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Evaluation of Anti-Hyperlipidemic Activity of Nimbin in Wistar Rat

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ABSTRACT

The present research work was to explore the anti-hyperlipidemic potential of Nimbin (2 miligram-kilogram /or 4 miligram-kilogram/, p.o). Hyperlipidemia is a largly growing disease. Hyperlipidemia is a disorder that is rapidly increasing in prevalence.Hyperlipidemia affects almost 30 million people in the United States, and current trends suggest that one out of every three adults will have diabetes by 2050 if current trends continue.Dexamethasone and Triton induced hyperlipidemia model was used to evaluate the anti-hyperlipidemic activity. In case of hyperlipidemia (4 miligram-kilogram/, p.o) is more effective comparison to 2 miligram-kilogram/ p.o. Research outcome indicates that Nimbin (2 miligram-kilogram/ p.o or 4 miligram-kilogram/ p.o) is having significant anti-hyperlipidemic potential.

Keywords- Hyperlipidemia, Triton, Dexamethasone, Diabetes, Nimbin

INTRODUCTION

Hyperlipidemia is described by raised serum levels of total cholesterol (TC), low- density lipoprotein (LDL), Very low -density lipoprotein (VLDL), and diminished serum level of high density- lipoprotein (HDL). According to American heart incorporation, a high level of fats known as hyperlipidemia. These fats consist of cholesterol and triglyceride. Lipids and fatty substances in the blood and is a greater risk factor in the growth of atherosclerosis and heart diseases. [1, 2] Hyperlipidemia is a dangerous substitute for gall stone, pancreatitis and xanthomas, or coronary artery disease (CAD), myocardial infarction (MI), hypertension. CAD could be contemplated as the most common source of death globally, including India, by 2020. Hyperlipidemic, being one of the major intricacies of CAD

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inflammatory disorder rising 8from the excessive inflammatory response to various forms of injurious stimuli to the artery wall. [3, 4]

Azadirachtaindica is a fast-growing, annual plant native to India, Africa, and Central America.The importance of the Neem tree was recognised by the US Scientific Community, which published a paper titled "Neem tree for resolving global concerns" in 1992.The progress of Neem research has now been previously published. Neem has been discovered to have a wide range of naturally dynamic combinations that have been artificially mixed and have enormous beneficial effects.Neem has been shown by German researchers to prevent tooth decay and periodontal disease, resulting in better oral health.Enterococcusfaecalis& Candida albicans are anti-microbially affected by neem leaf extract.[5,6]

MATERIALS AND METHODS

Experimental Rodents

Wistar rodents of either sex weighing between 150-200g were used for this examination. They were obtained in the AIBPS creature house Kanpur perceived by the Institutional Animal Ethics Committee (IAEC). Polypropylene limits were utilized to house (3 for each pen) the creature at body level of warmth of 28 ±50C and 12 hours light/dull cycle. Hindustan Lever chow pellets were used to take care of the creature and water not essential. The creatures were continued fasting medium – term going before the assessment and this examination was affirmed by IAEC for creature considers (1122/PO/Re/S/2007/CPCSEA) incorporate all structure utilized in the exploration.

Chemicals and Drugs

Nimbin and Triton was gotten from sigma Aldrich, Dexamethasone Phosphate Injection (Neon Laboratories Limited Andheri East Mumbai, Batch No.SLDS-313) and Gemfibrozil (Batch No. - 820280072) was purchased from Pfizer Pharmaceutical.

STUDY DESIGN

1-Dexamethasone –induced hyperlipidemia in rats

Hyperlipidemia will be raised using dexamethasone a glucocorticoid is known to evoke plasma lipid raise. Dexamethasone (10 mg/ kg/day, subcutaneous) was administered to wistar rats for 8 days to influence hyperlipidemia. The creatures were separate into five groups each group contains six (n= 6) wistar rodents.

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- Group1 (Normal control) - Administered normal saline solution
- Group 2 (Hyperlipidemic control) - Administered normal saline solution
- Group 3 (Standard group) – Gemfibrozil 10 miligram-kilogram/ day suspended in gum acacia in water
- Group 4 (Test group-I) – Nimbin 2 mg/ kg orally
- Group 5 (Test group- II) - Nimbin 4mg/kg orally

All the rodents in groups II, III, IV, and V were administered a subcutaneous injection of Dexamethasone (10 miligram-kilogram/day S.C) for 8 days to produced hyperlipidemia. The animals in normal hyperlipidemia control groups were taken normal saline, while Group III rodents are taken Gemfibrozil (10 miligram/kilogram/day I.P. suspended in gum acacia in water and Group IV and V rodents taken by oral route in doses of 2 miligram/kilogram/day and 4 milogram/kilogram/day, nimbin separately, throughout the 8 days experiments.After the experiment end, the overnight without food experimental rodents was sacrificed by decapitation under light ether anesthesia and blood was collected. Serum was isolated, and lipid profiles (biochemical parameters) were investigated [7].

2-Triton induced hyperlipidemia

The rats were divided into five groups of six rats in each group & were treated with single dose/ day (p.o.) of standard drug or test drug .

Group – I: Normal control.

Group – II: Hyperlipidemic control Triton (100 mg / kg)] i.p.

Group –III: Standard Gemfibrozil (10 mg / kg) p.o.

Group – IV: Test 1 Nimbin (2 mg / kg) p.o.

Group – V: Test 2 Nimbin (4mg / kg) p.o.

Hyperlipidemia was induced by single intraperitoneal injection of freshly prepared solution of Triton X-100 (100 mg/ kg) in physiological solution after overnight fasting for 18 hrs. This study was carried out for 7 days & the protocol of the present study was carried out for 7 day .After 8th day of treatment (after 18 hrs injection of triton X- 100), the blood was collected by retro orbital sinus puncture, under mild ether anesthesia. Serum obtained by immediate centrifugation of blood samples using ultra cooling centrifuge at 3000 rpm for 15 min at room temperature. Plasma was quantified using enzymatic kit. [8]

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Biochemical Estimation of Blood Serum

Plasma lipid levels include TC, TG, HDL, VLDL, LDL was determined by serum samples utilizing diagnostic commercial kits from Qualigens diagnostic Mumbai India. The samples were analyzed via utilizing semiautomatic analyzer.

Statistical Analysis

Graphpad 5.0 software has been used to do the statistical analysis. The statistical analysis was done standard error of the mean (S.E.M.).Frequency analysis (ANOVA) with Dunnett's test was used to examine the statistical significance of difference across groups. $P \leq 0.05$ differences were considered to be statistically significant.

RESULTS AND DISCUSSION

Dexamethasone induced hyperlipidemia results of total cholesterol and total TG

Total cholesterol levels in the hyperlipidemia- the induced group have importantly raised compared to normal rats. The values have increased to 118.71 ± 1.329 miligram/deciliter compared to Group I (normal rodent group), in which values fib in the range 65.43± 0.933 mg/dl. This shows hypercholesteremia. In the treatment group used with Nimbin (2mg/kg) or Nimbin (4mg/kg), the values are decreased to 85.23 ± 1.046 ($P< 0.001$) and 83.35 ± 0.885mg/dl ($P< 0.0001$), systematically. There is an important decrease in total cholesterol values in the nimbin treatment group. While Gemfibrozil also has importantly decreased serum total cholesterol levels to 74.70 ± 0.794 mg/dl ($P< 0.001$) [Table-1]. The TG levels have extended as 150.71 ± 0.518miligram/deciliter in dexamethasone-induced group relatively to normal rats where the values are 63.75 ± 0.507 mg/dl. This shows triglyceridemea. In the group treated with Nimbin (2mg/kg) or Nimbin (4mg/kg), the values are importantly reduced to 79.50 ± 0.526 miligram/kilogram ($P<0.001$) and 75.25 ± 0.641 mg/dl ($P<0.0001$), respectively. In the Gemfibrozil treated group (Std.Group), the values are reduced to 68.33 ± 0.572 mg/dl ($P<0.001$) [Table-1].

Dexamethasone induced results of high-density lipoprotein cholesterol

High density containing lipid protein cholesterol in a dexamethasone-induced group has importantly reduced relative to normal rats group. The values have decreased to 25.75 ± 0.410 miligram/kilogram relative to normal rat group, 41.68 ± 0.795 mg/dl. In the group prevented with Nimbin (2mg/kg) or Nimbin (4mg/kg). The values were 65.79 ± 0.602 ($P<0.001$) and 29.40 ± 0.517 mg/dl ($P<0.0001$), respectively. In the Gemfibrozil treated group (Std.Group), the values were 35.50 ± 0.665 mg/dl ($P<0.001$) [Table-1].

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Dexamethasone induced results of LDL-cholesterol and VLDL- cholesterol

LDL- cholesterol in a dexamethasone-induced group has importantly enhanced to 57..32 ± 0.811 mg/dl relative to normal rat group, 15.59 ± 0.495 mg/ dl. In the group prevented with Nimbin (2mg/kg) or Nimbin (4mg/kg).The values were diminished 34.67 ± 0.609(P<0.001) and 25.73 ± 0.551mg/dl (P<0.0001), respectively. There is an important decrease in LDL-cholesterol values in the nimbin treatment group. Gemfibrozil has importantly diminished LDL- cholesterol level to 24.35 ± 0.563 mg/dl (P<0.001) [Table-1].VLDL-cholesterol in the dexamethasone-induced group has importantly enhanced to 39.42 ± 0.650 mg/dl relative to normal rat group 14.42 ± 0.455 mg/ dl. In the group prevented with Nimbin (2mg/kg) or Nimbin (4mg/kg). The values were diminished 31.60 ± 0.441 (P<0.001) and 24.80 ± 0.505 mg/dl (P<0.0001), respectively. There is an importantly decreased nimbin treatment group. Gemfibrozil has importantly diminished VLDL- cholesterol level to 18.75 ± 0.527 mg/dl (P<0.001) [Table-1].

Dexamethasone induced results of atherogenic index

$$\text{Atherogenic index} = \frac{\text{Total serum cholesterol}}{\text{Total serum High density containing lipid protein-cholesterol}}$$

The atherogenic index in the dexamethasone-induced group hyperlipidemia control group is enhanced to 5.89 relatives to the normal rat group, 1.589 In the group prevent with Nimbin (2mg/kg) or Nimbin (4mg/kg), the values are importantly diminished to 2.26 and 1.89, respectively. Gemfibrozil has importantly diminished the values 3.13 [Table1]

Table 1 Effect of Nimbinin Dexamethasone injection induced Hyperlipidemia Wistar Rat

Group	Treatment /dose	Total cholesterol (milligram/deciliter)	Total TG (milligram/deciliter)	High density containing lipid protien (milligram/ deciliter)	Low density containing lipid protein (milligram/ deciliter)	Very low density containg lipid protein(milligram/deciliter)	Atherogenic index
I	Normal-group	65.43 ± 0.933	64.75 ± 0.711	41.68 ± 0.795	15.59±0.495	14.42 ± 0.455	1.59

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II	Normal-control group	118.71 ± 1.329	151.71 ± 0.518	25.75 ± 0.410	57.32 ± 0.811	39.42 ±0.650	5.89
III	Standard group Gemfibrozil (10mg/kg)	74.70 ± 0.794***	69.33 ± 0.572***	35.50 ± 0.665**	24.35 ± 0.563**	18.75 ± 0.527**	3.13
IV	Test group-I Nimbin (2mg/kg) –I	85.23 ± 1.046**	79.50 ± 0.526**	26.79 ± 0.602**	34.6 7± 0.609**	31.60 ± 0.441**	2.26
V	Test group-II Nimbin(50mg/kg)	83.35 ± 0.885***	76.25 ± 0.641***	29.40 ± 0.517***	25.73 ± 0.55***	24.80 ± 0.505***	1.89

Glucocorticoid hormonal level raises influence the plasma lipid concentration but varies from species to species. A small amount synthesis of triglyceride in the liver is encouraged by the injection of glucocorticoid in rodent and effectively may lead to the aggregation of fatty liver. The stimulation of the TG formation could lead to the raised secretion of VLDL. Raising VLDL secretion has been mentioned when dexamethasone is injected for several days in rodents. The rise in the TG level produces an imbalance in lipid metabolism shows to hyperlipidem.

Triton induced hyperlipidemia results of total cholesterol and total TG

Total cholesterol levels in the hyperlipidemia- the induced group have importantly raised compared to normal rats. The values have increased to 118.82 ± 1.329 miligram/kilogram compared to Group I (normal rodent group), in which values fib in the range 65.44± 0.933 mg/dl. This shows hypercholesteremia. In the treatment group used with Nimbin (2mg/kg) or Nimbin (4mg/kg). the values are decreased to 83.20± 1.046 ($P< 0.001$) and 82.36 ± 0.845mg/dl ($P< 0.0001$), systematically. There is an important decrease in total cholesterol values in the nimbin treatment group. While Gemfibrozil also has importantly decreased serum total cholesterol levels to 72.81 ± 0.794 mg/dl ($P< 0.001$) [Table-2]. The TG levels have extended as 151.72 ± 0.528 miligram/kilogram in dexamethasone-induced group relatively to normal rats where the values are 62.66 ± 0.507 mg/dl. This shows

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triglyceridemea. In the group treated with Nimbin (2mg/kg) or Nimbin (4mg/kg), the values are importantly reduced to 78.10 ± 0.521mg/dl (P<0.001) and 75.24 ± 0.631 miligram/deciliter (P<0.0001), respectively. In the Gemfibrozil treated group (Std.Group), the values are reduced to 69.30 ± 0.572 mg/dl (P<0.001) [Table-5.3].

Triton induced results of high-density lipoprotein cholesterol

HDL-cholesterol in a dexamethasone-induced group has importantly reduced relative to normal rats group. The values have decreased to 23.55 ± 0.410 mg/dl relative to normal rat group, 41.68 ± 0.784 mg/dl. In the group prevented with Nimbin (2mg/kg) or Nimbin (4mg/kg). The values were 24.29 ± 0.603(P<0.001) and 28.40 ± 0.527 mg/dl (P<0.0001), respectively. In the Gemfibrozil treated group (Std.Group), the values were 35.50 ± 0.675 mg/dl (P<0.001) [Table-2].

Triton induced results of LDL-cholesterol and VLDL- cholesterol

LDL- cholesterol in a dexamethasone-induced group has importantly enhanced to 55.20± 0.831 mg/dl relative to normal rat group, 14.58 ± 0.56mg/ dl. In the group prevented with Nimbin (2mg/kg) or Nimbin (4mg/kg). The values were diminished 31.76 ± 0.609(P<0.001) and 26.80 ± 0.551mg/dl (P<0.0001), respectively. There is an important decrease in LDL- cholesterol values in the nimbin treatment group. Gemfibrozil has importantly diminished LDL- cholesterol level to 23.34 ± 0.573 mg/dl (P<0.001) [Table-2].VLDL-cholesterol in the dexamethasone-induced group has importantly enhanced to 39.40 ± 0.650 mg/dl relative to normal rat group 12.91 ± 0.615 mg/ dl. In the group prevented with Nimbin (2mg/kg) or Nimbin (4mg/kg).The values were diminished 31.20 ± 0.431 (P<0.001) and 25.80 ± 0.525 mg/dl (P<0.0001), respectively. There is an importantly decreased nimbin treatment group. Gemfibrozil has importantly diminished VLDL-cholesterol level to 19.64 ± 0.527 mg/dl (P<0.001) [Table-2].

Triton induced results of atherogenic index

$$\text{Atherogenic index} = \frac{\text{Total serum cholesterol}}{\text{Total serum High density containing lipid protein}}$$

The atherogenic index in the dexamethasone-induced group hyperlipidemia control group is enhanced to 3.78 relatives to the normal rat group, 1.48. In the group prevent with Nimbin (2mg/kg) or Nimbin (4mg/kg), the values are importantly diminished to 3.04 and 2.88, respectively. Gemfibrozil has importantly

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diminished the values 2.22 [Table2]

Table 2- Effect of Nimbinon Triton induced Hyperlipidemia in Wistar Rat

Group	Treatment/ dose	Totalcholesterol (milligram/ deciliter)	Total TG (milligram/deciliter)	HDL-Cholesterol (milligram/deciliter)	LDL-Cholesterol (milligram/deciliter)	VLDL-Cholesterol (milligram/deciliter)	Atherogenic index
I	Normal-group	65.44 ± 0.943	62.66 ± 0.621	41.69 ± 0.784	14.58± 0.565	12.91 ±	1.48
II	Normal-control group	118.82 ± 1.329	151.72 ± 0.528	23.6 ± 0.410	55.20 ± 0.831	39.40 ±0.640	3.78
III	Standard group Gemfibrozil(10mg/kg)	72.81 ± 0.794**	69.30 ± 0.582**	35.50 ± 0.675**	23.34 ± 0.573**	19.64 ± 0.527**	2.22

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IV	Test group -I nimbin (2mg/kg) –I	83.20 ± 1.046**	78.10 ± 0.521**	24.28 ± 0.603***	31.76± 0.6088**	31.20 ± 0.431**	3.04
V	Test group -II nimbin (4mg/kg)	82.36 ± 0.845***	75.24 ± 0.631***	28.40 ± 0.527***	26.72 ± 0.405***	25.80 ± 0.525***	2.88

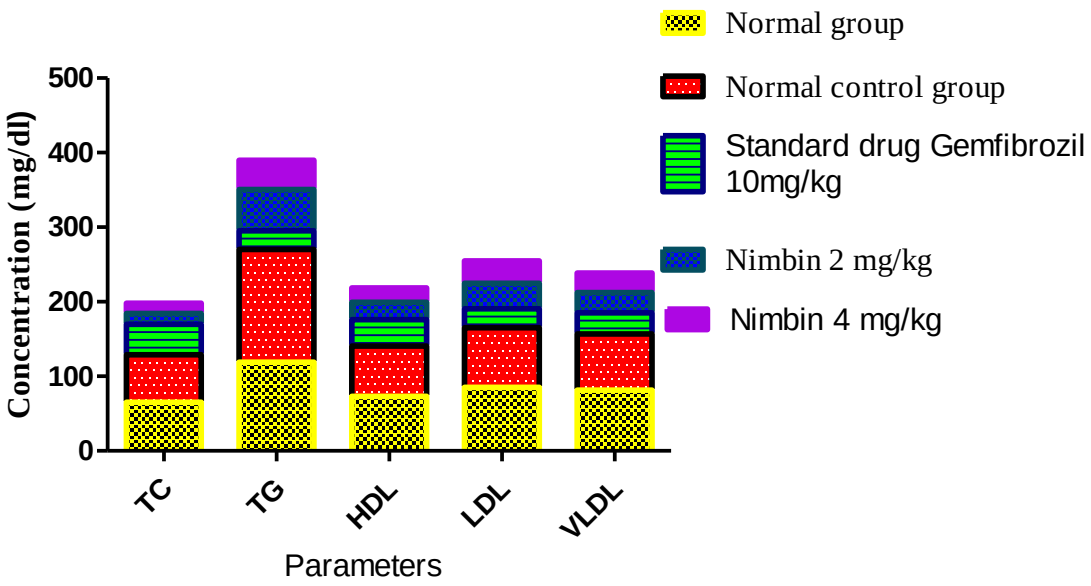

Figure-1 Column Graph Showing Effect of 2mg/kg and 4mg/kg against Dexamethasone Induced Hyperlipidemia

Patients suffering from hyperlipidemia, hypertension, and diabetes mellitus, and all these together, known as metabolic syndrome (syndrome-x) augment the rate of cardiovascular mortality and morbidity. Coronary heart disease resulting from growing atherosclerosis, remnants the most general origin of morbidity and mortality all over the world, influenced the level of total LDL and low level of HDL, many other agents including diabetes, hypertension, smoking, glucocorticoid, nutrient, and psychological agents are co-operating to its etiology .

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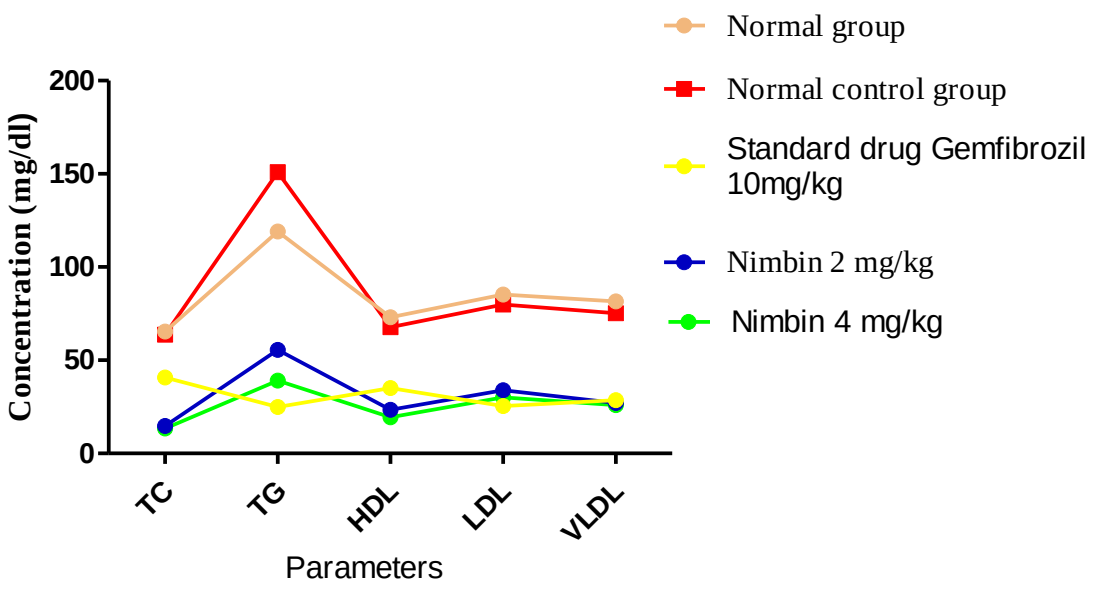

Figure-2Line Graph Showing Effect of 2mg/kg and 4mg/kg against Triton Induced Hyperlipidemia

Triton WR-1339 has already been frequently used to prevent TG-rich phospholipids from being cleared, resulting in transient hypertriglyceridemia in a variety of species.Parenteral treatment of Triton to mature rats caused hyperlipidemia, according to Schurr et al.Triton treatment causes a significant increase in plasma Total cholesterol & Triglyceride, that is mostly owing to an enhancement in very low density containing lipid protien synthesis by the hepatic, which is coupled by a decrease in very low density containing lipid protien and low density containing lipid protien catabolism .Triton is a detergent that inhibits phospholipids from blocking the absorption of lipoproteins from circulatory by peripheral tissues, resulting in higher lipid levels.

Whilst rodent Triton model of hyperlipidemia is widely utilised for a number of reasons, the rat analysis has been applied in particular for screening natural and manmade hypolipidemicmedicines.The investigation found that 4 milligrm/kilogram was the most efficacious dose for hyperlipidemia of the two dose levels evaluated.

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Author contribution

All author participated Equally.

Conflict of interest None

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