Research Article

Therapeutic potential of ethanolic extract of *Solanum nigrum* for lipofundin-induced hyperlipidemia in Rabbits

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Abstract

Attenuation of hyperlipidemia with extract of medicinally important plant and reversal of lipid disorders is clinically very important. The aim of present study was to identify the therapeutic potential of *Solanum nigrum* ethanolic extract in lowering the cholesterol level in lipofundin treated hyperlipidemic rabbits *in vivo*. 20% lipofundin was used to induce hyperlipidemia in rabbits @ 2 ml/kg body weight through slow intravenous administration in the marginal ear vein for 7 days. For next 2 weeks the control group was placed at standard diet, whereas the test group was given the ethanolic crude extract of *Solanum nigrum* at the dose of 300 mg/kg body weight. On the completion of treatment blood samples were collected from both control and test groups and were analyzed for the lipid profile values. It was observed in the test group after treatment with ethanolic extract of *S. nigrum*, the raised level of serum total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein were decreased towards normal values. Thus the present study demonstrated that *S. nigrum* possessed significant anti-hyper lipidemic activity in lipofundin induced hyperlipidemic rabbits.

Key words: Hyperlipidemia; Lipid profile; Medicinal plants; *Solanum nigrum*; Ethanolic extract

Introduction

Hyperlipidemia, including high serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) is an independent menace to atherosclerosis which can lead to coronary heart disease and ischemic stroke [1, 2]. Therefore, prevention and treatment of hyperlipidemia is very important. Commonly hyperlipidemia is treated with lipid-lowering drugs such as Hydroxy methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), phenoxy

aromatic acids (fibrates), nicotinic acid and its derivatives. All of the main lipid-lowering drugs including statins and fibrates have certain side effects. Moreover, the combination of statins and fibrates has a high risk of side effects such as muscle damage and liver damage.

The use of plant based medicines has been increasing gradually world-wide in recent years, as well as the search for new phytochemicals that could be potentially developed as useful drugs for the treatment

of various diseases. More than 2000 plants are used in traditional herbal or alternative medical systems and some may provide relief to patients with cardiovascular diseases, especially those with hyperlipidemia and ischemic heart disease. *Solanum nigrum*, a weed, also known as black-nightshade is a member of Solanaceae family found in the dry parts of Pakistan, India and other parts of the world. *S. nigrum* has been used traditionally as a folk medicine for curing many different disorders such as fever, pain, inflammation and liver disorders [3]. It contains minerals, proteins, vitamins, certain hormone precursors and several photochemicals such as tannins, alkaloids, flavonoids, phytic acid, saponins and hydrocyanic acid [4]. The presence of these phytochemicals makes it a nutritive herb [5]. *Solanum nigrum* also possesses many potential medical properties like anticancer [6], antioxidant [7], neuroprotective [8], cytoprotective [9], antiulcer [10], antimicrobial [11], antinociceptive and antipyretic [12]. However, detailed biochemical study of *Solanum nigrum* ethanolic extract against lipofundin induced hyperlipidemia has not been done. The present study was conducted to investigate the therapeutic potential of ethanolic extract of *Solanum nigrum* against lipofundin-induced hyperlipidemia in rabbits.

Materials and Methods

Plants materials

Solanum nigrum L. plant was collected from rural area from the vicinity of Jhelum River, District Jhelum, Pakistan. The herbs were shade dried at room temperature and at normal humidity level. After complete drying, the aerial parts of the herb were spliced carefully and then pulverized into powder. The powder was stored in air tight amber glass bottle.

Extract preparation

About 200gm of dried powder of aerial parts of *Solanum nigrum* was extracted by maceration with 1000ml of absolute ethyl alcohol and kept at room temperature for 10 days. The macerate was shaken by manual stirring 3 to 4 times daily. In order to remove the vegetative plant material, the soaked solution was passed through dried muslin cloth and the filtrate was obtained. The pooled extract of plant was concentrated in vacuum by using a rotary evaporator. A semi-solid material was obtained which was air dried and weighed. The extract thus obtained was stored in an air tight closed container.

Animal used

A local breed of rabbits weighing between 1 to 2 kg was used in the present study. The animals were housed in specially designed well ventilated animal cage and the animals had free access to water and food. A 12 Hr light and dark cycle was maintained. All the experiments on animals were performed in compliance with the guidelines of NIH.

Induction of hyperlipidemia

Hyperlipidemia was induced in all the rabbits with intravenous administration of lipofundin 20% as previously reported [13]. Briefly, lipofundin was slowly administered by intravenous injection over approximately 10 minutes @ 2 ml/kg body weight of the animal. This procedure is repeated for seven consecutive days.

Experimental design

Animals were divided in following three groups (n=5):

1. Control group: Lipofundin induced + No treatment
2. SN Treatment group: Lipofundin induced + Treated with *S. nigrum* L. extract

All the rabbits were weighed and tagged separately. To establish baseline values of different markers of lipid profile, about 3ml blood samples from marginal ear vein of

overnight fasted rabbits of both groups were collected and immediately placed in EDTA tubes. After centrifugation, the serum was separated and stored at -20°C for further analysis. Hyperlipidemia was induced in all the rabbits. For next two weeks the control group was placed at standard diet whereas the treatment groups were given the semi-solid plant extract at the dose of 300mg/kg body weight. At the end of treatment that is at 23rd day, the blood samples of each rabbit were taken for lipid profile. Computerized Metro Lab 1600 DR Semi Automated Chemistry Analyzer along with the commercial kits available for determining serum total cholesterol, serum triglycerides, high density lipoprotein cholesterol and low density lipoprotein cholesterol was used to determine lipid profiles.

Statistical analysis

All the data collected was transferred to Microsoft Excel 2010 sheet and was tabulated. All the graphs were plotted in Microsoft Excel 2010. The values were calculated for their Mean, Standard Error of Mean and Standard Deviation. P-value was calculated for each group to measure the degree of change occurring in parameters observed, for each animal of control group and test group as well.

Results

Measurement of total cholesterol

Measurement of total cholesterol (TC) is an important biomarker for the evaluation of hyperlipidemia and it was measured in this study to evaluate anti-hyperlipidemic potential of *S. nigrum* ethanolic extract. The results showed TC values of normal (baseline), control group at day1, control group at day23 and treatment group as 52.4 ± 3.2 , 76 ± 3.7 , 71.4 ± 4.4 and 48.6 ± 3.1 respectively as shown in figure 1.

Figure 1. Effect of treatment of *Solanum nigrum* extract on total Cholesterol levels. Concentrations of total Cholesterol of normal, control and treatment groups are shown. There is a marked decrease in total Cholesterol of treatment group as compared to control group. All values are expressed as Mean $\pm$ SEM

Thus, the ethanolic extract of *S. nigrum* has decreased TC levels of treated group significantly as compared to the control group.

Measurement of serum triglycerides

To assess the antihyperlipidemic activity of ethanolic extract of *S. nigrum*, the serum TG values were measured as a part of lipid profile of different groups. The serum TG values of normal (baseline), control group at day1, control group at day23 and treatment group were 48 ± 2.5 , 65.8 ± 3.1 , 61.4 ± 2.8 and 49.8 ± 3.0 respectively as shown in figure 2.

Figure 2. Potential of *Solanum nigrum* extract treatment for triglycerides. The values of triglycerides of normal, control and treatment groups are shown. A relatively lower value of triglycerides can be seen in treatment group as compared to control group. All values are expressed as Mean $\pm$ SEM

There was a decrease in the ethanolic extract of *S. nigrum* in serumTG levels of treated group as compared to the control group.

Measurement of high-density lipoprotein-cholesterol

The high-density lipoprotein-cholesterol is another important biomarker of hyperlipidemia. The results showed HDL-C values of normal (baseline), control group at day1, control group at day23 and treatment group 15.8 ± 0.8 , 25 ± 1.1 , 22.8 ± 0.9 and 14.4 ± 1.3 respectively as shown in figure 3.

Figure 3. Effect of *Solanum nigrum* extract treatment on HDL-C levels. Concentrations of HDL-C of normal, control and treatment groups are shown. There is a significant decrease in HDL-C in treatment group as compared to control group. All values are expressed as Mean $\pm$ SEM.

A significant decrease in high-density lipoprotein-cholesterol was observed in treated group as compared to the control group.

Measurement of low-density lipoprotein-cholesterol

The results showed LDL-C values of normal (baseline), control group at day1, control group at day23 and treatment group 25.2 ± 2.1 , 34 ± 2.6 , 30.8 ± 2.3 and 22.2 ± 1.2 respectively as shown in figure 4.

A significant decrease in low-density lipoprotein-cholesterol was observed in treated group as compared to the control group.

Figure 4. Potential of *Solanum nigrum* extract treatment for LDL-C. The values of LDL-C of normal, control and treatment groups are shown. There is no prominent difference between treatment group and control group. All values are expressed as Mean $\pm$ SEM.

Discussion

The present study showed that lipofundin 20% administration produces hyperlipidemia in rabbits and *S.nigrum* ethanolic extract supplementation decreased hyperlipidemia to the normal levels suggesting the therapeutic potential of *S.nigrum* ethanolic extract for hyperlipidemia. *S. nigrum* aerial parts ethanolic extract resulted in significant decrease in levels of biomarkers such as total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol. Antioxidant activity of the extract probably has this protective effect by blocking the free radicals production and lipid peroxidation inhibition. The anti-oxidant activity of drugs or other products is due to reduction of oxidative damage by inhibiting the generation of free radicals, ultimately leading to inhibition of lipid peroxidation [3]. Thus, it can be concluded from the present study that *S. nigrum* aerial parts ethanolic extract has anti-oxidant activity as it neutralized the oxidant biomolecules involved in oxidative damage to lipid membranes. *S. nigrum* extract has been reported as a potent antioxidant against various diseases and oxidative stress

induced degenerative processes [14, 15]. This antioxidant potential of the extract is might be due to the presence of excess phenolic compounds like flavonoids, Vitamin C, steroids and β -carotene. Strong scavenging activity of glycoprotein from *S. nigrum* against lipid peroxidation peroxyl radicals has been studied [16]. These studies suggest that the extract of *S. nigrum* regenerates the liver to near normal by positively modulating the antioxidant status. This is in accordance with a study carried out by Lin et al reporting that *S. nigrum* extract blocks the initiation of lipid peroxidation in CCl₄- administered rats [14]. Lipids are group of diverse active metabolic substances which play an important role in causing pathogenesis alcohol liver disease. Hypercholesterolemia and hypertriglyceridemia are the most common lipid abnormalities arise due to the chronic alcohol consumption [17, 18]. Liver; being a major antioxidant defense system organ plays a major role in lipoprotein transport regulation in plasma and cholesterol biosynthesis. During lipoprotein transport, LDL and HDL seem to be particularly important. LDL is regarded as bad cholesterol, is actually converted form of VLDL, containing cholesterol and cholesterol esters as major constituents. HDL on the other hand contains relatively little cholesterol, as high levels are associated with hyperlipidemia. Lipodundin elevates cholesterol levels in tissues and plasma. LDL-cholesterol level was increased significantly in plasma while HDL-cholesterol level was reduced. Triglycerides accumulation mainly results in fatty liver [16]. Lipofundin induced elevated level of trygrycerides are possibly due to the increased availability of FFA, low activity of triglyceride lipase activity, glycerophosphates and decreased fatty oxidation.

Based on above mentioned observations, it was found that *S. nigrum* extract supplement had antihyperlipidemic activity as it restored the lipid levels to nearly normal. It has been reported that the *S. nigrum* ethanolic extract's glycoprotein possesses hypolipidemic potential might be due to an increase in the activity of antioxidant enzyme in lipofundin induced rabbits. This decrease in the cholesterol level may be due to the loss of activity of HMG CoA reductase through phospholigation by cAMP-dependent protein kinase (PKA) activation by *S. nigrum* glycoprotein [16].

Conclusion

Therapeutic potential of medicinal plants has made alternative medicine research one of the most promising fields in biomedical research. There is a growing concern to find alternative sources of drugs to cure different diseases. Plant extracts have emerged as a new therapeutic tool in alternative medicine. *In vivo* study demonstrated reversal of hyperglipidemia in *Solanum nigrum* extract treated rabbits.

Abbreviations

TC: Total Cholesterol, TG: Triglycerides, LDL-C: Low density lipoprotein-cholesterol, HDL-C: High density lipoprotein cholesterol, HMG-CoA: Hydroxy methylglutaryl coenzyme A. SEM: Standard error of the mean, NIH: National health institute, USA, FFA: Free fatty acid.

Authors' contributions

Conceived and designed the experiments: I Ali, Performed the experiments: W Ahmed, Analyzed the data: I Ali, Contributed reagents/ materials/ analysis tools: M Tariq, R Asghar & MA Hussain, Wrote the paper: I Ali.

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