities\(^3\). Hypolipidemic effect of fruit extract of *W. coagulans* at a dose of 1000 mg/kg body weight has been reported in high fat diet induced hyperlipidemia in rats\(^4\). Antihyperlipidemic and antioxidant effects of low dose of aqueous extract of *W. coagulans* fruit (aqWC; 250 mg/kg body weight) in nicotinamide-streptozotocin induced diabetes mellitus in rats have been reported\(^5-6\). With regard to lipoprotein metabolism, rabbits have several aspects similar to those of humans and develop hypercholesterolemia after a few days of receiving high fat diet\(^7\). However, there is no report available about the hypolipidemic and antioxidant effects of aqWC in diet induced hypercholesterolemia in rabbits till date. Therefore, using rabbit as experimental model may provide more relevant information on the harmful effects of hyperlipidemia and oxidative stress and the modulatory role of aqWC extract.

In the present work, the effect of cholesterol feeding with or without simultaneous administration of aqWC has been studied on lipid profile, metabolic enzymes of lipid metabolism and oxidant-antioxidant parameters in blood and tissue homogenates in high cholesterol-fed rabbits.

**Materials and Methods**

*Plant material and preparation of fruit extract—*
Fruits of *W. coagulans* (English: *Indian Cheese Maker*, Hindi: *Doda Paneer*) were purchased locally and were identified and authenticated by CSIR-National Institute of Science Communication and Information Resources, New Delhi (Voucher number: NISCAIR/RHMD/Consult/-2008-09/979/10). Whole fruits of *W. coagulans*, after removal of calyx and pedicle, were soaked in distilled water and kept overnight at 4 °C. Next day, the mixture was filtered through a filter paper/sterile muslin cloth. The filtrate was lyophilized and the sticky brown powder

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obtained (yield 16% w/w) was stored at 4 °C, which was re-dissolved in water and fed orally to animals by intragastric tube.

Quantification of total phenolic and flavonoid content—The phenolic content of water extract of *W. coagulans* fruit (aqWC) was estimated by Folin-Ciocalteu reagent method as described by Singleton and Rossi\(^8\) and expressed as gallic acid equivalents (mg/g dry weight of aqWC extract). Flavonoids were extracted and estimated by the method of Chang *et al.*\(^9\) and expressed as quercetin equivalents (mg/g of aqWC).

**Experimental animals and treatment regimes**—Male albino rabbits weighing 1.5 ± 0.2 kg obtained from central animal house of University College of Medical Sciences and GTB Hospital, Delhi were housed in an air-conditioned room at 22±2 °C with 50 ± 5% RH: 12 h L:D cycle and these conditions were maintained throughout the experimental period. Rabbits were fed standard laboratory diet and water *ad libitum*. Ethical clearance was obtained from the Institutional Animal Ethics Committee for Animal Research (IAEC-AR) at University College of Medical Sciences and GTB Hospital, Delhi (UCMS/IAEC/16-17/30th December 2009) and experiments were carried out as per the guidelines of the committee.

Rabbits were divided into following three groups of 5 each. Gr. I: healthy control; Gr. II: cholesterol-fed (100 mg/kg body weight suspended in groundnut oil) daily for six weeks as standardized by Shukla *et al.*\(^2\), whereas Gr. III: animals were fed with cholesterol (100 mg/kg body weight) + aqWC (250 mg/kg body weight) orally for 6 weeks.

Collection of blood and tissues—After 6 weeks of treatment, rabbits were fasted overnight and their blood for the estimation of biochemical parameters was withdrawn from marginal ear vein/artery through 23G butterfly needle\(^10\) in EDTA coated vials and centrifuged at 1300 \(\times\) g for 10 min to obtain plasma. Subsequently animals were anesthetized by single intraperitoneal injection of pentobarbital at a dose of 150 mg/kg body weight and sacrificed and liver and heart excised promptly. Both tissues were washed with cold 0.15M KCl solution and stored at -70 °C till use. Lipid profile, activities of lipid metabolizing enzymes, lipid peroxidation, and antioxidant parameters were determined in blood and various tissues homogenates.

Biochemical parameters—Total cholesterol (TC), triacylglycerol (TAG) and HDL-cholesterol (HDL-C) were estimated in fasting plasma samples by using kits from Accurex Biomedical Pvt. Ltd, Mumbai, India. Low density lipoprotein (LDL-C) was calculated by Friedwald’s and Fredrickson’s Equation\(^11\). HMG-CoA and mevalonate levels in liver and heart tissue homogenates were estimated spectrophotometrically and the ratio of two was taken as an index of HMG CoA reductase activity, with decreased ratio indicating increased activity and vice versa\(^12\). Acetyl CoA carboxylase (ACC) activity in liver and heart homogenates was measured as per Nakanishi and Numa\(^13\). Estimation of reduced glutathione (GSH) in whole blood and tissue homogenates was carried out by the method of Beutler *et al.*\(^14\) and Ellman\(^15\) respectively using dithionitrobenzene (DTNB). The extent of lipid peroxidation in blood and tissue homogenates was estimated by measuring thiobarbituric acid reactive substances (TBARS) as described by Satoh\(^16\) and Wills\(^17\) respectively. Superoxide dismutase activity (SOD) was measured in erythrocytes and tissue homogenates by the method of Nandi and Chatterjee\(^18\). Total lipids from tissues were extracted and estimated by the method of Folch *et al.*\(^19\). Hemoglobin content of the blood was estimated by cyanmethemoglobin method using Drabkin’s reagent\(^20\) and protein content was determined by Lowry’s method\(^21\).

Histopathological study—For the histological study, tissues were fixed in 10% formalin and embedded in paraffin wax; 3-4 µm thickness sections were cut using a microtome, dehydrated in graded alcohol and stained with hematoxylin and eosin. The specimens were evaluated with a light microscope. All histopathological changes were examined by the pathologist.

Statistical analysis—All values were expressed as mean ± SD. The significance of difference between the mean of treated and untreated groups was calculated by one way ANOVA followed by Tukey’s multiple comparison test for one time-point studies using SPSS software 17.0 and value of \(P<0.05\) was considered significant.

Results

Total phenolic and flavonoid content in aqWC extract—The total phenolic content and flavonoid content were quantified in crude extract of aqWC, phenolic content was found to be 21.6±2.1 mg (gallic acid equivalent/g) of aqWC extract and flavonoid...
content was 10.3±1.7 mg/g aqWC (quercetin equivalent).

**Effect of aqWC on biochemical parameters**—Rabbits fed cholesterol up to six weeks showed gradual and significant increase in TC, TAG and LDL-C levels (310, 196 and 686% respectively) and decreased HDL-C levels (35%) as compared to healthy control (P<0.05). Whereas rabbits receiving cholesterol + aqWC showed significantly decreased TC, TAG, LDL-C levels and increased HDL-C levels (P<0.05), thus showing the beneficial effects of aqWC. However these levels were still significantly higher from healthy controls (P<0.05) (Table 1). The body weight of cholesterol fed rabbits was significantly increased whereas it was decreased in cholesterol + aqWC-treated rabbits (P<0.05) (data not shown).

**Effect of aqWC on liver histology**—Rabbits fed with cholesterol for 6 weeks developed high degree of steatosis with severe cytoplasmic vacuoles and swelling of hepatocytes (Fig. 1b) simultaneous administration of aqWC along with cholesterol resulted in prevention of hepatic fatty deposition in hepatocytes (Fig. 1c).

**Effect of aqWC on total lipid content in tissue homogenates**—The lipid content in liver and heart homogenates of cholesterol-fed rabbits was significantly increased by 65 and 70% respectively as compared to healthy controls (P<0.05). Whereas, cholesterol + aqWC fed rabbits showed significantly decreased lipid content in liver and heart (27% and 20% respectively) as compared to only cholesterol-fed rabbits (P<0.05) (Table 2).

**Effect of aqWC on lipid metabolizing enzymes in liver and heart homogenates**—The effect of aqWC on the activities of lipid metabolic enzymes are demonstrated in Table 2. HMG CoA reductase activity is inversely proportional to HMGCoA/mevalonate ratio, and this ratio increased significantly in liver and heart tissue homogenates of cholesterol-fed rabbits, thus showing that the activity of HMG CoA reductase decreased significantly in cholesterol-fed rabbits as compared to healthy controls (P<0.05), whereas HMG CoA reductase activity increased in cholesterol+aqWC-fed rabbits as compared to only cholesterol-fed animals (HMG CoA/mevalonate ratio decreased). Acetyl CoA carboxylase activity in liver and heart homogenates of cholesterol-fed rabbits increased significantly as compared to healthy controls. However after treatment with aqWC, rabbits showed decreased ACC activity in tissue homogenates as compared to only cholesterol-fed rabbits (P<0.05).

**Effect of aqWC on oxidant/antioxidant levels**—The cholesterol-fed rabbits showed significantly increased lipid peroxide and decreased GSH and SOD as compared to healthy controls (P<0.05). However, rabbits receiving cholesterol + aqWC showed significant reversal in the levels of above biochemical parameters as compared to only cholesterol-fed rabbits (P<0.05). Cholesterol-fed rabbits also showed significant increase in the lipid peroxide and decrease GSH as well as SOD activity in blood, liver and heart homogenates as compared to healthy controls (P<0.05). Whereas, rabbits receiving cholesterol + aqWC showed significant improvement in the levels of above biochemical parameters (P<0.05) (Table 2).

**Discussion**

Liver plays a key role in lipid metabolism however, excessive accumulation of lipids within hepatocytes due to imbalance between lipid formation and lipid degradation and excretion leads to fatty liver and hepatic steatosis. Clinically it is known that hypercholesterolemic/dyslipidemic patients show elevated LDL-C and HDL-C in plasma. Acetyl CoA carboxylase in liver and heart homogenates of cholesterol-fed rabbits increased significantly as compared to healthy controls. However after treatment with aqWC, rabbits showed decreased ACC activity in tissue homogenates as compared to only cholesterol-fed rabbits (P<0.05).

### Table 1—Effect of 6 weeks aqWC treatment on lipid profile and oxidant-antioxidant parameters in blood of cholesterol-fed rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>88.2 ± 7.3</td>
<td>362.1 ± 17.8*</td>
<td>157.8 ± 13.8**</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dL)</td>
<td>73.7 ± 6.8</td>
<td>219.0 ± 9.7*#</td>
<td>132.5 ± 14.5*#</td>
</tr>
<tr>
<td>High density lipoprotein (mg/dL)</td>
<td>36.0 ± 3.3</td>
<td>23.3 ± 3.6*#</td>
<td>32.1 ± 3.2*</td>
</tr>
<tr>
<td>Low density lipoprotein (mg/dL)</td>
<td>37.5 ± 2.8</td>
<td>294.9 ± 12.2*#</td>
<td>98.4 ± 10.6*#</td>
</tr>
<tr>
<td>Lipid peroxides (nmol/mL)</td>
<td>1.2 ± 0.9</td>
<td>6.1 ± 0.9*#</td>
<td>3.4 ± 0.7*#</td>
</tr>
<tr>
<td>Reduced glutathione (mg/g Hb)</td>
<td>4.8 ± 0.9</td>
<td>1.4 ± 0.3*#</td>
<td>4.2 ± 0.7*</td>
</tr>
<tr>
<td>Superoxide dismutase (U/g Hb)</td>
<td>2066 ± 86.0</td>
<td>1231 ± 71.2*#</td>
<td>1802 ± 46.8*</td>
</tr>
</tbody>
</table>

P<0.05* vs Group I; * vs Group II. Group I: healthy control; Group II: cholesterol-fed control; Group III: cholesterol+aqWC treated.
Many medicinal plants have been shown to improve lipid profile, decrease cholesterol and modulate the activity of various enzymes. Decrease in plasma cholesterol and liver lipid content may be mediated through increased excretion of cholesterol in bile and decreased enterohepatic circulation, resulting in increased synthesis of bile acids using endogenous cholesterol which ultimately leads to lower hepatic cholesterol and TAG accumulation as has been reported in Osmium sanctum fed rats. Further, modulation of enzymes of lipid metabolism is also achieved through various mechanisms.

Treatment with flavanoids and polyphenols has been reported to increase HDL-C level followed by decrease in LDL-C and TAG in hyperlipidemic rats. Fruit of W. coagulans has been reported to possess various phenolic compounds, flavonoids and steroidal lactones (Withanolides) etc which may lead to increase in HDL-C and decreased TC, LDL-C and TAG levels as well as lipid accumulation in the liver.

HMG CoA reductase is a rate limiting enzyme of cholesterol biosynthesis which is controlled by feedback regulation. HMG CoA reductase also increases the mRNA expression of LDL-receptors and increases the excretion of cholesterol from liver. The aqWC+cholesterol treated rabbits showed decreased LDL-C level and this effect may be mediated through increased hepatic clearance of cholesterol, by increased activity of HMG CoA reductase. Acetyl CoA carboxylase plays an essential role in regulating fatty acid synthesis. In abundance of cholesterol, ACC activity increases which helps to increase fatty acid biosynthesis whereas polyphenols can phosphorylate ACC and inhibit the activity of this enzyme, thereby inhibiting fatty acid synthetic pathways, thus preventing fat accumulation.

Recent findings indicate that some medical herbs have both a lipid-lowering ability and antioxidative parameters to suppress lipid peroxide production and then eventually may contribute to their effectiveness in preventing atherosclerosis and in protecting various organs at risk from hyperlipidemia. Free radical generated oxidative stress is a causative factor for atherosclerosis and CVD. It is well known that plants have natural antioxidants and are shown to protect from oxidative stress induced cell injury. Polyphenols and flavonoids, which are effective proton donors, have an important role in stabilizing lipid oxidation and are thus responsible for antioxidant activity. Polyphenols might also be effective in reducing lipid peroxidation due to the arrangement of hydroxyl groups on the benzene.

Fig. 1—Histopathology of liver tissue in different groups A: Healthy control, B: Cholesterol-fed C: Cholesterol+aqWC fed. HE×40)
Several studies have suggested that feeding with high cholesterol diet depresses the antioxidant defense mechanisms due to increased lipid peroxidation and formation of free radicals. During oxidative stress, increased lipid peroxidation results in increased formation of malondialdehyde. In the present study, cholesterol-fed rabbits showed increased lipid peroxidation, which were reduced significantly in rabbits receiving cholesterol + aqWC. Glutathione (GSH) is a major non enzymatic endogeneous antioxidant, which counteracts free radical-mediated tissue damage. Depletion of GSH in blood, liver and heart suggests increased oxidative stress. The cholesterol-fed rabbits showed significantly decreased GSH levels whereas cholesterol + aqWC treated rabbits showed significantly increased GSH and suppressed oxidative stress. Superoxide dismutase metabolizes the superoxide anions and provides an effective defense against endogenous and exogenous generation of the free radicals. Restoration of SOD activity in cholesterol + aqWC fed animals may be due to the presence of phytochemicals which are known to possess antioxidant activity.

**Conclusions**

In the present study, the lipid lowering activity of aqueous extract of *W. coagulans* and amelioration of oxidative stress have demonstrated in hypercholesterolemic rabbit model. These results suggest that aqueous extract of *W. coagulans* may improve dyslipidemia caused due to high cholesterol/fat consumption. The phytochemicals present in aqWC may decrease the levels of cholesterol and triglycerides and modulate the activity of enzymes of lipid metabolism. In addition, it also has antioxidant properties which protect from oxidative stress, particularly in hyperlipidemic state suggesting that aqWC may have potential benefits in humans. Further studies are necessary to isolate active phytochemicals and explore the potential use of aqWC as an alternative drug for lipid disorders in humans.

**Conflict of interest**

Author(s) declare that they have no competing interests.

**Acknowledgment**

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**References**


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**Table 2**—Effect of 6 weeks aqWC treatment on total lipid content, enzyme activities and oxidant - antioxidant parameters in liver (L) and heart (H) of cholesterol-fed rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipid content (mg/g tissue)</td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
</tr>
<tr>
<td>L</td>
<td>0.37 ± 0.03</td>
<td>0.61 ± 0.06</td>
<td>0.44 ± 0.10</td>
</tr>
<tr>
<td>H</td>
<td>0.24 ± 0.02</td>
<td>0.41 ± 0.03</td>
<td>0.33 ± 0.07</td>
</tr>
<tr>
<td>HMG CoA Reductase (HMGCoA/mavalonate ratio)</td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
</tr>
<tr>
<td>L</td>
<td>5.15 ± 0.95</td>
<td>8.34 ± 1.02</td>
<td>6.72 ± 1.10</td>
</tr>
<tr>
<td>H</td>
<td>2.90 ± 0.33</td>
<td>6.33 ± 0.79</td>
<td>4.54 ± 0.85</td>
</tr>
<tr>
<td>Acetyl CoA carboxylase (U/mg protein)</td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
</tr>
<tr>
<td>L</td>
<td>0.34 ± 0.03</td>
<td>0.66 ± 0.03</td>
<td>0.52 ± 0.04</td>
</tr>
<tr>
<td>H</td>
<td>0.21 ± 0.01</td>
<td>0.50 ± 0.01</td>
<td>0.41 ± 0.04</td>
</tr>
<tr>
<td>Lipid peroxidation (µmol/g wet tissue)</td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
</tr>
<tr>
<td>L</td>
<td>2.10 ± 0.30</td>
<td>3.58 ± 0.28</td>
<td>2.81 ± 0.31</td>
</tr>
<tr>
<td>H</td>
<td>1.13 ± 0.33</td>
<td>1.93 ± 0.05</td>
<td>1.56 ± 0.02</td>
</tr>
<tr>
<td>Reduced glutathione (µmol/g wet tissue)</td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
</tr>
<tr>
<td>L</td>
<td>7.88 ± 0.45</td>
<td>5.46 ± 0.28</td>
<td>6.67 ± 0.51</td>
</tr>
<tr>
<td>H</td>
<td>3.78 ± 0.30</td>
<td>2.35 ± 0.41</td>
<td>3.21 ± 0.14</td>
</tr>
<tr>
<td>Superoxide dismutase (U/mg protein)</td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
</tr>
<tr>
<td>L</td>
<td>118.7 ± 4.1</td>
<td>82.4 ± 9.8</td>
<td>109 ± 8.2</td>
</tr>
<tr>
<td>H</td>
<td>52.2 ± 7.4</td>
<td>31.6 ± 5.6</td>
<td>42.8 ± 4.9</td>
</tr>
</tbody>
</table>

P<0.05 vs Group I; * vs Group II. Group I: healthy control; Group II: cholesterol-fed control; Group III: cholesterol + aqWC treated.


