

INVESTIGATION OF LIPID LOWERING EFFECT OF GYNURA NEPALENSIS (LEAVES) IN FAT INDUCED HYPERLIPIDEMIC RATS.

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Abstract

Gynura nepalensis leaves have been reported to use traditionally in the treatment of diabetes as well as in indigestion and healing of wounds. The present study investigated the antihyperlipidemic potential of the leaf extract from *Gynura nepalensis* (GN). Experimental hyperlipidemia was induced by oral administration of animal fat for 30 days. After 30 days treatment with fat, hyperlipidemic rats were divided into different groups (n=6) and administered test extract along with animal fat for further 28 days. After 28 days treatment, rats were sacrificed, blood samples were collected; serum was separated and subjected to lipid profile test such as TC, TG, VLDL-C, LDL-C and HDL-C. Administration of extra animal fat along with normal diet in rats produced hyperlipidemia. After 28 days treatment of hyperlipidemic rats with GN leaves extract, GN 250mg/kg showed significant reduction of TC (p<0.01), TG (p<0.05) and LDL-C (p<0.01) level whereas GN 500mg/kg dose demonstrated significant reduction of TC (p<0.001), TG (p<0.001) and LDL-C (p<0.001) level. Also insignificantly reduced VLDL-C and insignificantly increased the HDL-C at both the doses as compared to the lipid control group. Based on this investigation, *G. nepalensis* leaves exhibited antihyperlipidemic effect by ameliorating lipid metabolism disorders in hyperlipidemic model rats. However, the specific mechanism (s) by which GN induces its effects on hyperlipidemia needs further investigation.

Keywords: Fat induced hyperlipidemia, antihyperlipidemic effect, *Gynura nepalensis*, lipid profile.

Introduction

Hyperlipidemia has been defined as abnormal elevation of serum lipids or lipoproteins. Hyperlipidemia is a major health problem nowadays. It is an important concern of health professionals because of it is a known major risk factor for the advancement of cardiovascular diseases including atherosclerosis [1-2]. Many studies have shown that elevated plasma cholesterol concentrations can cause coronary atherosclerosis [3] and that this effect is associated with heart disease, stroke, and death in both developed and developing countries [4]. American Heart Association (AHA) has identified the primary risk factor associated with progression of atherosclerotic lesions as elevated levels of total cholesterol (TC) and triglycerides (TG) in serum [5]. Therefore, prime consideration in therapy for hyperlipidemia and arteriosclerosis is to enervate the elevated plasma level of TC, TG and LDL along with increase in HDL lipid levels [6].

Plant based medicines are thoughtfully less harmful with less adverse reactions than synthetic medications. The potential therapeutic benefits of plant-based medicines exhibited significant pharmacologic activity producing compounds with hypoglycemic, hypolipidemic and antihypertensive properties [7-8]. Numerous herbal medicines have been recommended for the treatment of different diseases [9].

There are approximately 5000 plant species in Bangladesh of which about 1000 are thought to possess medicinal properties and are used in the traditional systems of medicine that serves as the primary healthcare for most of the people of Bangladesh. So, research in medicinal plants is a vital sector for the discovery of promising drugs in Bangladesh [10]. Therefore, investigation for new agents eligible for reducing serum lipids is a vital examination center.

***Gynura nepalensis* DC**, locally known as 'Diabetes Gach', in Bangladesh is a perennial herb. It is native to Nepal, distributed in India, China, Myanmar, Bhutan and Thailand and was first identified in Bangladesh in 2006 [11-12]. Phytochemical screening of *G. nepalensis* leaf extract indicated the presence of alkaloids, tannins, flavonoids, saponin and steroid [13-15]. It was reported that the plant is used in diabetes, hypertension, to heal cuts and wounds. It

is also used as hepatoprotective [11, 16-18]. The present study was undertaken to investigate the antihyperlipidemic potential of leaves extract from *Gynura nepalensis*.

Methods

Plant collection and Extraction: *Gynura nepalensis* (GN) leaves were collected from Savar area and was identified and authenticated by the Scientific officer, Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession No. 43206). The collected materials were thoroughly washed in water and shed dried at 35°-40° C for a week and pulverized in electric grinder to get extractable powder. Then powder was extracted in soxhlet apparatus with ethanol. Then the solvent washing the constituents of powder was dried with a rotary evaporator to get viscous substance. Finally a solid mass was obtained and preserved in a petridis in the refrigerator for further analysis.

Experimental animals: For the experiment SD rats of either sex, weighing 120 to 150gm were selected for the present study and acclimatized under standard conditions. The animals were bred at Pharmacology laboratory of Department of Pharmacy, Jahangirnagar University and was maintained on 12h light-dark cycle at 21±2 °C. A standard pellet diet and water were supplied *ad libitum* unless otherwise indicated. All protocols for animal experiment were approved by the institutional animal ethical committee.

Toxicity studies: Toxicity studies of the plant extract was carried out in Swiss Albino mice of either sex weighing between 25 and 30g. The extract was found to be safe till 5000mg/kg (p.o) [19].

Induction of hyperlipidemia and antihyperlipidemic study: The method of Shinnick FL *et. al.*, (1990) was followed with slight modification. Experimental hyperlipidemia was induced by feeding animal fat orally for 30 days except for the normal control group which was fed with standard pellet only. After 30 days treatment with animal fat, rats were divided into five groups of 6 animals each and treated with vehicle, standard drug and test extract respectively in following manner [20].

Group I (Normal Control): Normal rats, received water (10 mL/kg p.o.) and standard rat pellet for 28 days, and served as normal control.

Group II (Lipid Control): Hyperlipidemic rats, received water (10 mL/kg p.o.) and lipid (1ml/rat, p.o.) and normal food for 28 days.

Group III (STD): Hyperlipidemic rats, received standard drug atorvastatin (10 mg/ kg p.o.) and lipid (1ml/rat, p.o.) and normal food for 28 days.

Groups IV and V (GN 250mg/kg and GN 500mg/kg): Hyperlipidemic rats, received *G. nepalensis* (250 and 500mg/kg respectively, p.o.) and lipid (1ml/rat, p.o.) and normal food for 28 days.

Estimation of lipid profile: After a treatment period of 28 days, rats were anesthetized using ketamine (500 mg/kg, i.p.) After sacrifice, blood samples from each group of rats were collected in test tubes and the serum was separated by centrifugation (3000 rpm, 10 minutes). Serum samples were subjected to lipid profile test such as total cholesterol (TC), triglycerides (TG), LDL-C, VLDL-C and HDL-C. The tests were done in Erba Chem 5V3 semi auto biochemistry analyzer (Made in Germany).

Statistical Analysis: Statistical analysis for animal experiments was carried out by One way ANOVA following Bonferroni's post hoc comparison using SPSS 16.0 for windows. Data were presented as Mean±SEM. The results obtained were compared with the vehicle treated control group and $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered to be statistically significant, highly significant and very highly significant respectively.

Results

The acute toxicity studies demonstrated the nontoxic nature of the plant. No lethality or any toxic reactions or behavioral changes was found till the dose of 5g/kg bw for a period of 72 hours observation period.

Administration of high animal fat along with normal diet in rats caused hyperlipidemia with an abnormal increase in serum total cholesterol (TC), triglycerides (TG), LDL-C, VLDL-C and decrease in HDL-C levels when, compared to rats fed with a normal standard diet (Figure 1-5).

After 28 days treatment of hyperlipidemic rats with the ethanolic extract of *G. nepalensis* leaves at 250mg/kg showed significant improvement in the lipid profile. *G. nepalensis* at 250mg/kg highly significantly reduced the level of TC ($p < 0.01$), LDL-C ($p < 0.01$) level and significantly the TG ($p < 0.05$) level

whereas increased the HDL level insignificantly. However, at 500mg/kg dose, demonstrated very highly significant reduction of TC ($p < 0.001$), TG ($p < 0.001$) and LDL-C ($p < 0.001$) level. Also insignificantly reduced VLDL-C and insignificantly increased the HDL-C level as compared to the lipid control group (Figure 1-5).

Discussion

Asia has many species of medicinal plants and traditional medicines have been utilized in India and China to cure ailments for thousands of years. These medicinal plants play very important role in the treatment of rural people with few health facilities [21].

Various models are found in the literature for the study of hypercholesterolemia in rats. Diets containing high amount of fats or cholesterol lead to both hypercholesterolemia and hypertriglyceridemia which are major prognosis for cardiovascular diseases CVD [22]; and leading cause of death in developing and developed countries [23].

Cardiovascular diseases, (CVD), particularly coronary heart disease (CHD), have become a growing problem, especially in developing countries. Hypercholesterolemia is widely known as a dominant risk factor for the development of cardiovascular diseases [24].

Induction of hyperlipidemia by feeding rats with 30 days resulted in several alterations in the serum TC, TG, LDL-c, VLDL-c and HDL-c levels associated with a dramatic increase in the body weight. This effect resembles type IIa hyperlipidemia in humans [25] as shown in the present study. Dietary cholesterol is known to cause a temporary increase in the plasma cholesterol level and a marked increase in the liver cholesterol level, biliary excretion of bile acids and fecal excretion of sterols and bile acids [26]. The hypercholesterolemic effect induced by high fat diet (HFD) may be due to the activity of the rate-determining enzyme in cholesterol biosynthesis, HMG-CoA reductase; stimulating the cholesterologenesis rate [27]. On the other hand, development of hyperlipidemia may be also due to a decrease in catecholamine level which leads to low β_2 - adrenergic receptor function [28] and decrease lipolysis of fat cells [29].

Cholesterol is essential for the membranes of mammalian cell. It plays major roles in permeability and fluidity of the membrane and also serves as a precursor of bile acids, steroid hormones and fat-soluble vitamins [30-31]. Triglycerides are esters that play an important role in metabolism and transporters of dietary fat; they are a major component of very low density lipoproteins (VLDL-C) and chylomicrons [32-33].

Hypercholesterolemia and increased LDL-C levels have been found to be a major risk factor for the development of atherosclerosis [34-35]. Alteration in the serum lipid profile is also likely to increase the risk for coronary heart disease [36]. Scientists have concluded that overproduction of reactive oxygen species (ROS) (oxidative stress) plays a pivotal role in the oxidation of LDL-C molecules, which get accumulated in the layers of blood vessels. Lipid oxidation due to generation of ROS is considered as an important factor in the initiation and progression of several diseases [37].

A reduction in serum lipids, particularly of the LDL-C and VLDL-C fraction and TG should be considered as being beneficial for the long term prognosis of obese patients. Oral administration of GN extract caused significant declines in the blood levels of triglycerides, total cholesterol, LDL-cholesterol, but increased HDL-cholesterol which reflected that extracts had a hypolipidemic potential after a 28 days study period in hyperlipidemic model rats. Our study improved the lipid profile and was the most effective in ameliorating fat induced dyslipidemia and oxidative stress.

These findings could be interpreted by the inhibitory effect of GN on HMG CoA reductase enzyme which catalyzes the conversion of HMG-CoA to mevalonate, a rate-limiting step in the formation of endogenous cholesterol leading to the decrease in the intracellular stores of cholesterol. This in turn results in the up-regulation of LDL receptors on the cell membrane, thus increasing the clearance of LDL-c from plasma [38]. Another possible explanation of our results is that GN may lower LDL-c level by inhibiting hepatic cholesterol synthesis in very low density lipoprotein (VLDL) which is the source of LDL-c. Thus may impair VLDL particle assembly and secretion from liver, decrease the

VLDL levels in plasma, and further decrease the LDL level in plasma [39].

Conclusion

Results of the present experimental evaluation demonstrated the lipid lowering effect of *G. nepalensis* leaves extract with a significant improvement in the lipid profile such as significant reduction of TC, TG, VLDL-C and LDL-C as well as an insignificant increase in the HDL-C level on the fat induced hyperlipidemic model rats. Therefore, the plant may be used in the treatment of hyperlipidemia. Further investigations are recommended to find out the mechanisms of the effect.

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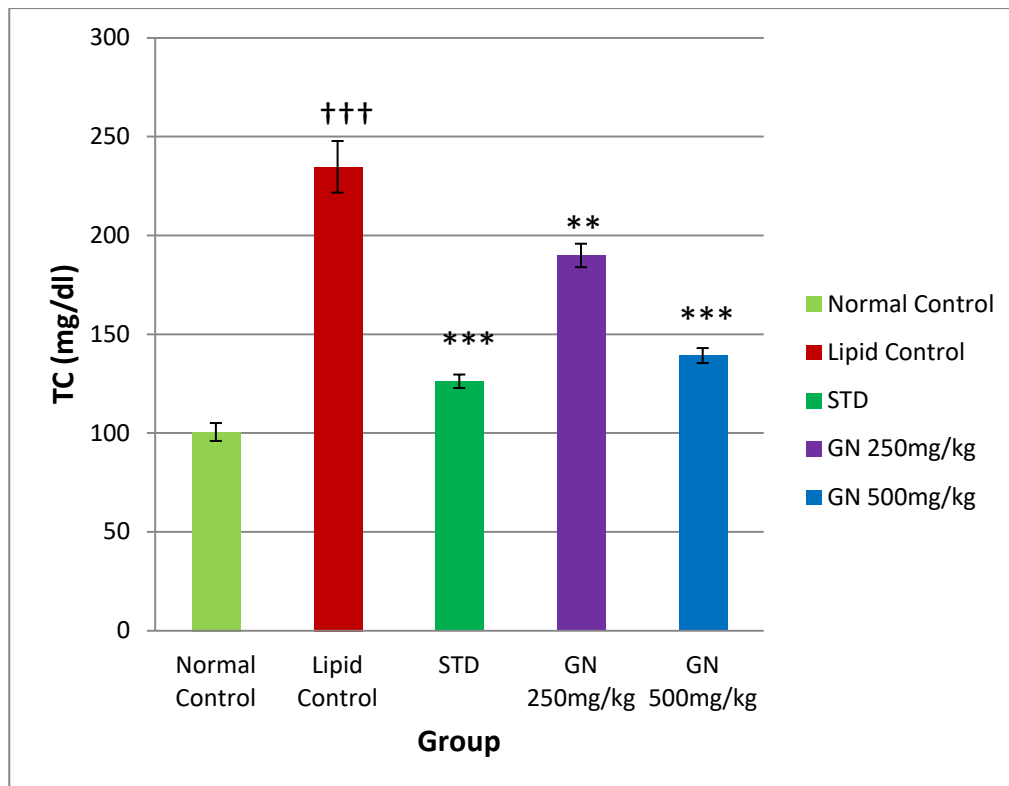


Figure 1: Effect of *G. nepalensis* leaves extract on serum total cholesterol (TC) level in fat induced hyperlipidemia in rats.

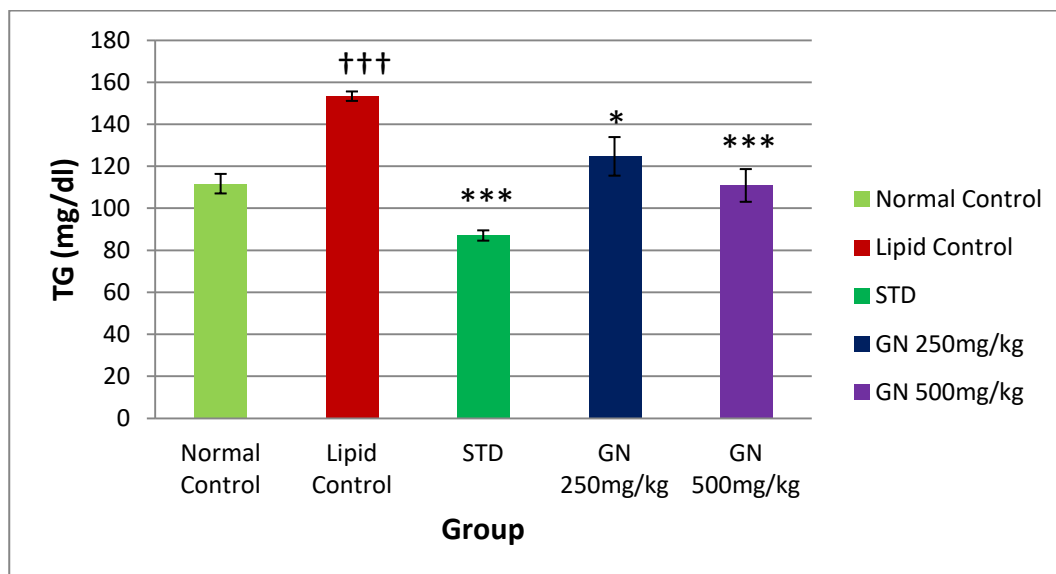


Figure 2: Effect of *G. nepalensis* leaves extract on serum triglyceride (TG) level in fat induced hyperlipidemia in rats.

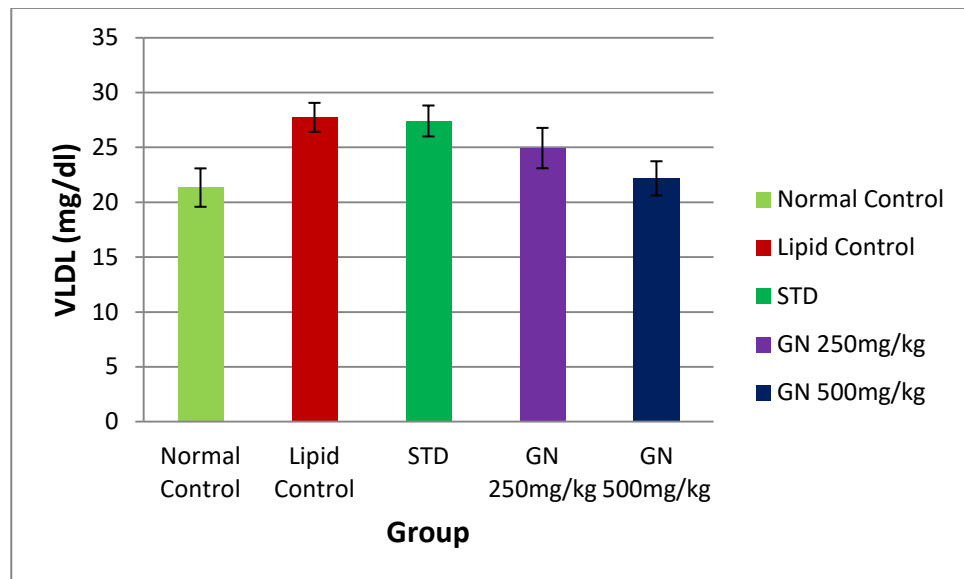


Figure 3: Effect of *G. nepalensis* leaves extract on serum VLDL-C level in fat induced hyperlipidemia in rats.

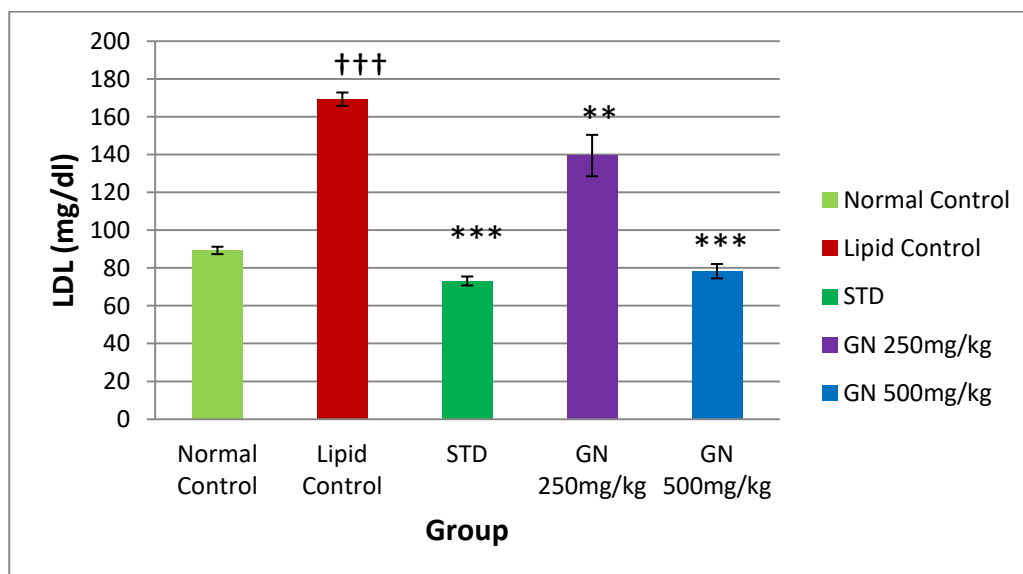


Figure 4: Effect of *G. nepalensis* leaves extract on serum LDL-C level in fat induced hyperlipidemia in rats.

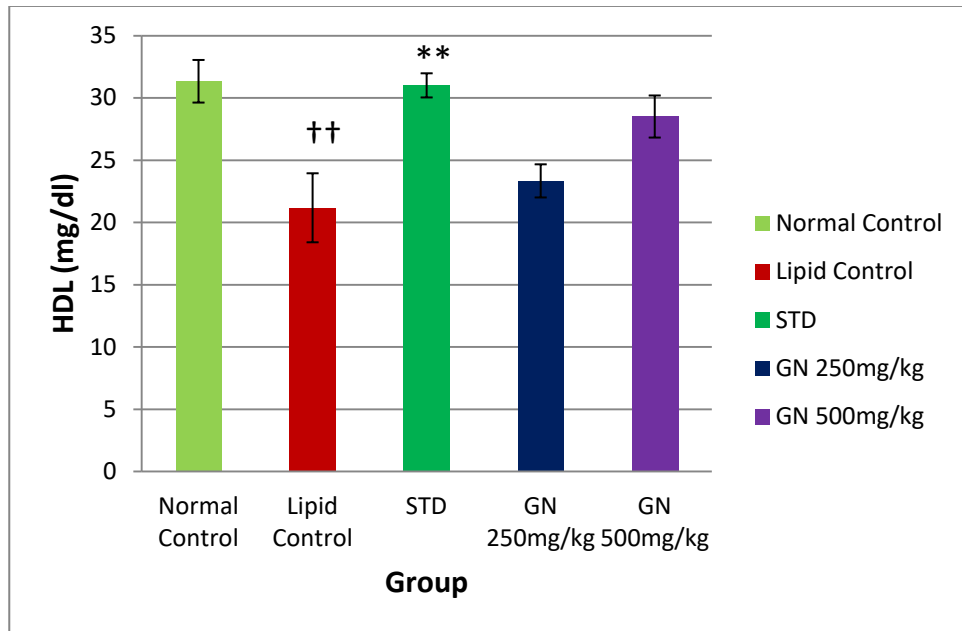


Figure 5: Effect of *G. nepalensis* leaves extract on serum HDL-C level in fat induced hyperlipidemia in rats.