Antihyperlipidemic activity of hydroalcoholic seed extract of *Cucumis sativus* L in triton X-100 induced hyperlipidemic rats

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**INTRODUCTION**: Hyperlipidemia is a condition in which there are increased levels of total cholesterol-TC, triglycerides-TG, low density lipoprotein cholesterol-LDL-C and very low density lipoprotein cholesterol-VLDL-C with a decrease in the levels of high-density lipoprotein cholesterol-HDL-C. It is based on the disorders in lipid metabolism leading to cardiovascular diseases (CVDs) (Firdous et al., 2021). The WHO has revealed that 40% of the population all over the world is suffering from higher levels of plasma cholesterol. Globally the most frequently health issues are diabetes mellitus (DM), cardiovascular diseases (CVDs) and myocardial infarction and these are much associated to hyperlipidemia. As hyperlipidaemia is linked to higher serum TC and TG levels, it may also result in major cardiac diseases like atherosclerosis. So, controlling this risk factor is crucial in preventing atherosclerosis. The use of synthetic therapeutic drugs like statins, bile acid sequestrants and fibrates can reduce the synthesis of lipids and their gastrointestinal absorption to treat hyperlipidaemia. However, the usage of these synthetic drugs may result a few persistent harmful side effects, mostly rhabdomyolysis, myopathy and increased risk of cancer. So the development of effective and novel natural hypolipidemic agents with minimal or undesired side effects is required on urgent basis (Aladaileh et al., 2019). Various herbal medicines have been shown to be effective for treating dyslipidemia due to the components like flavonoids, vitamins, antioxidant, and polyphenols substances (Soltani et al., 2014). WHO estimated that the population trust 80% of the use of herbal medicine in pathological conditions. This led to a tremendous increase in interest among the researchers in herbal and nutraceutical treatment with proved health benefits (Vogel, 2007). This plant (*Cucumis sativus* L) belonging to the *Cucurbitaceae* family is widely cultivated in the tropical areas (Minaian et al., 2011). Traditionally it has been utilized as diuretic, anti-diabetic, anti-inflammatory, anti-helminthic and lipid lowering agent (Saeedi et al., 2020). The seeds are rich in several constituents which includes fats (42.5%) and crude proteins (42%). The fatty acid components are stearic (16.2%), palmitic (0.63%), oleic acid (38.70%) and linoleic (40.11%). (Saeedi et al., 2020). Extracts of *C. sativus* L seeds are found to have analgesic (Kumar et al., 2010), antimicrobial (Begum et al., 2019), anti-inflammatory (Vetrivelan et al., 2013), anti-oxidant (Begum et al., 2019), anti-diabetic and anti-ulcer activities (Minaian et al., 2011). Hypoglycemic as well as hypolipidemic studies have been demonstrated on *C. sativus* L fruit in alloxan induced diabetic rats that reduced 72% triglyceride level (Sharmin et al., 2013).

**OBJECTIVES**: Although seeds of *C. sativus* L are used traditionally as a lipid-lowering agent but no previous study is present which confirms this effect. No previous report is present about hypolipidemic effect of the seeds of this plant in Triton X-100 induced hyperlipidemic rats. Also, no reports are present about HPLC analysis of hydroalcoholic extract of this plant. Therefore, the objectives of this study was (1) to analyse the phytochemical composition of hydroalcoholic extract of *C. sativus* L (var. pickling) seeds. (2) To investigate hypolipidemic activity of this extract in hyperlipidemic Wistar albino rats.

**MATERIALS AND METHODS**: Collection of plant: The fresh plant *C. Sativus* L was purchased from local arcade, Gulberg, Lahore, Pakistan. It was identified and authenticated by Professor Dr. Zaheer-ud-Din Khan, Department of Botany, G.C University, Lahore. A voucher specimen No. (GC. Herb. Bot. 3384) was submitted in the herbarium of same university.
Seeds were separated from ripened dried fruit of the plant and were properly dried.

**Preparation of plant seed extract:** Dried seeds (500 g) were reduced to fine powder with grinder. The powder was macerated in ethanol and water (hydroalcohol) in a ratio (75:25, v/v) respectively. Hydroalcoholic extract was passed through filter papers and dried using rotary (Heidolph Laborota 4002, Germany) under reduced pressure and extract was stored at -20°C in bottles after 72 h (Minaiyan et al., 2011).

**Phytochemical screening:** Hydroalcoholic extract of the plant was subjected to phytochemical screening by following the standard methods (Shah, 2009). The extract was tested for alkaloids, flavonoids, phenols, saponins and tannins.

**HPLC analysis:** RP-HPLC analytical study of hydroalcoholic extract was done for the identification of bioactive compounds.

**Preparation of mobile phase:** Methanol and water (60: 40, v/v) was used as mobile phase in HPLC analysis and was run through the membrane filter (Millipore bedfoed MA, USA) having a pore size of 0.45μm. It was sonicated for 15 min. to remove the gases from the mobile phase.

**Chromatographic conditions:** Reverse phase high performance liquid chromatographic (RP-HPLC) analysis was performed using HPLC (Shimadzu Prominence HPLC instrument), with liquid chromatograph (LC 20A), column C18 (250×4.6mm, 5μm) (Shimadzu Prominence) and a quaternary solvent pump system (LC 20AT-VP). UV-Vis spectrophotometer detector, a loop injector (Rheodyne 7725) having an injection capacity (20 μL). Degasser (DGU-20A-5R) and Column oven (CTO-20A). Equilibration of column was done by passing the pure methanol for 30 min through the column before injecting hydroalcoholic extract. This extract (10 μL) was passed through a reverse phase (RP) C18 column using isocratic system and maximum wavelength (238 nm). The detector used for the analysis was UV (Photo diode array). The column (CTO-20A) temperature for operating was 30°C. Flow rate of mobile phase was adjusted at 1μL/minute and total volume of injection was 10μL. Run time for the mobile phase was 10 min. Shimadzu Lab Solution with Version (6.43 SPI) was the software used during HPLC analysis.

**Antihyperlipidemic activity:** Animals required for the study: Experimental methods were approved under Approval No. IREC-2019-79 Dated 28/03/2019 by the Animal Ethical Committee (AEC), The University of Lahore. The rats were obtained from Post Graduate Medical Institute (PGMI) and were stored in the animal house of Faculty of Pharmacy, The University of Lahore. The animals were maintained at 25±1°C in cages and were placed for 20 days under the standard lab conditions. The animals were allowed to take the standard pellet diet and water freely. They were maintained at the lab conditions before starting the study.

**Acute toxicity studies:** Following Organization for Economic Co-operation Development (OECD) test guidelines 425 acute oral toxicity study was done (OECD., 1994). Hydroalcoholic extract of *C. Sativus* L (2000 mg/kg) was given to wistar albino rats and observed for signs of toxicity after 14 days.

**Induction of hyperlipidemia:** Single Triton X-100 solution was prepared freshly in the saline solution prepared normally and was administered by intraperitoneal (i.p.) injection at the dose (100 mg/kg) to all groups (B, C and D) after fasting for 18h. (Abdelgadir et al., 2020).

**Sample collection:** After the treatment protocols, anaesthesia was induced in all rats to collect samples. Blood samples were collected using cardiac puncture technique into tubes of gel clot activator for serum separation using centrifuge machine (3000 rpm/10min) (Keshetty et al., 2009) and stored at -20°C.

**Analysis of biochemicals:** The levels of biochemicals (TC, TG, HDL-C, AST, and ALT) were estimated after 72 hours using kits of chemical analyser (ROCHE diagnostics Roche, USA).

**Histopathological studies:** Liver and heart tissue samples were obtained and preserved in formalin (10%) solution for further histopathological studies. The tissues were fixed in formalin solution, paraffin embedded, consecutively cut with 5μm thickness, and treated for hematoxylin and Eosin staining (Abdelgadir et al., 2020).

**Statistical analysis:** The readings were taken as mean ± SEM. The data was analysed using one way ANOVA which was followed by Tukey's multiple comparison test (Version: Graph pad Prism 7.02) as post hoc test. P < 0.05 was considered as statistically significant.

**RESULTS AND DISCUSSION:** Phytochemical screening: The results showed the presence of tannins, flavonoids, phenols, saponins, oil, fats, sterols and the absence of alkaloids in hydroalcoholic extract of *S. sativus* L. Preliminary phytochemistry of *C. sativus* L seeds in literature revealed the presence of fixed and volatile oils, saponins, steroids, flavonoids, carotenes, amino acids, flavones, tannins, resins and alkaloids (Begum et al., 2019). The phytochemicals present in the seeds of plant justifies the medicinal use of this plant in treating different disorders. Phytochemical screening at preliminary level may be useful for detecting bioactive principles leading to the drug discovery. The plant may have different biological activities due to the phytochemicals constituents present in the plant and it is used
pharmacologically in the development of health benefitting novel compounds.

**HPLC analytical study of hydroalcoholic extract for determining different compounds:** The HPLC analysis of hydroalcoholic extract of *C. sativus* L seeds exhibited different peaks of compounds with various retention times (RT). The height and specific area with specific retention times of HPLC peaks are given in **table 1**.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Retention Time</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.60</td>
<td>294260</td>
<td>10606</td>
</tr>
<tr>
<td>2</td>
<td>2.13</td>
<td>86440</td>
<td>3615</td>
</tr>
<tr>
<td>3</td>
<td>2.63</td>
<td>41248</td>
<td>3507</td>
</tr>
<tr>
<td>4</td>
<td>2.82</td>
<td>62116</td>
<td>7439</td>
</tr>
<tr>
<td>5</td>
<td>3.26</td>
<td>1492131</td>
<td>41754</td>
</tr>
<tr>
<td>6</td>
<td>4.87</td>
<td>283337</td>
<td>5747</td>
</tr>
<tr>
<td>7</td>
<td>5.25</td>
<td>199836</td>
<td>5390</td>
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<td>8</td>
<td>5.98</td>
<td>300997</td>
<td>4866</td>
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<tr>
<td>9</td>
<td>7.43</td>
<td>213635</td>
<td>3227</td>
</tr>
<tr>
<td>10</td>
<td>9.03</td>
<td>238771</td>
<td>3205</td>
</tr>
<tr>
<td>Total</td>
<td>3212772</td>
<td>89357</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1:** The HPLC analysis of hydroalcoholic extract of *C. sativus* L. Different peaks of compounds with specific height and area are given with retention time.

The peaks with specific retention time show the presence of compounds in the extract. The area covered by each peak indicate the amount of compound present.

**Toxicological studies:** Single orally administered extract of *C. sativus* L did not cause clinical signs of toxicity or mortality in SD rats after 14 days. Thus, LD50 of hydroalcoholic extract of the plant was considered to greater than 2000 mg/kg and was unlikely to be toxic (*United Nations, 2011*).

**Effects of hydroalcoholic extract of the plant on hyperlipidaemia:** Effect of hydroalcoholic extract of the seeds of *C. sativus* L on biochemical parameters (mg/dl ± SEM) in hyperlipidaemic induced rats administered for 7 days are given in **table 2**. TC, TG-C, LDL-C, non-HDL-C, VLDL-C and alanine aminotransferase-ALT in serum levels of group D (hydroalcoholic extract of the plant) were shown to be decreased at significant (*P < 0.05*) level in comparison to hyperlipidemic control group. HDL-C levels were significantly (*P > 0.05*) increased in comparison to group B (hyperlipidemic control). There is non-significant (*P > 0.05*) decrease in the levels of aspartate transaminase-AST in serum of group D as compared to group B (hyperlipidemic control). The values are presented as mean ± SEM (n=3). Statistical analysis (one way ANOVA followed by Tukey Multiple Comparison test as post hoc test) of lipid profile of standard and plant treated group in comparison to hyperlipidemic control group. TC, Total Cholesterol; TG, Triglycerides; LDL-C, low-density lipoproteins; HDL-C, high density lipoprotein cholesterol; VLDL-C, Very low-density lipoprotein cholesterol; ALT, aspartate transaminase; AST, alanine aminotransferase. Both significant and non-significant results were obtained when lipid profiles were compared to hyperlipidemic control.

**Histopathological Examination of Liver:** The effect of hydroalcoholic extract of seeds of *C. sativus* L on the liver and normal configuration of liver in normal control rats is shown in **figure 1(a)**. There was severe congestion of blood vessels and fatty changes in hepatocytes in Triton X-100 induced rats (**figure 1b**). There were fatty changes in hepatocytes tissues by the fat accumulated in animals administered with standard simvastatin (**figure 1c**). *C. Sativus* L seed extract treated rats showed moderate congestion and there was accumulation of inflammatory cells of liver present in the portal areas. There were no hyperlipidaemic signs (**figure 1d**).

**Histopathological examination of heart:** Effect of hydroalcoholic extract of seeds of *C. sativus* L on the heart is exhibited in **figure 2**.

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**Figure 1:** Histopathological changes occurred in the liver of rats stained by Haematoxylin and Eosin. Normal configuration of the liver tissues of normal control rats (a). Clear congestion appeared in blood vessels (arrows) appeared in the liver tissues of untreated Triton X-100 induced hyperlipidaemic rats (b). Mild fatty changes (arrows) in hepatocytes of liver tissue of simvastatin treated rats (c). Moderate congestion of hepatic blood vessels (black arrows) and accumulation of inflammatory cells in the portal areas (blue arrow) in the liver tissue of *C. sativus* L treated rats (d).

**Figure 2:** Histopathological changes in transverse section of hearts of animals administered with hydroalcoholic extract of *C. sativus* L seeds (a). Pericardium, epicardium, myocardium, and coronary artery appear normal in normal control group. Coronary blood arteries appeared severely congested (black arrow), necrosis of cardiac myocyte (red arrow) with myocardia haemorrhages (blue arrow) of hyperlipidemic (diseased control) group (b). Normal mesothelium (red coloured arrow), slight congestion of coronary vessels in epicardium (black coloured arrow) and few number of necrotic foci (blue arrow) of simvastatin treated group (c). Mesothelium sloughed off indicated by blue coloured arrow, dilated blood...
vessels in epicardium indicated by black arrow, necrosis in myocardium as shown by red arrow with moderate haemorrhages as shown by green arrow were noticed in C. sativus L treated group (d).

Table 2: Effect of C. sativus L seeds on total cholesterol, triglycerides, HDL, LDL, VLDL, ALT and AST levels in Triton X-100 induced rats after 7 days of hydroalcoholic extract administration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A Normal Control rats</th>
<th>Group B Hyperlipidemic Control rats</th>
<th>Group C Hyperlipidemic standard Simvastatin</th>
<th>Group D Hyperlipidemic C. sativus L</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>55.6 ± 4.22</td>
<td>100.6 ± 7.83</td>
<td>55 ± 2.12b</td>
<td>63.6 ± 4.69b</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>79.2 ± 12.78</td>
<td>255.2 ± 58.69</td>
<td>81 ± 7.19b</td>
<td>86.4 ± 7.50b</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>30.04 ± 3.13</td>
<td>103.72 ± 21.66</td>
<td>27 ± 3.13b</td>
<td>39.68 ± 3.10b</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>15.84 ± 2.55</td>
<td>51.04 ± 11.73</td>
<td>16.2 ± 1.43b</td>
<td>17.28 ± 1.50b</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>41.4 ± 3.10</td>
<td>22.96 ± 5.97</td>
<td>44.2 ± 3.51b</td>
<td>41.2 ± 3.36a</td>
</tr>
<tr>
<td>Non-HDL-C (mg/dl)</td>
<td>14.2 ± 1.20</td>
<td>77.64 ± 11.50</td>
<td>10.8 ± 1.93b</td>
<td>22.4 ± 2.71b</td>
</tr>
<tr>
<td>AST (IU/dl)</td>
<td>122.8 ± 3.10</td>
<td>183.0 ± 14.60</td>
<td>138.6 ± 13.21 ns</td>
<td>193 ± 14.32 ns</td>
</tr>
<tr>
<td>ALT (IU/dl)</td>
<td>43 ± 3.36</td>
<td>70.6 ± 6.55</td>
<td>38.6 ± 3.48b</td>
<td>37.8 ± 2.78b</td>
</tr>
</tbody>
</table>

Severe hyperlipidaemic conditions i.e., severe congestion of blood vessels, necrosis of cardiac muscles was seen in hearts of group B rats in comparison to group A normal control rats. The rats administered with the standard in simvastatin exhibited congestion of blood vessels with mild hyperlipidemic condition. Group D treated with C. sativus L had diluted blood vessels with no lipidemic condition. There was no evidence of the storage of lipid droplets. The biologically active compounds that are present in seed extract of C. sativus L show various biological functions. To check whether the activity might be antihyperlipidemic, the effect of plant extract on the lipid profile was investigated compared to the standard simvastatin. Fruit extract of C. sativus L fruit was proved to have reducing effect on TG level up to 72% in alloxan induced diabetic rat model (Sharmin et al., 2013). Similar results were reported from adult hypolipidemic patients using clinical trial of randomized double blind placebo controlled where seeds extract of C. sativus elevated HDL concentration significantly (Soltani et al., 2017). This activity is due to the active phytoconstituents in the plant responsible for antihyperlipidemic activity. It has also been demonstrated that several plants with higher levels of unsaturated fatty acids, such as linoleic acid have lipid-lowering properties. The effects of fruit oil of Cornus wilsoniana containing unsaturated fatty acids on hyperlipidemia in rats were investigated by Fu et al. (2012). The findings demonstrated that the oil dramatically lowers serum TG, LDL, and total cholesterol. According to an analysis unsaturated fatty acids, such as linoleic acid and -linolenic acid, were found in significant numbers (69.12 %) in this oil by chemical analysis. A clinical investigation found that taking 400 mg of pomegranate seed oil twice daily had a positive impact on HDL and TG levels. This oil contains 6-7% linoleic acid (Mirmiran et al., 2010). Previously, a decrease in blood cholesterol, TG, and LDL was recorded (Berrougui et al., 2003) to evaluate the impact of oil of Argania spinosa (containing linoleic acid and oleic acid) on serum lipids. Fatty acids such as myristic, lauric, stearic, oleic, palmitic, and linoleic acids are also present in cucumber. The composition (%) of fatty acid in the plant is stearic (16.2%), palmitic (0.63%), oleic (38.70%) and linoleic acid (40.11%) (Saeedi et al., 2020). According to Rayees et al., (2013) cucumber seed is high in fatty acids like monounsaturated (oleic acid, 7%) and polyunsaturated (linoleic acid, 71%) supporting its potential application in the management of hyperlipidemia. Cucumis seed extract contains phytosterols, which may also be the cause of its hypolipidemic effects. Due to its hydrophobic shape, cholesterol is poorly absorbed by humans and is dependent on the bile salts’ emulsifying abilities to form tiny micelles. In order to prevent all of the cholesterol from being transported and expelled in the faeces, phytosterols with structures similar to those found in cholesterol have minimal enteric absorption (Kelshadi et al., 2016). Different kinds of hypercholesterolemia, such as familial hypercholesterolemia, familial mixed hypercholesterolemia, and undefined hypercholesterolemia, have been linked to the reducing effects of plant sterols (Garaiova et al., 2013). As per literature the plant contains a number of sterols like as codisteryl, 25 (27)-dehydro-porifersterol, clerosterol, stigmasteryl, isofucosterol, 22-dihydrobrassicasterol, campesterol, sitosterol, 25 (27)-hydrocondrillasterol, 25 (27)-dehydrofungisterol 24-β-ethyl-25 (27)-dehydrofungisterol,avenasterol, 24-methylenecolesterol, 22-dihydrispinosterol (Saedi et al., 2020). Abou-Zaid and colleagues previously analysed C. sativus seed. It has 5-sterols with structures related to cholesterol, such as 24-ethyl-cholest-5-en-3-ol, also 24-ethylcholesta7, 22-dien-3-ol and 4-ethylcholesta-7-en-3-ol.

CONCLUSION: The C. Sativus L exhibited a significant antihyperlipidemic activity in Triton X-100 induced hyperlipidaemic rats that is comparable to those of the common cholesterol-lowering medication simvastatin, which prevents the synthesis of cholesterol. Chemical compounds present in this plant are responsible for hypolipidemic activity. Cucumber seed may therefore be used as a food supplement to treat dyslipidaemia. In order to determine the effectiveness and completely understand the mechanism of action underlying the observed antihyperlipidemic effect of the plant, more research is strongly advised. It is needed to establish the safety and efficacy for more clinical study. Further investigation is recommended for isolation, purification and characterization of the compounds responsible for hypolipidemic activity.

CONFLICT OF INTEREST: Authors have no conflict of interest.

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