RESEARCH ARTICLE
Evaluation of Health Biomarkers in Hyperlipidemic Albino Rabbits after Treatment with Withania somnifera and Lactuca scariola
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ABSTRACT
The experiment was conducted to evaluate the efficacy of Withania (W.) somnifera (commonly known as Ashwagandha) and Lactuca (L.) scariola (commonly known as Kahu) on serum health markers of hyperlipidemic rabbits. Rabbits were randomly divided into five groups attributed as negative control, positive control, simvastatin treated group, W. somnifera treated group and L. scariola treated group. Except negative control group, all other groups were fed with atherogenic diet for first 90 days to induce experimental hyperlipidemia, followed by therapeutic regime after which the treatment (comprised of different plants extracts along with regular feed) was started to each group. Blood sampling was done thrice at days 30, 60 and 90 post treatment. The serum was separated to determine the total antioxidant capacity (TAC), total oxidant status (TOS), paraoxonase activity, arylesterase activity, ceruloplasmin activity and homocysteine concentration. Study revealed that both plants has significant efficacy (P<0.05) in reducing overall oxidative stress and improving health biomarkers of the body affected by the hypercholesterolemia however, L. scariola showed more promising results in comparison to W. somnifera. It is therefore recommended that L. scariola along with other plant formulations would be a good therapy in evading the drastic effects of obesity/hyperlipidemia with the minimal side effects as compared to the synthetic drugs.

INTRODUCTION
Hyperlipidemia is characterized by elevated serum total cholesterol, low density and very low density lipoprotein cholesterol levels. It is the most common risk factor for the development of atherosclerosis and its clinical consequences like coronary artery disease and myocardial infarction (Tietge, 2014). Approximately 12 million people reportedly die of cardiovascular disease each year worldwide (Yokozawa et al., 2003). Hence, a logical strategy to prevent or treat atherosclerosis and reduce the incidence of cardiovascular disease events is to target the hyperlipidemia by lipid-lowering drugs. Many commercially available drugs like atorvastatin, rosuvastatin and simvastatin are recommended for hyperlipidemic patients (Demir et al., 2013). However, along their desired effects, these drugs also have well known side effects including rhabdomyolysis, hepatotoxicity etc. (Speight, 1987). The development of new drugs with least side effects require considerable research and development beside being a time consuming and expensive project (Lee et al., 2011). However medicinal properties of many plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. More and more species are being gradually added in the Materia Medica (Ikram, 1983; Kala et al., 2006). On the same note, different medicinal plants like Terminalia pallida Linn (Sampathkumar et al., 2011) and Ocimum sanctum Linn (Triveni et al., 2013) have been reported for having their antihyperlcpidemic effects. However, there is still a great death of searching new lipid-lowering agents with minimal side effects from natural sources.
The seeds of the *Lactuca scariola* has been widely used in the traditional ethnomedical practices. The scientific analysis of *L. scariola* (Tukhme Kahu) proves many of the activities mentioned in Unani classical literature (Said, 1969). *L. scariola* has been used as antipyretic, anti-cancer, antibacterial, antifungal, spasmytotic, bronchodilator and vasorelaxant agent (Yadava and Jharbade, 2008, Janbaz et al., 2013, Mohammad, 2013). The antipyretic activity of the *L. scariola* is considered due to the presence of an alkaloid, lactucin in its seeds (Agarwal, 1997) while antibacterial activity is believed to be due to triterpenoid saponin present in its stem (Yadava and Jharbade, 2008).

The extract of *Withania somnifera* (commonly known as Ashwagandha) seeds have undergone many pharmacological studies to describe its multiple pharmacological properties (Dagenais, 2000). It has been extensively used plant drug especially its roots in the Indian system of medicine (Mohanty et al., 2004). Its therapeutic efficacy as an antioxidant, anti-inflammatory, antibacterial, hypoglycemic, cardioprotective, immunomodulatory, adaptogenic, and anticancerous agent has been reported in different studies (Bhattacharya et al., 2000; Dhuley et al., 2000, Gupta and Rana, 2007, Maliyakkal et al., 2013, Hill & Grubb, 2014). The main active constituent of *W. somnifera* from which its primary medicinal properties radiate, are based upon the actions of certain steroidal alkaloids and lactones as a class of constituents called withanolides (Lavie et al., 1965). However, it has been anticipated that its antioxidant property is due to the induction of a number of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, catalase, the oxidants such as glutathione and proteins like heat shock protein (Mohanty et al., 2004).

Keeping in view the importance of medicinal properties of these plants, the current study was designed to evaluate the effects of *W. somnifera* and *L. scariola* on the important physiological health markers in the hyperlipidemic rabbits. A little research is found in literature archive regarding antihyperlipidemic role of these agents in the diseases (Lalsare and Chutervedi, 2010) Therefore, present study was planned to elucidate effect of *W. somnifera* and *L. scariola* on oxidative and biochemical profile of hyperlipidemic Albino rabbits.

**MATERIALS AND METHODS**

Forty adult albino rabbits of either sex were procured from a local experimental animal supplier of Faisalabad, Pakistan and screened for any presence of disease and abnormality. The screened animals were kept under similar environmental conditions in individual iron cages at the Experimental Animal Room of Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. All guidelines by Society of Ethics of Animals, University of Agriculture Faisalabad, Pakistan were strictly followed during the whole experiment and approval was taken to conduct the experiment.

The rabbits were acclimatized for seven days, afterwards, the rabbits were fed atherogenic diet including cholesterol (Food grade) fed at 500 mg/kg b.wt. for 90 days to induce hyperlipidemia which was confirmed after 90 days by estimating serum lipid profile. Thereafter animals were randomly divided into five groups having 8 rabbits in each group attributed as Negative Control; NC (Normal diet i.e. atherogenic without any treatment); Positive Control; PC (Atherogenic diet including cholesterol (Food grade) fed at 500 mg/kg b.wt., without any treatment); Simvastatin group; SM (Atherogenic diet and treated with Simvastatin, survive*, Werrick Pharmaceuticals, Islamabad, Pakistan at 0.6 mg/kg b.wt); WS Group (Atherogeinc along with *W. somnifera* was given at a dose level of 0.5 g/kg b.wt.) and LS Group (Atherogeinc diet along with *L. scariola* was given at a dose level of 6.0g/kg b.wt.). The diet composition has been presented in the Table 1.

The negative control group was given normal lucern (*Medicago sativa*) feed, while rest of all groups were given atherogenic diet (Chen et al., 2010 and Woo et al., 2009) as presented in table 1. The feeding was done twice a day in the morning and evening. However, fresh drinking water was available *ad libitum*. The treatment was given for 90 days to onward after the rabbits were hyperlipidemized. The plants were procured from local market of Faisalabad and taxonomic identification was confirmed with the help of a botanist. The plants were thoroughly washed, dried, and grinded to make fine powder. The methanolic extract was taken and lyophilized. The lyophilized powder was mixed with water and ingested with stomach tube to the albino rabbits.

Blood samples were collected at day 30, 60 and 90 post treatment from jugular vein of the rabbits. Blood serum was extracted by centrifugation at 1900 × g for 5 minutes and stored at -20°C till further analysis. After thawing, serum samples were used to determine serum total antioxidant capacity (TAC), total oxidant status (TOS), homocysteine, arylesterase, paraoxonase and ceruloplasmin by calorimetric methods, using Biosoymat Semi Auto-Analyzer (BTS-330, Costa Brava, Barcelona, Spain). TOS were measured on the basis of oxidation of ferrous ion wherein hydrogen peroxide was used to make standard calibration curve (Anwar et al., 2012). The absorbance was taken at 444 nm wavelength. The delta change in the absorbance was used to calculate the actual concentrations in Trolox (Vit. E analogue) equivalent. TAC were measured using 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical cation as substrate using the
Table 1: Composition of atherogenic diet given to rabbits

<table>
<thead>
<tr>
<th>Component</th>
<th>Parts</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH2O</td>
<td>60%</td>
</tr>
<tr>
<td>Protein</td>
<td>15%</td>
</tr>
<tr>
<td>Fat</td>
<td>15%</td>
</tr>
<tr>
<td>Salts</td>
<td>4%</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3%</td>
</tr>
<tr>
<td>Fiber</td>
<td>2%</td>
</tr>
<tr>
<td>Vitamins</td>
<td>1%</td>
</tr>
</tbody>
</table>

(Ashok and Daradka, 1999).

method of Erel (2005) described by Anwar et al. (2012). Briefly the absorbance was taken at bichromatic wavelength, where main wavelength was 560 nm and secondary wavelength was 800 nm. The delta change in the absorbance was used to calculate the actual concentrations in terms of micromolar hydrogen peroxide equivalent per liter. Arylesterase and paraoxonase activities were assayed using phenyl acetate and paraoxon (Sigma Chemical Co., London UK) as substrate, respectively (Juretic et al., 2006). Ceruloplasmin activity was assayed using O-dianisidine di hydrochloride as substrate based upon its oxidizing activity (Anwar et al., 2012). Serum homocysteine concentrations were determined using standard protocol provided with kit procured from Diazyme Laboratories, San Diego, CA 92186, USA. Delta absorbance was used to determine the concentration of homocysteine from the standard curve constructed by the standards available with the kit. Absorbance was taken at 340 nm for both standards and samples.

Statistical Analysis

Two way Analysis of Variance technique was used to compare the significance between different groups. The significance between different days and different treatments of sampling was statistically analyzed by the Duncan Multiple Range test. The software package CoStat 6.4® and GraphPad Prism 5.04® were used to analyze the data. Results were accepted as significant at P≤0.05.

RESULTS

The mean TAC concentrations were higher (P<0.05) in the SM at day 30 and 90 compared with the other treatment groups. Furthermore, overall mean TAC value was also higher (P<0.05) in SM. The LS had higher (P<0.05) TAC concentrations at day 60 and 90 compared to the WS group. The mean TOS concentrations were higher (P<0.05) in PC at day 30 and 60 compared with the other treatment groups. The WS had higher TOS concentrations at day 30 and 90. However, overall mean TOS concentrations were higher in PC and LS group. SM had higher paraoxonase activity at day 30 and 60 compared with NC, PC and WS group, whereas, paraoxonase activity was higher in LS at day 30 and 90. The overall mean paraoxonase activity was higher in SM and LS groups when compared with other groups.

Arylesterase activity was higher in the SM at day 60 and 90. The overall mean arylesterase activity was higher in SM followed by LS group. Overall mean ceruloplasmin concentrations as well as day 30, 60 and 90 concentrations were higher in SM. Homocysteine concentrations were highest in WS group at day 60, whereas, overall mean homocysteine concentrations were higher in PC and WS groups when compared with the rest of the groups.

DISCUSSION

Hyperlipidemia is characterized by low anti-oxidants levels which may lead to poor body defense against oxidative species and lead to cardiovascular and metabolic ailments. In the current study, highest anti-oxidant concentrations were observed in simvastatin group followed by the LS and the WS groups. The results are quite similar to the recent findings of Urmila et al., (2013) and Stojakowska et al., (2013) who found significant 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide and hydrogen peroxide scavenging activity by the extracts of these plants. Furthermore, the observations by Mehrrota et al., (2011) and Manwar et al., (2013) also describe the anti-oxidant capacity of *Withania somnifera* extracts. It is assumed that the plant ingredients of *L. scariola* play a precursor role for different anti-oxidant enzymes or they might have boosted the immune potential by dietary supplementation of vitamins and minerals present in it (Anwar et al., 2015).

The ratio of oxidant and anti-oxidant enzymes is very important as this ratio determines the redox status of the body (Gwinner et al., 1997). Any change in its equilibrium may lead to the oxidative damage. In the current study, the oxidants status was reduced in the LS group, which may be in response to elevated anti-oxidant capacity. The higher level of oxidants in positive control group compared to the negative control group is due to linoleic acid found in atherogenic diet given (Berry et al., 1991; Bonanome et al., 1992; Tsimikas et al., 2003). The superoxide anion production by the endothelial cells has also been reported to increase in hypercholesterolemia (Ohara et al., 1993).

In the present study, trend in paraoxonase activity was seen similar to anti-oxidant status of different groups. It was found highest among simvastatin and LS groups. Similar to our findings, Aviram et al. (1999) reported that presence of dietary antioxidants is important to preserve serum paraoxonase activity because paraoxonase is inactivated by lipid peroxides. However, these findings are contradicted to the results of Silaste (2003), who reported that the natural antioxidants from vegetables, berries and fruits do not seem to increase or
preserve the serum paraoxonase activity. To the best of our knowledge no study has been reported earlier to investigate the efficacy of L. scariola on paraoxonase activity of hyperlipidemic patient. A suitable justification for higher paraoxonase activity in L. scariola supplemented group may be due to high level of natural antioxidants found in the L. scariola. Arylesterase is a paraoxonase related enzyme whose activity is actually an indication of the activity of paraoxonase against phenyl acetate as substrate (Juretic et al., 2006). An increase in the concentrations of arylesterase in the LS group can be related to the justification for higher paraoxonase activity in L. scariola.

In the current study, ceruloplasmin level was found highest in the simvastatin group. No literature reports are available regarding the ceruloplasmin activity in L. scariola treatment in any species. However, Vedi et al. (2014) found an increase in ceruloplasmin level by W. somnifera treatment in rats subjected to brome benzone induced oxidative damage. Furthermore, Eduardo et al. (1994) reported that the ceruloplasmin can also cause oxidation of LDL by increasing the TAC and lowering the TOS.

The concentrations of homocysteine were observed highest in the positive control and the WS groups. The reduction in serum homocysteine level in the LS group is assumed to be due to the analgesic and anti-inflammatory properties of the plant (Ahmad et al., 1992). Silaste (2003) observed enhanced serum and erythrocyte folate concentrations as well as reduced plasma total homocysteine concentrations in human in response to increased intake of vegetables, berries and citrus fruits rich in natural folate. Consequently, there has been a vast amount of interest in evaluating factors that influence the LDL oxidation, as well as development of pharmacological agents and antioxidants that could reduce the oxidative modification of LDL. These plants especially L. scariola is very effective in lowering the oxidative stress, may be through its antioxidant potentiation or through its pessimistic impact on the enzymes capable of anti-oxidation. This may have the LDL modification property which makes it possible to skip from the lipid

### Table 2: Serum biomarkers of rabbits in treated and control groups at different sampling intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>NC</th>
<th>PC</th>
<th>SM</th>
<th>WS</th>
<th>LS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAC (mmol/TOS)</td>
<td>Homocysteine (U/L)</td>
<td>Arylesterase (U/min/ml)</td>
<td>Ceruloplasmin (U/L)</td>
<td>Homocysteine (umol/L)</td>
</tr>
<tr>
<td>30</td>
<td>1.53±0.15</td>
<td>60±5.63</td>
<td>60±5.63</td>
<td>90±5.63</td>
<td>90±5.63</td>
</tr>
<tr>
<td>60</td>
<td>1.53±0.15</td>
<td>60±5.63</td>
<td>60±5.63</td>
<td>90±5.63</td>
<td>90±5.63</td>
</tr>
<tr>
<td>90</td>
<td>1.53±0.15</td>
<td>60±5.63</td>
<td>60±5.63</td>
<td>90±5.63</td>
<td>90±5.63</td>
</tr>
<tr>
<td></td>
<td>1.53±0.15</td>
<td>60±5.63</td>
<td>60±5.63</td>
<td>90±5.63</td>
<td>90±5.63</td>
</tr>
</tbody>
</table>

### Table 3: Serum enzyme and atherosclerosis marker in treated and control groups of rabbits at different days intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Negative Control</th>
<th>Positive Control</th>
<th>Simvastatin</th>
<th>WS</th>
<th>LS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arylesterase activity (U/min/ml)</td>
<td>30</td>
<td>86.37±2.98</td>
<td>44.11±6.04</td>
<td>169.87±11.24</td>
<td>75±3.01</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>86.37±2.98</td>
<td>78.25±4.01</td>
<td>177.25±13.92</td>
<td>66.63±2.93</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>86.37±2.98</td>
<td>65.63±4.81</td>
<td>177.63±8.63</td>
<td>77.5±2.93</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>86.37±2.98</td>
<td>62.67±4.04</td>
<td>174.92±6.37</td>
<td>73.04±2.79</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>99.25±5.14</td>
<td>73.63±14.14</td>
<td>179.12±15.34</td>
<td>28.38±2.54</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>99.25±5.14</td>
<td>54.25±5.52</td>
<td>174.5±11.28</td>
<td>58.75±3.66</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>99.25±5.14</td>
<td>52.13±5.85</td>
<td>156.63±9.37</td>
<td>65.75±3.66</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>99.25±5.14</td>
<td>60±5.63</td>
<td>170.08±7.06</td>
<td>50.96±4.03</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>99.25±5.14</td>
<td>10.87±0.29</td>
<td>4.67±0.46</td>
<td>8.23±0.71</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>99.25±5.14</td>
<td>9.48±0.68</td>
<td>4.48±0.45</td>
<td>11.84±0.96</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>99.25±5.14</td>
<td>9.49±0.96</td>
<td>4.23±0.49</td>
<td>8.57±0.96</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>99.25±5.14</td>
<td>9.95±0.41</td>
<td>4.45±0.26</td>
<td>9.54±0.56</td>
</tr>
</tbody>
</table>

**Note:** Similar alphabets in a row do not differ significantly (P > 0.05); **Similar alphabets do not differ significantly (P ≤ 0.05).**
Effect of *Withania somnifera* and *Lactuca Scariola* on health biomarkers

peroxidation reactions. Therefore, these plants have beneficial effects on the health biomarkers of hyperlipidemic subjects and are the matter of concern as these plant formulations could serve as an alternative therapy for hyperlipidemia with the minimal side effects as compared to the synthetic drugs. In conclusion, *L. scariola* and *W. somnifera* can improve antioxidant capacity of the hyperlipidemic subjects, though commercially available simvastatin is comparatively better medication for hyperlipidemia.

REFERENCES


Anwar et al


Woo MN, SH Bok and MS Choi, 2009. Hypolipidemic and body fat-lowering effects of Fatclean in rats fed a high-fat diet. Food and Chemical Toxicology, 47: 2076-2082.
