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Raj Shree
Department of Biochemistry, Patna University, Patna, Bihar, India

Asish Kumar Ghosh
a) Department of Biochemistry, Patna University, Patna, Bihar, India
b) Department of Chemistry, Patna University, Patna, Bihar, India

Sudhanshu Kumar Bharti
Department of Biochemistry, Patna University, Patna, Bihar, India

Correspondence
Raj Shree
Department of Biochemistry, Patna University, Patna, Bihar, India

Effect of dietary Withenia somnifera and Commiphora weightii on induced hyperlipidemic wistar rats

Raj Shree, Asish Kumar Ghosh, Sudhanshu Kumar Bharti

Abstract
This study evaluates the whole plant ethanolic extract of two plants' *i.e., Withania somnifera* Dunal (Ashwagandha) and *Commiphora wightii* (Arnott.) Bhandari (Guggul) for their hypolipidemic effects in high fat diet induced hyperlipidemic Wistar rats. After determining lethal dose (LD_{50}), two defined doses of the two extracts i.e., 250 mg and 500 mg of extract per kg rat body weight, serum glucose, total cholesterol, triglyceride as well as plasma creatinine, urea, glutamic oxaloacetic transaminase, aspartate aminotransferase, glutamic pyruvic transaminase and alanine transaminase were evaluated. The main finding of the study demonstrated that both the extract perform effectiveness compared to atorvastatin in a dose dependent manner in prevention of hyperlipidemic condition and could promote a better health conditions. When comparing therapies for hyperlipidemia, the higher dose of *W. somnifera* and *C. wightii* plant extracts have shown improvements in body weight, lipid profile, glucose levels, liver and kidney function.

Keywords: Withania somnifera Dunal, Commiphora wightii (Arnott.) Bhandari, antihyperlipidemia, plant extract, liver function, kidney function

1. Introduction
Hyperlipidemia comprises a heterogeneous group of disorders which causes greater morbidity and mortality, in both young and old people. Its characteristic expression is an elevation in the plasma concentration of lipids (triglyceride and cholesterol) and lipoproteins fractions [low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL)] (Park, 2013, Bharti *et al.*, 2018) [45, 13]. The world health organization (WHO) has drawn attention to the fact that hyperlipidemia is considered to be one of the major modifiable risk factors for atherosclerosis and coronary heart disease (CHD) contributing to the prevalence and severity of cardiovascular diseases (Park, 2013) [45]. Moreover, current predictions estimate that by the year 2020 cardiovascular diseases, notably atherosclerosis will become the leading global cause of total disease burden. The risk factors for CHD include hypercholesterolemia, smoking, hypertension, obesity, a strong family history of cardiovascular events and a sedentary lifestyle (Park, 2013) [45].
The dietary cholesterol during its metabolism is delivered to the hepatic cells where substantial amounts of reactive oxygen species are generated. This process is believed to generate highly toxic products, including lipid peroxides as aldehydes, epoxides and carbonyls, and cause rapid consumption of antioxidants such as vitamin E or vitamin C. Further, high cholesterol diet increases serum LDL levels, and, due to oxidative stress, the LDL is oxidized increasingly thereby facilitating atherosclerotic plaque formation (Bharti *et al.*, 2017) [6].
Management of dyslipidaemia forms an important part of strategies for preventing cardiovascular disease. Most of the current guidelines reflect the results of the five major statin trials published between 1994 and 1998. Overall, statins reduced the risk of CHD by 31% and total mortality by 21% (Bharti *et al.*, 2017) [6]. Dietary therapy together with hypolipidemic drugs is central to the management of hyperlipidemia. For the same reason, several hypolipidemic drugs have been already introduced in mainstream medicine such as HMG-Co A (simvastatin, atorvastatin), nicotinic acid (niacin), bile acid sequestrates, fenofibrates etc. But long term use of these drugs have been reported for several adverse effects, such as statins have been reported to elevate amino-transferase three times greater than normal levels, hepatotoxicity, myopathy-diffuse muscle pain, renal insufficiency etc. Nicotinic acid (niacin) has been reported to produce hepatic dysfunction, hyper-urecemia, acanthosis nigricans and gastritis (Katzung, 2012) [31]. Besides the adverse effects of the hypolipidemic drugs, the high cost of these drugs is also a main drawback especially for the developing countries.

Besides the adverse effects of the hypolipidemic drugs, the high cost of these drugs is also a main drawback especially for the developing countries. It has long been known that plant phenolic compounds inhibit the absorption of cholesterol, with which they are closely related structurally. These compounds compete with cholesterol for incorporation into mixed micelles, thereby impairing its absorption from the intestine, but their limited lipid solubility makes it difficult to dissolve them in fat spreads in effective concentrations. This was overcome by esterifying them with long chain fatty acids, which increases their lipid solubility and facilitates their incorporation into foods (Blair *et al*., 2000) [17].

Currently, the use of complimentary/alternative medicine and especially the consumption of herbal medicines have been rapidly increasing worldwide. Over the past 25 years, 50% of prescription drugs have been developed from natural products and their derivatives. Many Unani drugs have demonstrated favourable effects in modifying lipid risk factors for CHD. Many scientific studies have proven the antihyperlipidemic effect of some unani drugs like Tukhme karafs (Apium graveolens Linn.) (Mansi *et al*., 2009) [40], Tukhme suddab (Ruta graveolens Linn.) (Parray, 2010) [46], lac (Coccus lacca) (Ghufran *et al*., 2011) [26] filfile siyah (Piper nigrum Linn.) (Vijaykumar, 2002) [60] etc.

India has a long history as far as the use of medicinal plants for management of dislipidemia is concerned. The Ayurvedic management of hyperlipidemia emphasizes dietary and lifestyle recommendations and herbal preparations, in accordance with the stage and type of disease as well as the psychophysiologic constitution of the patient. Ayurvedic treatments known as Apatarpana (balanced diet with restricted calories) and Santarpana (highly nutritious, high-calorie diet intended to increase weight) are recommended for patients with hyperlipidemia. There are several reports on effective herbal treatment of anti-hyperlipidemia and their subsequent secondary complications. Various Ayurvedic herbs are utilized based on the stage and type of disease as well as the psychophysiologic constitution of the patient.

Based on folkloric usage and reported literature the present study endeavoured to investigate the whole plant extract of two plants' i.e., Withania somnifera Dunal (family: Solanaceae), also known as Ashwagandha, Indian ginseng, or winter cherry and Commiphora wightii (Arnott.) Bhandari (family: Burseraceae) also known as Guggul or Gugglu for their multiple pharmacological activities like hypolipidemic, antioxidant, and antiinflammatory effects. The W. somnifera is an important medicinal plant that has been used in indigenous medicinal system for over 3,000 years. The aqueous extract of the fruits of W. somnifera exert antiangiogenic and antihyperlipidemic activity. The extract of W. somnifera exhibited hypoglycaemic/ hypolipidemic activity due to presence of alkaloids and steroids (Bharti *et al*., 2012, 13 & 15) [4, 10, 16, 8, 9, 11, 15, 5, 14]. They showed that the aqueous extract of Withenia fruits, in high fat diet induced hyperlipidemic rats, significantly reduced elevated serum cholesterol, triglycerides, lipoprotein and the lipid peroxidation levels. The Withanolides obtained from Withania are potent inhibitors of pro-inflammatory transcription factors NF-kB and AP-1 and therefore, holds promise as a novel agent for the treatment of inflammatory cascade of cardiovascular diseases (Prince *et al*., 2008) [49].

Commiphora wightii (Arnott.) Bhandari is mentioned as early as from 3000 to 10,000 years ago in the Vedas, the holy scriptures of India for treating human illnesses. In 1986, with proven efficacy and safety, guggul was approved for marketing in India as a hypolipidemic drug (Satyavati, 1991) [52]. The hypolipidemic effect of gugulipid and guggulsterone has been consistently demonstrated in various animal species, including rat, mouse, rabbit (Satyavati 1991; Chander *et al*., 2002; Kumari and Augusti 2007; Urizar *et al*., 2003) [52, 19, 35, 59], chicken (Baldwa *et al*. 1981) [3], domestic pig (Khanna *et al*. 1969) [33], dog and monkey (Dixit *et al*. 1980) [23]. A recent study by Deng *et al*. (2007) [22] demonstrated that guggulsterone upregulates the expression of the bile salt export pump, a rate-limiting efflux transporter for eliminating cholesterol metabolites, bile acids from the liver as possible mechanisms for the hypolipidemic effect of guggulsterone.

Consistent with the preclinical data, most of the clinical trials studies demonstrated hypolipidemic activity of guggul or gugulipid with an average of 10–30% and 10–20% decrease in total cholesterol and triglyceride, respectively (Urizar and Moore 2003; Ulbricht *et al*. 2005) [59, 58].

Although recent progress has been made in understanding the underlying mechanisms of Witahnia and guggul or guggulsterone-mediated diverse activities, further studies are required to firmly establish the action mechanisms. The present study evaluates the antihyperliidemic activity of the ethanolic extract of Withania somnifera Dunal (family: Solanaceae) and Commiphora wightii (Arnott.) Bhandari (family: Burseraceae), based on folkloric use and efficacy of its phytochemicals reported so far.

2. Materials and methods

The whole plant of Withania somnifera Dunal and Commiphora wightii (Arnott.) Bhandari were harvested from Patna Science College Campus, Patna University, Patna, Bihar, India. They were further prepared after identification and botanical authentication according to the relevant monographs of Indian Pharmacopoeia (2012). Freshly harvested plant materials were washed under running tap water, blotted with filter paper, and was dried in the shade at room temperature. They were then grounded in a mortar. Each of the dried plant samples (2.6 kg each) was then soaked with absolute methanol under reflux condition for the ethanolic extract preparation. Both the samples were then homogenized with extraction buffer and the supernatant were collected after three rounds of extraction. The solvent from both the samples were evaporated under reduced pressure in a rotary evaporator at 400 C. To this thick paste of both the samples, colloidal silicon dioxide was added and dried in vacuum tube dryer. The obtained plant extracts were stored in freezer at _200 C until further test. The quality control sample was prepared as 5 mg/ml of each extract in ethanol. A voucher specimen of plant extracts have been deposited in the Chemistry Department, Patna University, Patna, Bihar, India.

2.1 Chemicals

Heparin, glucose oxidase and peroxidase were purchased from Sigma Chemical Co. (St Louis, MO, USA). Atorvastatin calcium tablets were purchased from Pfizer Pharmaceuticals Limited. Blood glucose diagnostic kit was purchased from Span diagnostic Limited, Surat, India. The biochemical kits for measuring the lipid profiles like total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) assay kits etc were procured from Biosys, Bangalore and Ketamine from Neo Pharmaceuticals, Bangalore, India. All other chemicals and solvents used in this study were obtained from Merck, India and were of analytical grade.

2.2 Animal model

Adult Wistar rat weighing around 220–280 gram with 7.5 ± 1.0 cm length are selected for experiments. The rats were housed in shoe-box type cages under good hygienic conditions in the departmental animal house during experimental period. The rats were allowed to acclimatize for 15 days in an environmentally controlled room under standard environmental conditions (21± 2°C, 55± 5% humidity, 12 hr Light: Dark cycle). The rats were fed on "In laboratory prepared enriched bread" having the standard composition {wheat grains (1000 g), choker wheat (250 g), grams grains (200 g), maize grains (200 g), soyabean grains (250 g), sundrop oil (50 g), milk powder (2 table spoon) and jaggery (50 g) per 2 kilogram} and water ad libitum to ensure proper growth and reproduction. The compositions of high fat diet are cholesterol (2%), sucrose (80 g), glucose (20 g), starch (50 g), coconut oil (5 ml/ 100 g), casein (30 g), cellulose (100 g), vitamin mix (20 g), sodium cholate (1%), propylthiouracil (0.2%), lard (15%) and yolk powder (5%). In order to make the feed more enriched with vitamins, minerals, etc ~2 g of each of spinach, carrot, and sprouted grams were also given to rats. Coconut oil was used in the preparation of hyperlipidemic diet because it contains more saturated fatty acids which aggravate the atherogenic profile in the rats. All the above mentioned constituents were mixed properly and pellets of equal size and weight (10 g each) were made manually. In each cage one pellet of feed per rat was given. The diet was palatable to the animal as evidenced by feeding success. It has been observed that an adult rat normally intakes about 7 to 10 g of diet per day depending upon the physiological and health status of rats as well as the environmental temperature.

For induction of hyperlipidemia the weight of normal Wistar rats were taken. The rats were then allowed to access the respective food and water ad libitum. At 8:00 AM weekly, 1.5 ml of blood was collected via retro-orbital bleeding from each rat under fasting conditions (water freely for 12 h). The blood samples were centrifuged at 4,000 ×g for 10 min at 4°C, and the supernatant was subjected to test the levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) to validate the model. A parallel set of control rats (normo-lipidemic) were fed on in laboratory prepared enriched bread only. Once daily, the rats were observed for changes in their skin fur, eyes and nasal mucous membrane and also respiratory rate and circulatory (heart rate and blood pressure) changes.

2.3 Phytochemical screening of plant extracts

The screening of chemical constituents of each extract was carried out qualitatively with ethanol by using chemical methods. The different solvent extracts of two plants were analyzed for the presence of alkaloid, flavonoid, saponin and tannins according to standard methods (Harborne, 1973) [28]. Briefly, for alkaloid 100 mg of each extract was dissolved in dilute hydrochloric acid separately. Each solution was clarified by filtration. Filtrate was tested with Dragendorff's reagent (potassium bismuth iodide) and Mayer's reagent (potassium mercuric iodide). The treated solution was observed for any precipitation. For flavonoids, 5ml of ethyl acetate was added to a solution of 0.5 g of the extract in water. The mixture was shaken, allowed to settle, and inspected for the production of yellow colour in the organic layer, which is taken as positive for free flavanoids. For saponins, 0.5 g extracts were dissolved in 10 ml of distilled water for about 30 seconds. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over a period of 30 minutes. If a "honey comb" froth above the surface of liquid persists after 30 minutes the sample is suspected to contain saponin. For tannins and phenolic compound, to a solution of about 0.5g extract in 5ml water, three drops of formaldehyde and six drops of dilute hydrochloric acid were added. The resulting mixture was heated to boiling for 1min and then cooled. The precipitate formed (if any) was washed with hot water, warm alcohol, and warm 5% potassium hydroxide successively. A bulky precipitate, which leaves a coloured residue after washing, indicated the presence of tannins.

2.4 Pharmacological evaluation

2.4.1 Preparation and test doses and acute toxicity study

The acute toxicity and lethality (LD50) of two plants' extracts i.e., Withania somnifera Dunal and Commiphora wightii (Arnott.) Bhandari in rats (n = 13 for each plant's extract) were estimated using the method described by Lorke (1983) [38]. The study was carried out in two stages for each plant's extract. In stage one, rats (n=3) received oral administration of 10, 100, or 1000 mg/kg of each of the two plants' extracts (suspended in 20% Tween 80) and were observed for 24 h for number of deaths. At the end of 24 h, only the 1000 mg/kg dose caused death in treated rats. Consequently, a fresh batch of rats (n= 1 for each plant's extract) received 150, 250, 450 and 650 mg/kg of each plant's extract in the second stage of the test and were observed for 24 h for deaths. Death occurred only in the 650 mg/kg dose group. The LD50 was calculated as the geometric mean of the highest non-lethal dose (450 mg/kg) and the lowest lethal dose (650 mg/kg) i.e., LD50= Square root of the product of minimum toxic dose and maximum tolerated dose.

2.4.2 Animal groupings and experimental design

For the experiment, all the rats were fed on in laboratory prepared enriched bread for initial 2 weeks, and then randomly divided into two groups. One group continued to receive in laboratory prepared enriched bread and constituted the NLC group (normolipidemic control rats); the other was fed with hyperlipidemic diets, in order to induce hyperlipidemia. All the rats had free access to food and water. The hyperlipidemic rats were then divided into four groups having six rats in each group: one HLC group (hyperlipidemic control rats) and three HLT group (hyperlipidemic rats treated with two different doses of extract (HLT250 and HLT500) as well as atorvastatin (HLTAVT) at a defined dose per kg of body wt). The rats in the atorvastatin treatment group were administered atorvastatin calcium tablets at a dose of 2.0 mg kg-1day-1 (Pfizer pharmaceuticals limited). Body weights were recorded weekly during the experimental period. Treatment with extracts was started after eleventh week of hyperlipidemic diet treatment, which was considered as the 1st day of treatment. Blood samples were taken after 8 h fasting from the retro-orbital sinus vein prior to the administration of test substances or the buffer and 3 weeks after the treatment under mild ether anesthesia and allowed to clot for 30 min at room temperature. Blood samples were centrifuged at 3000 rpm for 20 min. Serum was separated and stored at -20°C until biochemical estimations were carried out. The animals in the control groups received the same volume of normal saline as the drug-treated groups, and all of

the administrations were performed by oral gavages.

2.5 Body weight
The body weights were measured on day 1 before dosing and weekly during experimental period. To reduce the error originating from feeding, all animals were fasted (water was not restricted) about 8 h before weighing.

2.6 Serum glucose (Glucose oxidase/peroxidase method)
The glucose in the serum is estimated based on the glucose oxidase/peroxidase (GOD-POD) method (Trinder, 1969) [55]. Briefly, glucose is oxidized by glucose oxidase to produce gluconate and hydrogen peroxide. The hydrogen peroxide then oxidatively couples with 4-aminoantipyrine and phenol to generate quinoneimine. This coloured complex produced is proportional to the glucose concentration in the sample and can be measured photometrically at 500 nm with a V-670 research grade UV-Vis spectrometer (M/s. Jasco international co. Ltd., Japan).

2.7 Serum total cholesterol (cholesterol oxidase/ peroxidase method)
Cholesterol is a fatty substance found in the blood, bile and brain tissues, mainly as cholesterol ester. It serves as a precursor to bile acids, steroids, and vitamin D. The amount of cholesterol ester indirectly indicates the total cholesterol concentration in the blood. There are various sub-fractions of cholesterol present in the blood stream. Cholesterol ester is hydrolyzed by the enzyme cholesterol esterase to give cholesterol and fatty acid. This free cholesterol participates in two coupled reactions (shown below), that permits its measurement in a V-670 research grade UV-Vis spectrometer (M/s. Jasco international co. Ltd., Japan) at a wavelength of 500 nm.

2.8 Serum triglyceride (Glycerol phosphate oxidase/ peroxidase method)
Triglyceride is hydrolyzed to glycerol and free fatty acids by lipase. The amount of glycerol produced corresponds to the amount of triglycerides. In the presence of ATP and glycerol kinase, glycerol is converted to glycerol-3-phosphate, which is then oxidized by glycerol-3-phosphate oxidase to yield H2O2 and dihydroxyacetone phosphate in the presence of O2. H2O2 in the presence of peroxidase reacts with chromogens (4-chlorophenol and 4-aminoantipyrine) to yield a coloured complex. The intensity of the colour developed is proportional to the triglyceride concentration which is measured photometrically at 520 nm (V-670 research grade UV-Vis spectrometer by M/s. Jasco international co. Ltd., Japan).

2.9 Serum HDL-cholesterol (Phosphotungustic method)
High density lipoprotein (HDL) contains free and esterified cholesterol, triglycerides, phospholipids, and apoproteins. HDL-cholesterol is estimated as described by GidezL *et al*., (1982) [25]. Chylomicrons, LDL and VLDL are precipitated from serum by phosphotungustate in the presence of divalent cations including magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated photometrically at 505 nm (V-670 research grade UV-Vis spectrometer, Jasco international co. Ltd., Japan) with EBRA reagent (Erba diagnostic kits, GmbH, Germany).

2.10 Serum LDL-cholesterol and VLDL
In the absence of direct method, indirect approach is adopted to estimate LDL and VLDL cholesterol in the serum (Friedewald *et al*., 1972) [24]. VLDL cholesterol can be indirectly ascertained as one fifth of the triglyceride value (TG/5), keeping in mind that the ratio of triglyceride to cholesterol in VLDL is 5%. The amount of LDL cholesterol in the serum is then calculated as shown below from the values of triglyceride, total cholesterol and HDL which was measured using enzymatic method as described above.

2.11 Plasma creatinine (Alkaline picrate method)
Measuring plasma creatinine is an inexpensive method of evaluating the renal dysfunction. Creatinine is a non-protein waste product of creatine phosphate metabolism by skeletal muscle tissue. Creatinine production is continuous and is proportional to the muscle mass. Creatinine is freely filtered and therefore the plasma creatinine level depends on the Glomerular Filtration Rate (GFR). Renal dysfunction diminishes the ability to filter creatinine and hence the plasma creatinine rises. Creatinine reacts with picric acid in alkaline conditions to form a yellow complex which absorbs at 500 nm (M/s. Systronics, Ahmedabad, Gujarat, India; Model: 119). The rate of the color formed is proportional to the creatinine concentration in the sample.

2.12 Plasma urea (Nitroprusside method)
It is a measure of the amount of nitrogen present in the blood in the form of urea, which is a measure of renal function. Urea is a substance which forms in the liver, as a waste product of the protein digestion, and is secreted out from the blood by the kidney. Urease hydrolyses urea to form ammonia and CO2. The ammonia liberated, reacts with salicylic acid in the presence of nitroprusside to produce indophenol, which is measured spectrophotometrically at 600 nm (M/s. Systronics, Ahmedabad, Gujarat, India; Model: 119). Increased plasma urea level indicates impaired renal function or increased tissue protein catabolism and, decreased level indicates liver damage or pregnancy.

2.13 Plasma glutamic oxaloacetic transaminase (SGOT) or Aspartate aminotransferase (AST)
SGOT or AST is an enzyme majorly present in the heart muscle, liver tissues, skeletal muscles, and kidneys. Any infection or injury to these organs results in the release of the enzyme into the blood stream. An elevated level of SGOT is observed in the case of myocardial infarction, cardiac operation, hepatitis, acute pancreatitis, and acute renal disease, whereas decreased level indicates pregnancy, beriberi and diabetic ketoacidosis.
The principle of the SGOT measurement is as follows and it involves two step biochemical reactions. In the presence of SGOT, L-aspartate and α-ketoglutarate gets converted to oxaloacetate and L-glutamate. The oxaloacetate formed reacts with 2,4-dinitrophenyl hydrazine in alkaline medium to produce a brown coloured complex 2,4-dinitrophenyl hydrazone, which is measured with a spectrophotometer at 505 nm (M/s. Systronics, Ahmedabad, Gujarat, India; Model: 119).

2.14 Plasma glutamic pyruvic transaminase (SGPT) or Alanine transaminase (ALT)

SGPT or ALT is present in a number of different tissues, but the major source is liver. Abnormality in the SGPT level indicates problem with the functioning of liver. Increased level indicates hepatitis, cirrhosis, obstructive jaundice, and other hepatic injuries. L-alanine and α-ketoglutarate transaminase, in the presence of SGPT enzyme get converted to pyruvate and L-glutamate. The former reacts with 2,4-dinitrophenyl hydrazine in alkaline medium to produce a brown colored complex, 2,4-dinitrophenyl hydrazone, whose intensity is measured in a spectrophotometer at 505 nm (M/s. Systronics, Ahmedabad, Gujarat, India; Model: 119).

2.15 Statistical analysis

Data were expressed as the mean ± S.E.M. For statistical analysis of the data, group means were compared by one-way ANOVA with Post Hoc analysis. The Tukey–Karmer Post Hoc test was applied to identify significance among groups. Graphs are plotted using MATLAB version 7.8.0 R2009a, Natick, Massachusetts: The Mathworks Inc. 2009. The p-value 0.05 was considered to be statistically significant.

3. Results

3.1 Phytochemical determination of plant extracts

Phytochemical screening of two selected plant extracts of Withania somnifera Dunal and Commiphora wightii (Arnott.) Bhandari showed the presence of active phytochemicals such as alkaloid, flavonoid, saponin, tannin etc. (Table 1).

3.2 Acute toxicity and lethality (LD50) test of plant extracts

The acute toxicity testing of both the plant extracts in rats gave an oral LD50 of 469.04 mg/kg of body weight.

3.3 Effect of two plant extracts on body weight (BW)

The effect of treatment on BW changes in assorted rat groups have been shown in Table 2. The hyperlipidemic control rats (HLC) presented significantly higher BW ($p<0.05$) when compared with the normolipidemic control rats (NLC). The groups of hyperlipidemic treated rats (HLT250, HLT500 and HLTAVT) either with the extract of two plants' i.e., W. somnifera and C. wightii or with atorvastatin showed significant BW reductions when compared to the HLC rats. However, the reduction in BW was found to be more in atorvastatin treated rats (HLTAVT) than extract treated rats of either dose of both the plants i.e., HLTWS-250, HLTWS-500, HLTCW-250 and HLTCW-500. The rats of W. somnifera treatment i.e., HLTWS-250 and HLTWS-500 groups showed a decrease of 27.38 g (8.22%; $p<0.05$) and 37.89 g (11.37%; $p<0.05$) in BW respectively after 25 days of treatment. In the same way, the C. wightii treated rats i.e., HLTCW-250 and HLTCW-500 groups showed a decrease of 35.88 g (10.77%; $p<0.05$) and 36.69 g (11.00%; $p<0.05$) in BW respectively after 25 days of treatment. Contrary to this, HLTAVT group rats showed a decrease of 38.71 g (11.61%; $p<0.05$) in BW after 25 days of treatment.

3.4 Effect of two plant extracts on blood glucose level

The changes in the blood glucose levels before and after receiving the treatment in normal, hyperlipidemic and hyperlipidemic treated rats are listed in Table 3. As expected, the hyperlipidemic control (HLC) rats showed significantly ($p<0.05$) higher level of glucose (+280.53%) which is about 375.13 mg/dl more than normal control rats (NLC). Hyperlipidemic treated rats groups i.e., HLTWS-250, HLTWS-500, HLTCW-250 and HLTCW-500 of both the plants showed a significant ($p<0.05$) reduction in glucose levels, when compared to NLC rats. In the last week of post-treatment the atorvastatin treated rats (HLTAVT) showed a reduction of 243.03 mg/dl of glucose which is about −47.77% when compared to HLC rats. When put side by side, the reduction in blood glucose level was found to be more in HLTAVT rats than extract treated rats of either dose of both the plants i.e., HLTWS-250, HLTWS-500, HLTCW-250 and HLTCW-500. The rats of W. somnifera treatment groups i.e., HLTWS-250 and HLTWS-500 showed a significant decrease of 181.67 mg/dl (−35.75%) and 201.46 mg/dl (−39.67%) in blood glucose levels respectively at the end of the experimental period. Correspondingly, the C. wightii treated rats i.e., HLTCW-250 and HLTCW-500 groups showed a decrease of 188.78 mg/dl (−37.02%; $p<0.05$) and 207.32 mg/dl (−40.75%; $p<0.05$) in blood glucose levels respectively during the 4-week treatment program.

3.5 Effect of two plant extracts on lipid profile

The effect of two plant extracts on serum lipid levels on experimental rat groups have been presented in Table 4. When compared with normolipidemic control rats (NLC), the hyperlipidemic control rats (HLC) had moderately higher total cholesterol (TC) values by about 132.91 mg/dl (+174.92%; $p<0.05$) and TGs values by about 354 mg/dl (+585.90%; $p<0.05$). The atorvastatin treated rats (HLTAVT) showed a reduction of 111.91 mg/dl of serum TC level and 295.66 mg/dl of serum TGs level which are about −53.57% and −71.34% respectively when compared to HLC rats. These changes in biochemical parameters are as expected, as when the uncontrolled hyperlipidemic condition progresses, considerable alterations in total cholesterol and triglycerides values are predictable.

The HLTWS-250 rats showed significantly lower values of serum TC by 43.04 mg/dl (−20.60%; $p<0.05$) and TGs by 209.99 mg/dl (−50.66%; $p<0.05$), when compared to the hyperlipidemic control (HLC) counterparts. Contrary to this, the higher dose of W. somnifera extract treatment (HLTWS-500) showed superior lowering effects on serum TC by about 100.02 mg/dl (−47.88%; $p<0.05$) and on serum TG by about 274.46 mg/dl (−66.22%; $p<0.05$) when compared to the HLC counterparts. In the same way, treatment with lower dose of C. wightii extract (HLTCW-250) showed significantly lower values of serum TC by 50.07 mg/dl (−23.96%; $p<0.05$) and TGs by 197.20 mg/dl (−47.58%; $p<0.05$), when compared to the HLC rats (Table 4). In contradiction of this, the higher dose of C. wightii extract treatment (HLTCW-500) showed better-quality lowering effects on serum TC by about 102.07 mg/dl (−48.86%; $p<0.05$) and on serum TGs by about 273.13 mg/dl (−65.91%; $p<0.05$) when compared to the HLC counterparts. Relative to normal control (NLC), the HLC rats had higher value of low density lipoprotein (LDL) by about 27.17 mg/dl (+30.225%; $p<0.05$) while diminished value of high density lipoprotein (HDL) by about 23.57 mg/dl (-59.10%; $p<0.05$). This is because when the unrestrained hyperlipidemic condition advances, considerable changes in these biochemical parameters are as expected and predictable.

Hyperlipidemic rats treated with lower dose of W. somnifera extract (HLTWS-250) showed significantly lower values of serum LDL by 13.03 mg/dl (−14.49%; $p<0.05$) and higher value of HDL by 19.41 mg/dl (−48.67%; $p<0.05$), when

compared with the HLC counterparts. Contrary to this, the higher dose of W. somnifera extract treatment (HLTWS-500) showed even better lowering effects on LDL by 20.00 mg/dl (−22.25 %; p<0.05) when compared to the hyperlipidemic control (HLC) counterparts as well as lower dose of W. somnifera extract (HLTWS-250) treated rats and improved level of HDL by 16.77 mg/dl (+42.05%; p<0.05).

In the same way, treatment with lower dose of C. wightii extract (HLTCW-250) showed significantly lower values of serum LDL by 15.11 mg/dl (−16.80%; p<0.05) and higher value of HDL by 5.63 mg/dl (14.11%; p<0.05), when compared to the HLC rats. In contradiction of this, the higher dose of C. wightii extract treatment (HLTCW-500) showed better-quality lowering effects on serum LDL by about 21.01 mg/dl (−23.37%; p<0.05) and higher value of serum HDL by about 17.25 mg/dl (−43.25%; p<0.05) when compared to the HLC counterparts (Table 4). In contrast, treatment of hyperlipidemic rats treated with atorvastatin (HLTAVT) at a dose of 2.0 mg/kg of body wt showed a considerable diminished level of LDL by 25.10 mg/dl (-27.92%; p<0.05) while improved level of HDL by 19.41 mg/dl (+48.67%; p<0.05) compared with HLC rats. The HDL to TC ratio is a number that is helpful in predicting atherosclerosis, the process of fatty buildup in the walls of the arteries. A low ratio indicates a higher risk of heart attack while a high ratio indicates a lower risk. High total cholesterol (an indicator that our body has a lot of the lipoproteins that contribute to atherosclerosis) and low HDL cholesterol increases the ratio, so that scenario is undesirable. Conversely, low total cholesterol and high HDL cholesterol lowers the ratio and is good news. It has been shown that HLC rats have only 19.09% of HDL/ TC ratio while NLC rats have shown about 83% of this ratio. A dose dependent recovery trend has been found in treated rats (Table 4).

3.6 Effect of two plant extracts on kidney function and liver function markers

Hyperlipidemic control rats (HLC) have higher levels (approximately twice) of blood urea, creatinine, ALT and AST which have been shown in Table 5. All the four markers decrease considerably in hyperlipidemic rats treated with atorvastatin (HLTAVT) at a dose of 2.0 mg/ kg of body wt when compared to hyperlipidemic control rats. The NLC rats showed the values of blood urea, blood creatinine, serum ALT and serum AST as 39.65 mg/dl, 0.91 mg/dl, 29.31 IU/L and 67.4 IU/L respectively. In HLTAVT rats the parameters, blood urea, blood creatinine, serum ALT and serum AST were reduced by 131.2%, 84.61%, 108.93% and 72.60% respectively. Treatment with W. somnifera extract and C. wightii extract decreases the values of all the four markers in a dose dependent manner when compared to hyperlipidemic control rats. The maximum efficacious dose was found to be 500 mg/kg body weight of rats and have been shown in Table 5.

Hyperlipidemic rats treated with lower dose of W. somnifera extract (HLTWS-250) showed significantly lower values of serum urea by 42.39 mg/dl (−46.23%; p<0.05), serum creatinine by 0.77 mg/dl (−45.83%; p<0.05), serum ALT by 21.35 IU/L (−34.86%; p<0.05) and serum AST by 33.95 IU/L (−29.18%; p<0.05) when compared with the HLC counterparts. Contrary to this, the higher dose of W. somnifera extract treatment (HLTWS-500) showed even better lowering effects on serum urea by 48.31 mg/dl (−52.65%; p<0.05), serum creatinine by 0.65 mg/dl (−38.69%; p<0.05), serum ALT by 28.46 IU/L (−46.47%; p<0.05) and serum AST by 44.56 IU/L (−38.29%; p<0.05) when compared to the HLC counterparts.

In the same way, treatment with lower dose of C. wightii extract (HLTCW-250) showed significantly lower values of serum urea by 42.81 mg/dl (−46.69%; p<0.05), serum creatinine by 0.39 mg/dl (−23.21%; p<0.05), serum ALT by 22.98 IU/L (−37.52%; p<0.05) and serum AST by 33.24 IU/L (−28.56%; p<0.05) when compared with the HLC counterparts.

In contradiction of this, the higher dose of C. wightii extract treatment (HLTCW-500) showed better-quality lowering effects on serum urea by 48.70 mg/dl (−53.11%; p<0.05), serum creatinine by 0.64 mg/dl (−38.09%; p<0.05), serum ALT by 30.00 IU/L (−48.98%; p<0.05) and serum AST by 45.40 IU/L (−39.01%; p<0.05) when compared with the HLC counterparts. In contrast, treatment of hyperlipidemic rats treated with atorvastatin (HLTAVT) at a dose of 2.0 mg/ kg of body wt showed a considerable diminished level of serum urea by 51.51 mg/dl (−56.18%; p<0.05), serum creatinine by 0.69 mg/dl (−41.07%; p<0.05), serum ALT by 27.93 IU/L (−45.61%; p<0.05) and serum AST by 46.70 IU/L (−40.13%; p<0.05) when compared with the HLC counterparts.

4. Discussions

Hyperlipidemia is a potent risk factor for atherosclerosis and coronary heart disease and is present in a substantial proportion of world populace. According to data from the National Health and Nutrition Examination Survey, 12% of adults aged 20 to 39 and 42% of adults aged 40 to 64 had elevated low-density lipoprotein cholesterol (LDL-C) levels, but only 10.6% of adults aged 20 to 39 and 48% of adults age 40 to 64 with hyperlipidemia were receiving treatment. Obesity rates are also increasing in children and adolescents around the world, predisposing them to poor health from an early age (Bharti *et al*., 2014-18) [7, 13].

The current lifestyle has driven obesity prevalence to epidemic proportions with a substantial genetic contribution of 40-70 percent approximately. Since human obesity occurs due to sedentary lifestyle, epigenetics also play an important role in its establishment. Because the natural history of atherosclerosis is prolonged, the risk of clinical events rises exponentially late in life. As a result, the new cholesterol guidelines led to a high number of older adults aged ≥60 years to be recommended for statin therapy, with relatively fewer younger adults meeting statin recommendation thresholds (Park, 2013) [45].

The CHD risk can be reduced by cholesterol lowering, changes in lifestyle, such as smoking cessation, exercise and the use of cholesterol-lowering diets, along with non-cholesterol drug treatments, including aspirin and antihypertensives. A diet for those with hyperlipidemia should also help achieve and maintain a normal body weight as well as prevent heart and vascular disease, which are frequent complications of hyperlipidemia (Park, 2013; Bharti *et al*., 2016) [45, 12].

Multiple lipid lowering medications have been developed that effectively reduce fasting concentrations of low density lipoprotein- cholesterol (LDL-C) and triglycerides (TG). Although several of these medications, particularly the statins, routinely reduce CVD risk by 25-35%, there remains substantial residual and absolute risk in higher CVD risk populations due to elevation in lipids. In fact, in contemporary post-industrialized societies most individuals spend the

majority of non-sleeping hours in the postprandial state. The adverse effect of postprandial triglycerides is thought to be mediated by proatherogenic lipolysis products of nascent triglyceride-rich lipoproteins, such as remnant lipoproteins and fatty acids and even a transient increase in these factors may worsen vascular function (Bharti *et al*., 2018) [13].

Man has used plants extensively for treating various kinds of diseases since ancient times. Herbal plants and plant-derived medicines have been widely used in traditional cultures all over the world and have gained popularity in modern society as natural alternatives to produce new potential therapeutic compounds for combating diseases. Plants and plant extracts were used to combat the diseases as early as 1550 B. C., with as many as 400 before the development earlier this 21st century of effective medications to control hyperlipidemia. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. Sixty percent of today's available anti-hyperlipidemic drugs originated from natural products, and their derivatives. This has resulted in greater confidence in natural products as important sources for the development of effective anti-hyperlipidemic agents (Bharti *et al*., 2012-17) [4, 10, 16, 6].

Presence of active phytochemicals such as alkaloid, flavonoid, saponin, tannin and phenolic compounds are secondary metabolites that are derivatives of various metabolic pathways in plants (Randhir *et al*., 2004) [50]. These active phytochemicals exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Manach *et al*., 2005) [39]. It has been well documented that phytochemicals from natural products possess potent antioxidant activity that are capable in the prevention of the onset and/or progression of many human diseases by counteracting reactive oxygen species (ROS) (Liu *et al*., 2007; Bharti *et al*., 2013) [35, 8, 9, 11, 15]. In our present investigation phytochemical screening of two selected plant extracts of W. somnifera Dunal and C. wightii (Arnott.) Bhandari showed the presence of active phytochemicals such as alkaloid, flavonoid, saponin and tannin (Table 1).

In this study the effectiveness of the bioassay of two selected plant extracts of W. somnifera Dunal and C. wightii (Arnott.) Bhandari for predicting the toxicity of plant extracts was evaluated through comparison with LD50 value-results obtained from oral acute toxicity tests in experimental rats. These rats are sensitive to toxic substances occurring in plants. The administration of the extracts in increasing amounts enables the evaluation of the toxicity limits, and the test should be carried out in two ways, for three doses, and for both sexes, taking into account such factors as age, sex, weight, species, diet, and environmental conditions (Cáceres, 1996). The acute toxicity testing of both the plant extracts in rats gave an oral LD50 of 469.04 mg/kg of body weight.

Detailed descriptions regarding their actions, uses and indications as well as the varieties of W. somnifera and C. wightii have been described in numerous Ayurvedic treatises including Charaka Samhita (1000 BC), Sushruta Samhita (600 BC and Vagbhata (7th century AD) (Satyavati, 1991) [52]. In addition, various medical lexicons were written between the 12th and 14th centuries AD. These plants have been found responsible for reducing fat, indicated for healing bone fracture to inflammation, arthritis, atherosclerosis, obesity, and hyperlipidemia (Sushruta Samhita, Chapter 15, verse 37-38). Traditionally, W. somnifera and C. wightii have been given in the form of Yog, wherein the extracts are mixed with other drugs along with castor oil or Indian clarified butter. The Yog could also be prepared by cooking the extracts with water and other herbal drug powder (Mishra, 2000) [41].

Protective and antioxidant properties of W. somnifera and C. wightii also play a part in its lipid lowering activity and reduce lipid peroxides, xanthine oxidase, and increases superoxide dismutase. W. somnifera and C. wightii have been found to have the capacity to enhance productions of thyroxin which also account for their lipid lowering activities (Panda and Kar, 1999) [44]. Thyroid hormones increase metabolism of carbohydrates, enhance protein synthesis, and stimulate use and breakdown of lipids. A keto-steroid, 2-gugglusterone was found to counteract the thyroid suppressant activity of carbimazole (Tripathi *et al*., 1984) [56]. Preclinical studies have reported their effect on biogenic amines, catecholamine and dopamine liable to attribute to its lipid lowering properties. These have been noted for helping the hypercholesterolemic rabbits to recover the decrease in catecholamine synthesis. W. somnifera and C. wightii significantly lowers serum triglycerides and cholesterol as well as LDL and VLDL cholesterol (Gorsek, 2002) [27].

It is of common consensus that excess adipose tissue increases the workload of the cardiovascular system, adversely alters immune function, and dramatically increases the risk of heart disease, non-insulin-dependent diabetes mellitus, obstructive pulmonary disease, arthritis and a variety of cancers (Conway and Rene, 2004) [21]. Although once considered a major health problem isolated to developed countries, obesity is now recognized as a global problem with potentially catastrophic consequences for health economics. This dramatic change reflects the social, nutritional, and lifestyle changes involved in rural-urban migration and the urbanization of rural areas. Worthy of note in this context are the changes from traditional high fibre diets and high-activity lifestyles to diets rich in saturated fat and low in fibre, further compounded by reduction in physical activity levels (Lee and Sobal, 2003) [36]. Hyperlipidemia, caused by the concomitant, complex interaction of environment (e.g. diet, activity level, elevated body mass, excess visceral adiposity) and genetic factors, is a fundamental pathophysiologic cause of the metabolic syndrome, a recognized risk factor for cardiovascular disease. Nutritional management, especially caloric restriction for the purpose of weight reduction, can improve insulin action on target tissues like skeletal muscle, hepatocytes, and peripheral and visceral adipocytes (Park, 2013) [45].

In our experiment the hyperlipidemic control rats (HLC) presented significantly higher body weight compared to normolipidemic control rats (NLC) while the treated groups of hyperlipidemic rats (HLT250, HLT500 and HLTAVT) either of the consumption of extracts of two plants' i.e., W. somnifera and C. wightii showed significant body weight reductions when compared to the HLC rats (Table 2). The rats of W. somnifera treatment i.e., HLTWS-250 and HLTWS-500 groups showed a decrease of 8.22% and 11.37% in body weight respectively after 25 days of treatment. With aqueous fruit extract of W. somnifera, Ojha and Arya (2009) [42], Hoda *et al*., (2010) [29], Prasad *et al*., (2010) [48] and Jaiswal *et al*., (2010) [30] have reported similar results that W. somnifera has profound hypocholesteremic, hypolipidemic and antiatherogenic activity.

In the same way, the C. wightii treated rats i.e., HLTCW-250 and HLTCW-500 groups showed a decrease of 10.77% and

11.00% in body weight respectively after 25 days of treatment (Table 2). Correspondingly, hypolipidemic effect of gugulipid and guggulsterone have been consistently demonstrated by Satyavati, (1991) [52], Baldwa *et al*., (1981) [3], Chander *et al*., (2002) [19]. All of them have findings that animals receiving guggul had normal serum cholesterol and lipid levels with no fatty streaks or plaque deposits in their arteries.

Atherosclerosis or hardening of the arteries results from build up of cholesterol on the interior blood vessel walls. It is the LDL that leads to this build-up and HDL takes the cholesterol back to the liver. Guggulipids from C. wightii have been found having capacity to lower the VLDL, LDL and triglycerides with simultaneously raising the HDL revealing that C. wightii is useful in providing protection against atherosclerosis. The effect is proclaimed to resulting out from the C. wightii's action on liver and thyroid, wherein, thyroid is stimulated to increase body's metabolic rate and the liver is stimulated to metabolize LDL cholesterol. The C. wightii being antioxidant, helps stop the oxidation of cholesterol and subsequent hardening of the arteries. Moreover, C. wightii has also been shown to reduce the stickiness of platelet, another effect that lowers the risk of coronary artery disease (Singh *et al*., 1997) [54].

Diet changes and exercise implementation may also be part of a treatment plan for diabetes. In our findings the hyperlipidemic control (HLC) rats showed significantly higher level of glucose (+280.53%) than normal control rats (NLC) (Table 3). Hyperlipidemic treated rats groups i.e., HLTWS-250, HLTWS-500, HLTCW-250 and HLTCW-500 showed a dose dependent reduction in glucose levels. The rats of W. somnifera treatment groups i.e., HLTWS-250 and HLTWS-500 showed a decrease of 35.75% and 39.67% in blood glucose levels respectively at the end of the experimental period. Similar findings were observed by other coworkers; Ojha and Arya (2009) [42], Hoda *et al*., (2010) [29], Prasad *et al*., (2010) [48] and Jaiswal *et al*., (2010) [30]. Likewise, the C. wightii treated rats i.e., HLTCW-250 and HLTCW-500 groups showed a decrease of 37.02% and 40.75% in blood glucose levels respectively during the 4-week treatment program (Table 3) in line with other workers; Satyavati *et al*., (1991) [52], Baldwa *et al*., (1981) [3], Chander *et al*., (2002) [19].

It has been well documented that when the uncontrolled hyperlipidemic condition progresses, considerable alterations in total cholesterol and triglycerides values are predictable (Kumar *et al*., 2010; Colledge *et al*., 2010) [20]. In our study, the hyperlipidemic control rats (HLC) had moderately higher total cholesterol (TC) values by about 174.92% and TGs values by about 585.90% when compared with normolipidemic control rats (NLC) (Table 4).

Hyperlipidemic rats treated with lower dose of W. somnifera extract (HLTWS-250) showed significantly lower values of serum TC by 20.60%, TGs by 50.66% and serum LDL by 14.49% and higher value of HDL by 48.67% (Table 4) while the higher dose of W. somnifera extract treatment (HLTWS-500) showed superior lowering effects on serum TC by about 47.88%, on serum TG by about 66.22% and serum LDL by 22.25% and improved level of HDL by 42.05% when compared to the hyperlipidemic control (HLC) counterparts (Table 4). In similar findings, Ojha and Arya (2009) [42], Hoda *et al*., (2010) [29] and Prasad *et al*., (2010) [48] demonstrated that W. somnifera possess antioxidant, anti-hypertensive and hypolipidemic effects which may contribute to its cardio-protective properties.

Guggul and gugulipid have a long history in the treatment of cardiovascular diseases including hypercholesterolemia and atherosclerosis. In our study, treatment with lower dose of C. wightii extract (HLTCW-250) showed significantly lower values of serum TC by 23.96%, TGs by 47.58% and serum LDL by 16.80% and higher value of HDL by 14.11% (Table 4) while the higher dose of C. wightii extract treatment (HLTCW-500) showed better-quality lowering effects on serum TC by about 48.86%, serum TGs by about 65.91% and serum LDL by about 23.37% and serum HDL by about 43.25% when compared to the hyperlipidemic control (HLC) counterparts (Table 4). The hypolipidemic effect of gugulipid and guggulsterone has been consistently demonstrated in various animal species, including rat, mouse, rabbit (Baldwa *et al*., 1981; Khanna *et al*., 1969; Dixit *et al*., 1980) [3, 33, 23]. Guggul markedly inhibits liver cholesterol biosynthesis (Orten and Neuhaus, 1982) [43]. This causes interference in lipoprotein formation and lipid turnover. Guggul increases fecal excretion of bile acids (cholic and deoxycholic acids) and cholesterol and lowers intestinal absorption of fat and cholesterol. Guggul stimulates the LDL receptor binding activity in hepatocytes and enhances its catabolism. It also inhibits oxidative modification of LDL due to its constituent gugglusterone (Wang *et al*., 2004) [61].

Guggulsterones may also lower serum cholesterol by enhancing hepatic reuptake of cholesterol by stimulating hepatic LDL receptors. According to Shishodia *et al*., (2008) [53] most clinical human trials show a considerable decrease in serum TGs and cholesterol, with positive benefits being seen in patients. In addition, Guggul increases thyroid stimulation, improves digestion, and accelerates metabolism to pass the food along the GIT tract quickly. It also prevents the transformation of undigested carbohydrates into triglycerides and reduces cholesterol in blood by metabolizing the existing fatty acid. Guggul is considered to be a well-established fat burning agent (Policappelli *et al*., 1995) [47]. Guggul has antioxidant effect because of its active constituent gugglusterone, which inhibits the generation of oxygen free radical (Chander *et al*., 2002; Singh *et al*., 1994) [19]. Several other studies have revealed cardioprotective abilities of guggul including increased fibrinolytic activity and decreased the platelet adhesive index (Tripathi *et al*., 2003; Baldeva *et al*., 1980; Kaul and Kapoor, 1989) [2, 32]. Guggulipid is effective against myocardial infarction and known to cause thyrogenic effect.

The HDL/TC ratio is helpful in predicting atherosclerosis. A low ratio indicates a higher risk of heart attack while a high ratio indicates a lower risk. High total cholesterol and low HDL cholesterol increases the ratio, with the intention that situation is detrimental. On the contrary, low total cholesterol and high HDL cholesterol lowers the ratio and is fine reports. It has been shown that hyperlipidemic control rats (HLC) have only 19.09% of HDL/TC ratio while normolipidemic control rats (NLC) have shown about 83% of this ratio. In our experiment, dose dependent recovery trend have been found in treated rats (Table 5).

Healthy kidneys remove wastes and excess fluid from the blood. Blood urea and creatinine are good markers for the regular functioning of the kidney. Urea is a waste product that is created by protein metabolism and excreted in the urine. Creatinine is a waste product of muscle energy metabolism and is produced at a constant rate that is proportional to the muscle mass of an individual. The body does not recycle it, so the quantity filtered by the kidneys in a given amount of time

is excreted with the urine, making creatinine clearance a specific measure of the kidney function. In this study we have observed that hyperlipidemic control rats (HLC) have approximately twice the level of blood urea, creatinine, ALT and AST. All these four markers have been found to decrease considerably in hyperlipidemic rats treated with W. somnifera extract and C. wightii extracts in a dose dependent manner when compared to hyperlipidemic control rats (Table 5).

Hyperlipidemic rats treated with lower dose of W. somnifera extract (HLTWS-250) showed significantly lower values of serum urea by 46.23%, serum creatinine by 45.83%, serum ALT by 34.86% and serum AST by 29.18% when compared with the hyperlipidemic control (HLC) counterparts (Table 5). Contrary to this, the higher dose of W. somnifera extract treatment (HLTWS-500) showed even better lowering effects on serum urea by 52.65%, serum creatinine by 38.69%, serum ALT by 46.47% and serum AST by 38.29% when compared to the hyperlipidemic control (HLC) counterparts. The hypolipidemic effect of W. somnifera fruits were found to be comparable to Ayurvedic product containing Commiphora mukkul shown by Prasad *et al*., (2010) [48]. They concluded that the extracted coagulin L from fruits of W. somnifera has antidyslipidemic effect in mice.

In the same way, treatment with lower dose of C. wightii extract (HLTCW-250) showed significantly lower values of serum urea by 46.69%, serum creatinine by 23.21%, serum ALT by 37.52% and serum AST by 28.56% when compared with the hyperlipidemic control (HLC) counterparts. In contradiction of this, the higher dose of C. wightii extract treatment (HLTCW-500) showed better-quality lowering effects on serum urea by 53.11%, serum creatinine by 38.09%, serum ALT by 48.98% and serum AST by 39.01% when compared with the hyperlipidemic control (HLC) counterparts.

Table 1: Qualitative determination of active ingredients in ethanolic extracts of two plants

Plant constituents	Ethanolic plant extracts	
	Withania somnifera	***Commiphora wightii***
Alkaloids	++	++
Flavonoids	++	++
Saponins	++	++
Tannins	++	++

Table 2: Effect of treatment on body weight changes in various rat groups

Treatment Days	Body weight (in gram) of Various Treatment Groups of Rats						
				Withania somnifera		***Commiphora wightii***	
	NLC	**HLC**	**HLT_{AVT}**	**HLT_{WS-250}**	**HLT_{WS-500}**	**HLT_{CW-250}**	**HLT_{CW-500}**
Day 0	157.13 ± 2.30	325.50 ± 2.73*	323.58 ± 2.87	326.55 ± 2.78	323.37 ± 1.45	327.56 ± 2.75	324.48 ± 1.98
Day 7	159.86 ± 2.64	329.78 ± 2.96*	297.50 ± 2.54	313.53 ± 2.57	302.23 ± 2.73	311.24 ± 2.42	305.59 ± 2.78
Day 15	154.16 ± 2.58	331.23 ± 2.35*	295.50 ± 2.47	308.85 ± 2.69	297.75 ± 2.48	299.39 ± 2.97	298.58 ± 2.64
Day 25	152.15 ± 2.39	333.25 ± 2.68*	294.54 ± 2.44	305.87 ± 2.47	295.36 ± 2.87	297.37 ± 2.94	296.56 ± 2.66

*Values expressed as Mean ± S.E.M, n = 6 in each group; *Significant as compared to control.*
NLC: Normolipidemic control rats; HLC: Hyperlipidemic control rats; HLT_{250}: Hyperlipidemic rats treated with 250 mg of extract; HLT_{500}: Hyperlipidemic rats treated with 500 mg of extract; HLT_{AVT}: Hyperlipidemic rats treated with atorvastatin at a dose of 2.0 mg/ kg of body wt.

Table 3: Effects of treatment on blood glucose levels in various rat groups.

GROUPS		Blood glucose levels (mg/dl) in week				
		Pretreatment	**Post-treatment**			
		0	**1**	**2**	**3**	**4**
NLC		133.72 ± 2.08	131.75 ± 2.24	130.56 ± 2.87	132.22 ± 2.36	134.02 ± 2.10
HLC		508.85 ± 3.88*	512.89± 3.21*	520.97 ± 2.90*	518.87 ± 2.43*	519.89 ± 2.99*
HLT_{AVT}		509.59 ± 2.85	468.89 ± 3.85	411.81 ± 2.81	343.33 ± 2.44	266.56 ± 3.56
W. somnifera	HLT_{WS-250}	508.12 ± 2.32	498.86 ± 3.87	421.87 ± 2.87	373.36 ± 2.47	326.45 ± 3.45
	HLT_{WS-500}	507.87 ± 3.78	477.27 ± 2.92	418.84 ± 2.44	356.34 ± 2.44	306.41 ± 3.21
C. wightii	HLT_{CW-250}	509.91 ± 3.16	496.34 ± 3.35	425.83 ± 2.33	369.39 ± 2.49	321.13 ± 3.53
	HLT_{CW-500}	508.74 ± 2.71	469.91 ± 3.37	408.85 ± 2.55	353.35 ± 2.44	301.42 ± 3.42

*Values expressed as Mean ± S.E.M, n = 6 in each group; * p<0.05 as compared with normal control.*
NLC: Normolipidemic control rats; HLC: Hyperlipidemic control rats; HLT_{250}: Hyperlipidemic rats treated with 250 mg of extract; HLT_{500}: Hyperlipidemic rats treated with 500 mg of extract; HLT_{AVT}: Hyperlipidemic rats treated with atorvastatin at a dose of 2.0 mg/ kg of body wt.

Table 4: Effects of treatment on serum lipid levels in various rat groups.

GROUPS		TC (mg/dl)	TG (mg/dl)	HDL(mg/dl)	HDL/TC (%)	LDL(mg/dl)
NLC		75.98 ± 1.89	60.42 ± 0.78	63.45 ± 2.45	83.50 ± 2.08	62.72 ± 2.24
HLC		208.89 ± 1.67*	414.42 ± 3.42*	39.88 ± 2.82*	19.09 ± 3.84*	89.89 ± 3.35*
HLT_{AVT}		96.98 ± 1.58	118.76 ± 3.54	59.29 ± 3.21	61.14 ± 3.84	64.79 ± 3.76
W. somnifera	HLT_{WS-250}	165.85 ± 1.54	204.43 ± 3.34	44.84 ± 3.82	27.04 ± 3.84	76.86 ± 3.87
	HLT_{WS-500}	108.87 ± 1.37	139.96 ± 3.67	56.65 ± 3.85	52.03 ± 3.84	69.89 ± 3.91
C. wightii	HLT_{CW-250}	158.82 ± 1.32	217.22 ± 3.43	45.51 ± 3.32	28.65 ± 3.84	74.78 ± 3.76
	HLT_{CW-500}	106.82 ± 1.51	141.29 ± 3.86	57.13 ± 3.31	53.48 ± 3.84	68.88 ± 3.89

*Values expressed as Mean ± S.E.M, n = 6 in each group; * p<0.05 as compared with normal control.*
TC = Total Cholesterol; TG = Triglycerides; HDL = High density lipoprotein; LDL = Low density lipoprotein
NLC: Normolipidemic control rats; HLC: Hyperlipidemic control rats; HLT_{250}: Hyperlipidemic rats treated with 250 mg of extract; HLT_{500}: Hyperlipidemic rats treated with 500 mg of extract; HLT_{AVT}: Hyperlipidemic rats treated with atorvastatin at a dose of 2.0 mg/ kg of body wt.

Table 5: Effects of treatment on blood urea, creatinine, ALT & AST in various rat groups.

GROUPS		**Urea** (mg/dl)	**Creatinine** (mg/dl)	**ALT** (IU/L)	**AST** (IU/L)
NLC		39.65 ± 1.22	0.91 ± 0.037	29.31 ± 0.29	67.42 ± 1.46
HLC		91.68 ± 1.86*	1.68 ± 0.043*	61.24 ± 1.44*	116.37 ± 2.71*
HLT_{AVT}		40.17 ± 0.34	0.99 ± 0.027	33.31 ± 0.61	69.67 ± 0.74
W. somnifera	HLT_{WS-250}	49.29 ± 1.17	1.26 ± 0.046	39.89 ± 2.95	82.42 ± 1.72
	HLT_{WS-500}	43.37 ± 1.76	1.03 ± 0.023	32.78 ± 0.58	71.81 ± 1.42
C. wightii	HLT_{CW-250}	48.87 ± 1.15	1.29 ± 0.039	38.26 ± 2.47	83.13 ± 1.44
	HLT_{CW-500}	42.98 ± 1.45	1.04 ± 0.047	31.24 ± 0.54	70.97 ± 1.57

*Values expressed as Mean ± S.E.M, n = 6 in each group; * p<0.05 as compared with normal control.*
ALT: Alanine transaminase; AST: Aspartate aminotransferase; NLC: Normolipidemic control rats; HLC: Hyperlipidemic control rats; HLT_{250}: Hyperlipidemic rats treated with 250 mg of extract; HLT_{500}: Hyperlipidemic rats treated with 500 mg of extract; HLT_{AVT}: Hyperlipidemic rats treated with atorvastatin at a dose of 2.0 mg/ kg of body wt.

5. Conclusion

Therefore the current results demonstrated the cardiovascular therapeutic benefits of W. somnifera and C. wightii plants extracts compared to atorvastatin in a dose dependent manner in improving the kidney function and thus, in the prevention of hyperlipidemic condition and could promote a better health. The hyperlipidemia and its associated problems in this study could be ameliorated in different degrees by using W. somnifera and C. wightii plants extracts. The study also showed that the two plants extracts signifi¬cantly improved cholesterol, LDL triglycer¬ide concentrations, creatinine, urea etc resulting from the hyperlipidemic induction. So it also had hypolipi¬demic, renoprotective, hapatoprotective and antioxidant effect. Consequently, we can infer that application of W. somnifera and C. wightii plants extracts can be a supplement to the existing oral anti hyperlipidemic drugs.

6. References

1. Anonymous, Indian Herbal pharmacopoeia, revised edition, (Indian Drug Manufacturers Association, Mumbai), 2012, 134.
2. Baldeva VS, Sharma RC, Ranka PC, Chittera MD: Effect of Commiphora mukul (guggul) on fibrinolytic activity and platelet aggregationin coronary artery disease, Rajasthan Med J. 1980; 19:84.
3. Baldwa VS, Bhasin V, Ranka PC. Mathur KM: Effects of Commiphora mukul (Guggul) in experimentally induced hyperlipemia and atherosclerosis. J Assoc Physicians India. 1981; 29:13-17.
4. Bharti SK, Kumar A, Sharma NK, Krishnan S, Gupta AK, Padamdeo SR. Antidiabetic effect of aqueous extract of Withania coagulans flower in Poloxamer-407 induced type 2 diabetic rats. Journal of Medicinal Plants Research. 2012; 6(45):5706-5713.
5. Bharti SK, Krishnan S, Sharma NK, Kumar A, Prakash O, Gupta AK, Kumar A. In vivo and in silico investigation of antidiabetic activity of fruit of Withania coagulans Dunal. Current hypertension reviews. 2015; 11(2):143-158.
6. Kumar A, Bharti SK, Kumar A. Therapeutic molecules against type 2 diabetes: what we have and what are we expecting? Pharmacol Rep. 2017; 69:959-970.
7. Kumar A, Bharti SK, Kumar A. Type 2 diabetes mellitus: the concerned complications and target organs. Apollo Med. 2014; 11:161-166.
8. Bharti SK, Krishnan S, Gupta AK. Herbal formulation to combat type 2 diabetes mellitus. Germany: LAMBERT Academic Publishing, 2013.
9. Bharti SK, Krishnan S, Kumar A. Antidiabetic activity and molecular docking of fructooligosaccharides produced by Aureobasidium pullulans in poloxamer-407-induced T2DM rats. Food Chem. 2013; 136:813-821.
10. Bharti SK, Krishnan S, Kumar A. Antihyperglycemic activity with DPP-IV inhibition of alkaloids from seed extract of Castanospermum australe: investigation by experimental validation and molecular docking. Phytomedicine. 2012; 20:24-31.
11. Bharti SK, Kumar A, Sharma NK. Tocopherol from seeds of Cucurbita pepo against diabetes: validation by in vivo experiments supported by computational docking. J Formos Med Assoc. 2013; 112:676-690.
12. Bharti SK, Krishnan S, Kumar A. Phytotherapy for diabetes mellitus: back to nature. Minerva Endocrinol. 2016; 41:143-146.
13. Bharti SK, Krishnan S, Kumar A, Kumar A. Antidiabetic phytoconstituents and their mode of action on metabolic pathways. Ther Adv Endocrinol Metab. 2018; 9(3):81-100.
14. Bharti SK, Krishnan S, Kumar A, Gupta AK, Kumar A. Mechanism-based antidiabetic activity of Fructo- and isomalto-oligosaccharides: Validation by in vivo, in silico and in vitro interaction potential. Process Biochem. 2015; 50(2):317-327.
15. Bharti SK, Kumar A, Prakash O, Krishnan S, Gupta AK. Essential oil of Cymbopogon citratus against diabetes: Validation by in vivo experiments and computational studies. J Bioanalysis and Biomed. 2013; 5(5):194-203.
16. Bharti SK, Krishnan S, Kumar A, Sharma NK, Jaiswal SK, Gupta AK, Ghosh AK. Dipeptidyl Peptidase IV inhibitory activity of seed extract of Castanospermum australe and molecular docking of their Alkaloids. Topclass J Herbal Med. 2012; 1(1):029-035.
17. Blair SN, Capuzzi DM, Gottlieb SO, *et al*., Incremental reduction of serum total cholesterol and low-density lipoprotein cholesterol with the addition of plant stanol ester-containing spread to statin therapy. Am J Cardiol. 2000; 86:46-52.
18. Cáceres A. Plantas de Uso Medicinal en Guatemala. 1st ed. Editorial Universitaria: San Carlos de Guatemala. 61, 67, 167, 236, 283, 325, 328, 1996.
19. Chander R, Khanna AK, Pratap R. Antioxidant activity of guggulsterone, the active principle of guggulipid from Commiphora mukul, J Med Aromat Plant Sci. 2002; 24:370.
20. Colledge NR, Walker BR, Ralston SH. Davidson's Principles and Practice of Medicine. 21stedition. Churchill Livingstone Elsevier, 2010, 449-456.
21. Conway B, Rene A. Obesity as a disease: no lightweight matter. Obes Rev. 2004; 3:145-151.
22. Deng R, Yang D, Radke A, Yang J, Yan B. The hypolipidemic agent guggulsterone regulates the expression of human bile salt export pump: Dominance

of transactivation over farsenoid X receptor-mediated antagonism. J Pharmacol Exp Ther. 2007; 320:1153-1162.
23. Dixit VP, Joshi S, Sinha R, Bharvava SK, Varma M. Hypolipidemic activity of guggul resin (Commiphora mukul) and garlic (Alium sativum linn.) in dogs (Canis familiaris) and monkeys (Presbytis entellus entellus Dufresne). Biochem Exp Biol. 1980; 16:421-424.
24. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of lowdensity lipoprotein cholesterol in plasma, without the use of preparative centrifuge. Clin. Chem. 1972; 18:499-502.
25. GidezL Miller G, Burstein M, *et al*. Separation and quanufication of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. J Lipid Res. 1982; 23:1206-1223.
26. Ghufran A. The effect of luk maghsool (processed lac) on diet-induced hyperlipidemia in Albino rats. Hippocratic J Unani Med. 2011; 6(2):155-165.
27. Gorsek WF. Cholesterol treatment formulation, US Patent Appl. No 187614, 2002.
28. Harborne JB. Phytochemical Methods, Chapman and Hall, Ltd, London, 1973, pp. 49-188.
29. Hoda Q, Ahmed S, Akhtar M, Najmi AK, Pillai KK, Ahmed SJ. Antihyperglycaemic and antihyperlipidaemic effect of polyconstituents, in aqueous and chloroform extracts of Withania coagulans Dunal in experimental type 2 diabetes mellitus in rats. Hum. Exp. Toxicol. 2010; 29(8):653-658.
30. Jaiswal D, Rai PK, Watal G. Hypoglycemic and antidiabetic effects of Withania coagulans fruit ethanolic extract in normal and streptozotocin-induced diabetic rats. Ind. J. Clin. Biochem. 2010; 24(1):88-93.
31. Katzung BG. Basic and Clinical Pharmacology. 12th ed. International Edition; McGraw-Hill, 2012, 560-572.
32. Kaul S, Kapoor NK. Cardiac sarcolemma enzymes and liver microsomal cytochrome P450 in isoproterenol treated rats, Indian J Med Res. 1989; 90:62.
33. Khanna DS, Agarwal OP, Gupta SK, Arora RB. A biochemical approach to anti-atherosclerotic action of Commiphora mukul: An Indian indigenous drug in Indian domestic pigs (Sus scrofa). Indian J Med Res. 1969; 57:900-906.
34. Kumar V, Abbas AK, Fausto N, Robbins SL, Cotran RS. Robbins and Cotran pathologic basis of disease. 8th ed. Philadelphia: Elsevier Saunders Elsevier Inc, 2010, 514-525.
35. Kumari K, Augusti KT. Lipid lowering effect of S-methyl cysteine sulfoxide from Allium cepa Linn in high cholesterol diet fed rats. J Ethnopharmacol. 2007; 109:367-371.
36. Lee K, Sobal J. Socio-economic, dietary, activity, nutrition and body weight transitions in South Korea. Public Health Nutr. 2003; 6:665-674.
37. Liu F, Gan PP, Wu HN, Woo WS, Eng SO. A combination of metabolomics and metallomics studies of urine and serum from hypercholesterolaemic rats after berberine injection. Anal Bioanal Chem. 2012; 403:847-856.
38. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983; 54:275-87.
39. Manach C, Mazur A, Scalbert A. Polyphenols and prevention of cardiovascular diseases. Curr Opinions in Lipidology. 2005; 16:77-84.
40. Mansi K, Abu Shaffa AM, Disi A, Aburjai T. Hypolipidaemic effects of seed extract of celery (Apium graveolans Linn.) in rats. Pheog Mag. 2009; 5:301-305.
41. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of Withania somnifera (Ashwagandha): a review. Alt Med Rev. 2000; 5:334-346.
42. Ojha SK, Arya DS. Withania somnifera Dunal (Ashwagandha): A Promising Remedy for Cardiovascular Diseases. World J. Med. Sci. 2009; 4(2):156-158.
43. Orten JM., Neuhaus OW. Human Biochemistry. C.V. Mosby Company, St. Louis, Mo., U.S.A. Cited by Sabinsa Corp, 1982-2000.
44. Panda S, Kar A. Gugulu (Commiphora mukul) induces triiodothyronine production: possible involvement of lipid peroxidation. Life Sci. 1999; 65(12):137.
45. Park K. Park's Textbook of Preventive and Social Medicine.22th ed. M/S Banarasidas Bhanot, 2013, 302-309.
46. Parray AS. An experimental study of Tukhme Suddab to evaluate its efficacy in Diet Induced Hyperlipidaemia and Atherosclerosis in Rabbits. Bangalore: Dissertation submitted to RGUHS, 2010.
47. Policappelli E, Garzone R, Russo C. Dietary supplement, US Patent Appl No 426677, 1995.
48. Prasad SK, Kumar R, Patel DK, Hemalatha S. Wound healing activity of Withania coagulans in streptozotocin-induced diabetic rats. Pharm Biol. 2010; 48(12):1397-404.
49. Prince PSM, Selvaraju S, Devika PT, Vaithianathan. Cardioprotective effect of 'Marutham' a polyherbal formulation on isoproterenol induced myocardial infarction in Wistar rats. Fitoterapia. 2008; 79:433-438.
50. Randhir R, Lin YT, Shetty K. Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. Asia Pacific J Clin Nutri. 2004; 13:295-307.
51. Sahni S, Hepfinger CA, Sauer KA. Guggulipid use in hyperlipidemia: case report and review of the literature. American Journal of Health-System Pharmacy. 2005; 62(16):1690-1692.
52. Satyavati GV, Guggulipid. A promising hypolipedemic agent from gum guggul (Commiphora wightii), Econ Med Pl Res. 1991; 5:47.
53. Shishodia S. The guggul for chronic diseases: ancient medicine, modern targets. Anticancer Res, 2008.
54. Singh K, Chander R, Kapoor NK. Guggulsterones, a potent hypolipedemic prevents oxidation of low-density lipoprotein. Phytother Res. 1997; 11:291-294.
55. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor, Ann. Clin. Biochem. 1969; 6(5):24-27.
56. Tripathi YB, Malhotra OP, Tripathi SN. Thyroid stimulating action of Z-guggulsterone obtained from Commiphora mukul, Planta Med, 1984, 78.
57. Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A, Dandona P. Elevation of Free Fatty Acids Induces Inflammation and Impairs Vascular Reactivity in Healthy Subjects. Diabetes. 2003; 52(12):2882-2887.
58. Ulbricht C, Basch E, Szapary P, Hammerness P, Axentsev S, Boon H, Kroll D, Garraway L, Vora M, Woods J. Guggul for hyperlipidemia: A review by the Natural Standard Research Collaboration. Complement

Ther Med. 2005; 13:279-290.
59. Urizar NL, Moore DD. GUGULIPID A natural cholesterol-lowering agent. Annu Rev Nutr. 2003; 23:303-313.
60. Vijaykumar RS, Surya D, Senthil kumar R, Nalini N. Hypolipidemic effect of Black Pepper in rats fed high fat diet. J Clin Biochem Nutr. 2002; 32:31-42.
61. Wang X, Greilberger J, Ledinski G, Kager G, Paigen B, Jurgens G. The hypolipidemic natural product Commiphora mukul and its component guggulsterone inhibit oxidative modification of LDL. Atherosclerosis. 2004; 172(2):239-246.