Preclinical Evaluation of Anti-Hyperlipidaemic Activity of Nutraceutical Formulation NC30 in Sprague Dawley Rats

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ABSTRACT

The antihyperlipidemic exertion of Nutraceutical formulation (NC30) whole formulation was studied in NC30 and high fat diet (HFD) convinced models in Sprague Dawley rats. The present study was designed to probe the antihyperlipidemic exertion of polyherbal formulation excerpt in NC30 and High fat diet convinced hyperlipidaemic rats. Treatment with formulation excerpt (200 and 400 mg/ kg, p. o body weight) was suitable to significantly drop the situations of TC, TG and LDL. Also, the excerpt was set up to beget a significant increase in the HDL situations. thus, it can be concluded that polyherbal formulation is suitable to effectively suppress hyperlipidaemia and High fat diet convinced hyperlipidaemia in rats, suggesting the implicit defensive part in coronary heart complaint. The hypolipidemic exertion of Nutraceutical expression was compared with a standard medicine Fenofibrate (20 mg/ kg. p. o body weight), a given lipid- lowering agent in both models. This result is considered as important for the treatment of hyperlipidaemia convinced atherosclerosis and supposedly validates the folk medicinal use of hyperlipidaemic cases in India

Key words: Anti-hyperlipidaemic, Lipid profile, SD rats, High fat, *Nutraceutical formulation*, NC30.

1) INTRODUCTION

Health is defined as complete absence of physical, internal or moral condition, especially freedom from fleshly pain or complaint, but true health is further than that. It includes the joy of living, the power and capability to lead a satisfying and purposeful life. Hyperlipidaemia is a term used to describe various conditions in which there is a high concentration of lipids in the blood and is caused by abnormalities in lipid metabolism or tubular transport of lipids or a complaint of tubular lipoprotein mixing and declination (Azezli Aetal., 2005). Hyperlipidaemia is a common problem in developed countries and is a leading cause of coronary heart disease caused by high blood fat levels. These fats include cholesterol and triglycerides. They are important for our body, but when they are high, they can cause heart problems and strokes. Heart disease and stroke are usually caused by atherosclerosis of the large and medium-sized arteries. Hypercholesterolemia is the most important factor in the pathogenesis of atherosclerosis. Hyperlipidaemia manifests as hypercholesterolemia and/or hypertriglyceridemia. however, hypercholesterolemia is the most common hyperlipidaemia. The lipids associated with hypercholesterolemia are cholesterol, an important element of cell membranes and a precursor of steroid hormone synthesis. As a result of hyperlipidaemia, it can lead to atherosclerosis over time and therefore increases the risk of coronary artery disease and stroke.

However, hypercholesterolemia is more common. As a result of hyperlipidaemia, it can lead to atherosclerosis over time and therefore increases the risk of coronary heart disease and stroke. Hyperlipidaemia associated with risk factors such as atherosclerosis, hypertension, type 2 diabetes, round, myocardial infarction, congestive heart failure, angina pectoris, gall bladder disease, degenerative general diseases, sleep apnea and gravity. Allopathic medicines are available to counteract liver damage and hyperlipidaemia, but the side effects and costs associated with these allopathic medicines require preparedness that does not cause side effects. To maintain normal body functions, it is important to reduce elevated serum cholesterol to an acceptable level. However, due to the growing concerns about health promotion, by-products of cholesterol-lowering drugs have gradually appeared. For example, studies have reported that statin therapy is not tolerated in some cases due to musculoskeletal symptoms and other side effects (Raderetal., 2001 BallantyneC. Metal., 2003).

No previous reports on the antihyperlipidemic activity of Nutraceutical Formulation NC30 were found in the literature review. In addition to the multi-herbal formulation, (i.e., jowar,foxtailmillet,littlemillet,fingermillet,pearlmillet,saggubhiyam,barley,barnyardmillet,porosomillet,greengram,soyabean,blackeyedbean,bengalgram,horsegram,redgram,blackgram,toordal,maize,sunflowerseeds,pumpkinseeds,flaxseeds,almonds,groundnut,cashewnuts,drieddates,elachi,pepper) ,as we generate a lot of verifiable evidence through many pharmacological tape styles, we can also expect to include this NC30 regularly in our natural straightening leaves and as a dietary supplement to help and slow down the progression of hyperlipidaemia and others. related conditions. metabolic conditions.

2.MATERIALS & METHOD:

2.1 Collection, Identification & Authentication of polyherbs:

Multi herbal formulations is procured from local Ayurvedic formulary.

2.2 Preparation of formulations and phytochemical constituents:

- The test material was dried under shade for few weeks, later powdered, sieved into fine particles & stored in an air tight container.
- The Fenofibrate (Dr. Reddy's pvt, ltd.) was brought from local medicals store.
- All chemicals were procured by Teena Bio Laboratories, Hyderabad.

2.3 Animal study

Sprague Dawley rats of 180-250 g were obtained from the Teena lab for experimental purpose. The animals were maintained under controlled conditions of tempera- ture (28 ± 2 C), humidity (51 ± 5%) and 12 h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. Habituation period for animals prior to study was 48 hrs. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Teena Research and training institute, Hyderabad (REF- TBPL/AHLA/009/23) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg No: 177/PO/RcBi/2000/CPCSEA), Govt. of India.

2.4Determination of Acute Toxicity (LD_{50})

2.4.1Animals

SD rats weighing 180-250g were used for the study. They were nulliparous and non-pregnant. These were acclimatized to laboratory condition for one week prior to start of dosing.

2.4.2 Preparation of dose

The formulation was dissolved in suitable solvent, to prepare a dose of 200 and 400 mg/kg. The doses were selected according to the OECD guideline.

2.4.3 Procedure

The procedure includes fasting of rats for a whole night and then divided into three phases.

- ❖ PHASE I: High fat diet was induced and observed in rats for about 20days.
- ❖ PHASE II: Observed the animals for next 20days of test drug administrated.
- ❖ PHASE III: A set of disease control rats were administered with standard drug Fenofibrate (200 mg/kg. p. o).

EXPERIMENTAL ANALYSIS

SD rats weighing 180-250g were divided into five groups of six in each group.

Experimental Design

- **Group-I:** Distilled water was administered and served as normal control.
- **Group-II:** Distilled water was administered and served as disease control.
- **Group-III:** Standard drug (Fenofibrate 20mg/kg, p. o) was administered.
- **Group-IV:** NC30 test drug I was administered at a dose rate of 200mg/kg, p. o body weight.
- **Group-V:** NC30 test drug II was administered at a dose rate of 400mg/kg, p. o body weight.

BIOCHEMICAL ANALYSIS

On 8th day after fasting for 18hrs the creatures were anesthetised with ether and blood was pulled back by retro orbital sinus cut. Serum was isolated by centrifugation of blood at 3000rpm per 10min for estimation of biochemical parameters such as TC, TG, HDL-C and LDL-C was calculated. TG and TC were measured with enzymatic packs. HDL-C was decided after precipitation of LDL with phosphotungstic corrosive and magnesium chloride. The LDL cholesterol concentrations were calculated from the browned Wald's equation.

2.5.3 BIOCHEMICAL PARAMETERS

2.5.3.1 Estimation of serum triglycerides

Lipoprotein lipase is the protein which hydrolyses tri- glycerides to glycerol's and free greasy acids. The glycerol shaped with ATP within the nearness of glycerol kinase shapes glycerol 3 phosphate which is oxidized by the enzyme glycerol phosphate oxidase to make hydrogen peroxide. The hydrogen peroxide assist responds with phenolic compound and 4-aminoantipyrene by the catalytic activity of peroxidase to

create ruddy coloured quinonimine colour complex. The colour shaped is directly relative to the sum of triglycerides pre- sent within the test. Screen the absorbance at 505 nm (Herbert K et al., 1984).

cholesterol oxidase at that point changes over the cholesterol in to hydrogen peroxide and cholesterone. Within the nearness of peroxidase, hydrogen- peroxide responds with 4-amino antipyrine and phenol to create a quinonimine colour. The absorbance of quinonimine measured spectrometric- ally at 505nm was relative to cholesterol concentration within

$$\text{Triglycerides mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

the example (Nader R et al., 1994).

$$\text{Cholesterol mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

2.5.3.1 Estimation of serum HDL-Cholesterol

Low Density Lipoprotein (LDL) Cholesterol, Very Low-Density Lipoproteins (VLDL) Cholesterol and Chylomicron fractions are precipitated by addition of polyethylene Glycol 6000 (PEG). After centrifugation, the High-Density lipoprotein (HDL) fraction remains in the supernatant and is determined with CHOD-PAP method (Siedel J *et al.,* 1983).

$$\text{HDL - Cholesterol mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 50 \times 2$$

Procedure

Mix well and keep at room temperature (15-30^{0}C) for 10 min. Centrifuge for 15 min at 2000 rpm and separate clear supernatant. Use the supernatant for HDL- Cholesterol estimation.

2.6 Statistical analysis

Effect of NC30 on Body Weights in HFD Induced Hyperlipidaemic Rats

S.NO	GROUP	Body weights (gm)		
		Day 0	Day 25	Day 50
1	Normal control	189.170 ±0.04	238.16 ±1.30	269.67 ±0.12***
2	Disease control	210.52 ±0.175	245.86 ±1.081**	387.25 ±0.13
3	Fenofibrate (20 mg/kg)	180.48 ±0.088**	297.94 ±1.48*	347.68 ±1.14***
4	Test drug I (200 mg/kg)	212.79 ±0.230*	295.61 ±0.95*	362.94 ±1.15**
5	Test drug II (400 mg/kg)	198.26 ±0.37**	268.87 ±1.84*	316.33 ±1.68**

HFD: High Fat Diet, NC30: Nutraceutical formulation

Effect of NC30 on Lipid Profile in HFD Induced Hyperlipidaemic Rats

S. NO	Group	Serum Lipid Profile mg/dl			
		TC	TG	HDL	LDL
1	Normal Control	147.1±0.82	176±0.16	37.85±0.072	68.67±0.326
2	Disease Control	196.5±0.382***	321.3±1.528	32±0.397***	116.1±0.173**
3	Fenofibrate (20 mg/kg)	154.1±0.81**	177.4±0.7192	36.74±0.132**	98±0.6774**
4	Test-I (200 mg/kg CE)	195.1±0.2613*	298.3±0.9811	25.87±0.153**	63.98±0.06594*
5	Test-II(400mg/kg CE)	264.45±0.217**	348.2±0.9908	36.37±0.306**	84.42±0.686

All values are compared with HFD control. HFD: High Fat Diet; NC30: Nutraceutical formulation

Effect of NC30 on Lipid Profile in formulation induced Hyperlipidaemic Rats

GROUP	Serum lipid profile mg/dl			
	TC	HDL	LDL	TGs
Normal control	20.42±0.008	21.45±0.279	5.737±0.0338	22.5±0.1705
Disease control	49.32±0.25***	3.476±0.06***	37.31±0.085***	102.3±2.05***
Fenofibrate (20mg/kg)	31.81±0.263**	9.48±0.06***	25.71±0.3**	25.75±0.132***
Test drug I (200mg/kg)	20.72±0.311**	7.038±0.02*	17.41±0.2241**	43.22±0.0811*
Test drug II (400 mg/kg)	22.93±0.064**	9.658±0.280**	19.72±0.1910**	30.28±0.1625**

All values are compared with positive control. NC30: Nutraceutical formulation

The values are expressed as Mean ± SEM. The data was analysed by using one way ANOVA followed by using Graph Pad Prism software 6.01 version. Statistical significance was set at $P \leq 0.05$.

3.RESULTS & DISCUSSION

3.1 Effect of NC30 on HFD Induced Hyperlipidaemic Rats

A study of 50 days antihyperlipidaemic was done in HFD induced hyperlipaemic rats with NC30 and the results were tabulated.

3.2 Acute toxicity studies

In both procedures, no animal showed toxicity with a single dose of NC30 (200 mg/kg, p.o.). Therefore, the procedure is repeated increasing the dose of the extracts (400 mg/kg, p.o.). No animal showed toxicity. Thus, doses of 200, 400 mg/kg body weight were chosen for this study.

3.3 Effect of NC30 on Physical Parameters of HFD induced hyperlipidaemic rats

- **Body Weight**

During the 50-day hyperlipidemia induction and treatment, animal weights were monitored every 25 days from day 0 and the results were tabulated.

During the first 25 days, ie. from day 0 to day 25, the animals of group II-V increased their weight very significantly (238.16±1.30, 297.94±1.48, 295.61±0.95, 268.87± 1.84) compared to (diseased control group) 245.86±1.081).

From day 25 to day 50, animals in all groups lost weight. However, animals treated with 200 mg/kg p.o. NC30 did not show significant weight loss until day 50. Animals treated with 400 mg/kg, p.o., showed significant weight loss from day 41 and extreme weight loss on days 45, 50. importance of weight loss . $P<0.001$.

Rosuvastatin effectively demonstrated an antihyperlipidemic effect on body weight, with very significant weight loss from day 25 to day 50.

- **Serum Lipid Profile**

Lipid profile was assessed by assessing triglycerides (TG), total cholesterol (TC), HDL cholesterol (HDL-c), LDL cholesterol (LDL-c) and VLDL cholesterol (VLDL-c) in normal and hyperlipidemic subjects & animals

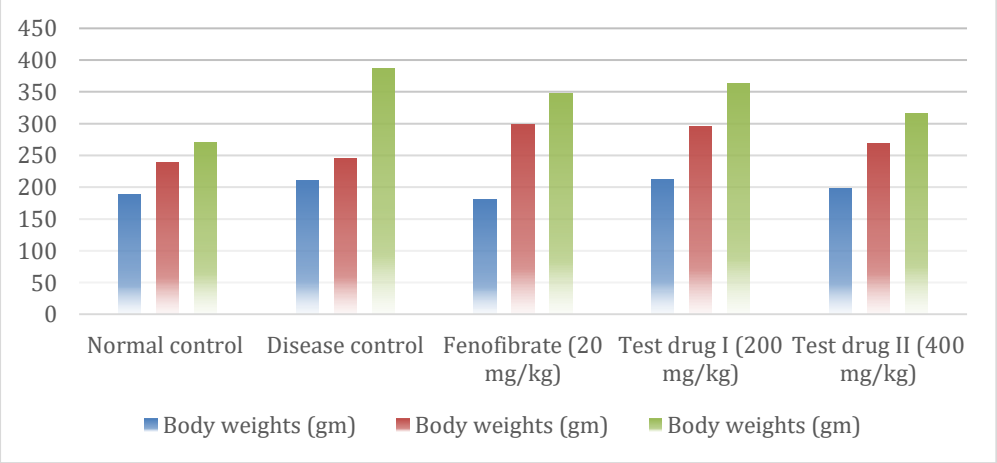


Figure 1: Effect of NC30 on Body Weights in HFD Induced Hyperlipidemic Rat

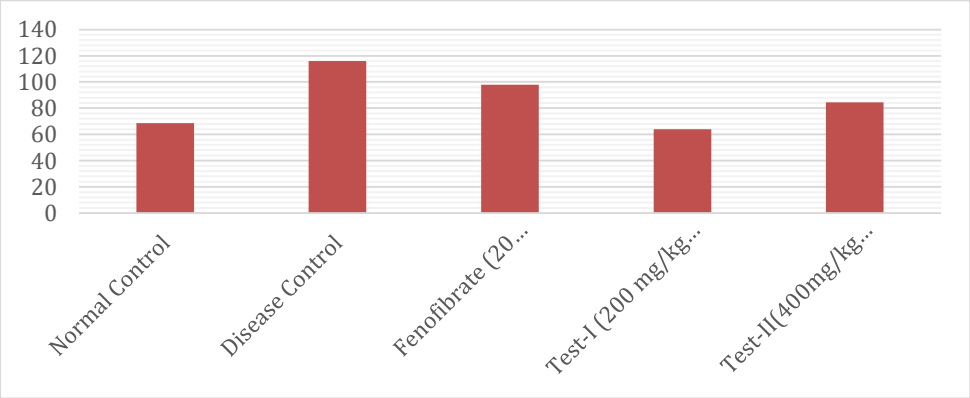


Figure 2: Effect of NC30 on HDL in HFD Induced Hyperlipidemic Rats

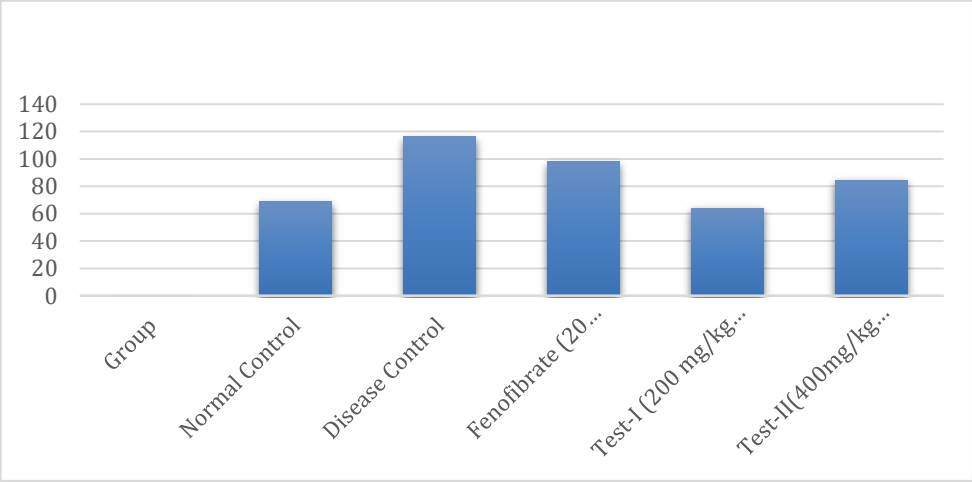


Figure 3: Effect of NC30 on LDL in HFD Induced Hyperlipidemic Rats

High Density Lipoprotein (HDL)

The level of HDL decreased very significantly (3.476±0.02 mg/dl) in animals in the HFD group compared to the normal control group (21.45±0.279 mg/dl).

Hyperlipidemic animals treated with 200 mg/kg p.o NC30 showed no significant increase in HDL, animals treated with 400 mg/kg p.o NC30 significantly increased HDL by 36.37 ± 0.306 mg/dl P<0.001.

I. Low Density Lipoprtein (LDL)

In the animals of the HFD group, the level of LDL increased very significantly (116.1±0.173 mg/dl) compared to the normal control group (68.67±0.326 mg/dl).

Hyperlipidemic animals treated with 200 mg/kg and 400 mg/kg p.o NC30 showed no significant reduction in LDL levels P<0.001. The values were tabulated.

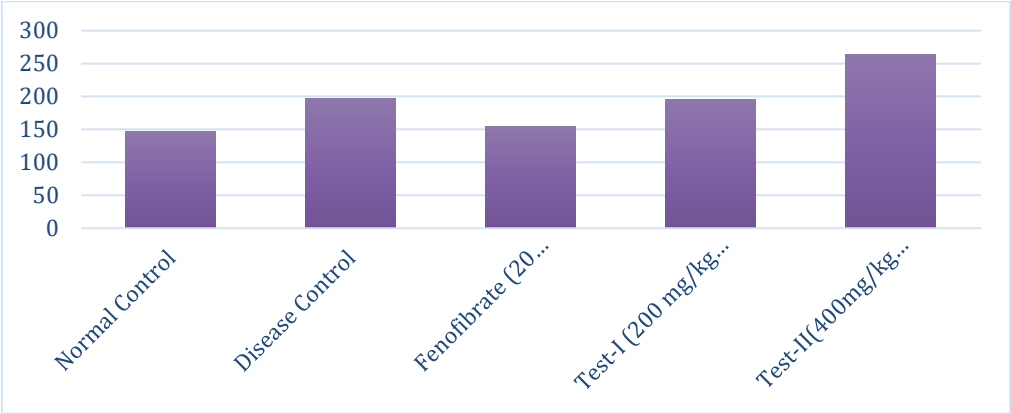


Figure 4: Effect of NC30 on Total glycerides level in HFD induced Hyperlipidaemic Rats

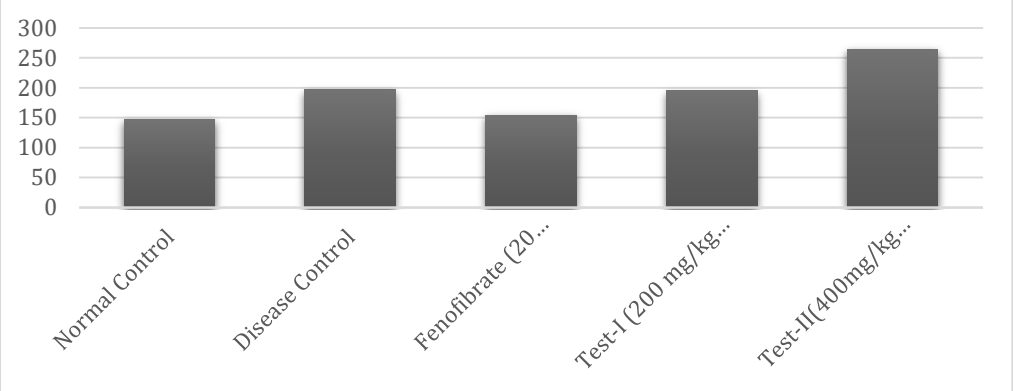


Figure 5: Effect of NC30 on Total Cholesterol level in HFD induced Hyperlipidaemic Rats

II. Total Cholesterol (TC)

In the HFD group animals, the total cholesterol level increased very significantly (196.5±0.382 mg/dl) compared to the normal control group (147.1±0.82 mg/dl).

Hyperlipidemic animals treated with 200 mg/kg p.o NC30 showed no significant decrease in total cholesterol, animals treated with 400 mg/kg p.o NC30 showed a very significant decrease in total cholesterol 264.45 ± 0.217 mg/dL P <0.01.

III. Triglycerides (TG)

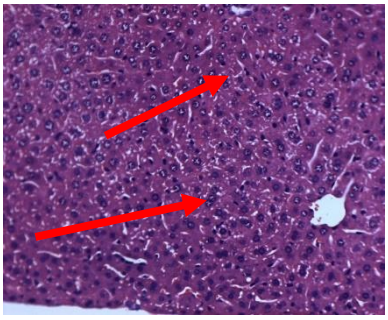
In the HFD group animals, triglyceride levels increased very significantly (321.3±1.528 mg/dl) compared to the normal control group (176±0.16 mg/dl).

Hyperlipidemic animals treated with 200 mg/kg, p.o. NC30 showed a significant decrease in triglycerides by 298.3 ± 0.9811 mg/dL $P<0.05$, animals treated with 400 mg/kg, p.o. NC30, triglycerides the level dropped very significantly 348.2±0.9908 mg/dl $P<0.01$.

3.4 Histopathology Reports

- **Photomicrogragh of Normal Liver:**

Normal morphology of hepatocytes were observed in portal, peri portal and centri lobular region – Red arrow



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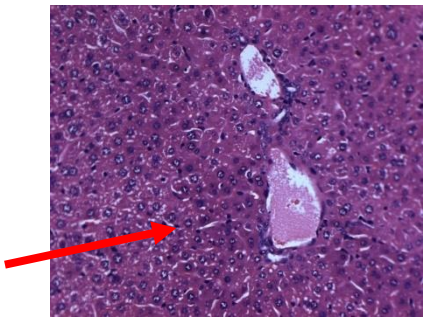

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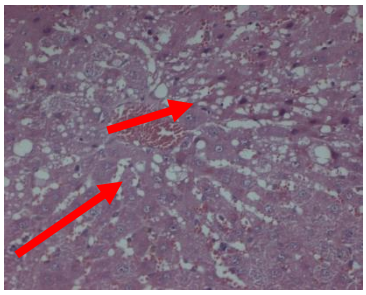
- **Photomicrograph of Diseased Liver:**

Macro vesicular steatosis – lobular pattern[+] in peri portal and centri lobular region of liver was observed Red arrow ; Moderate sinusoidal hemorrhages was noticed in sinusoidal spaces of liver – green arrow



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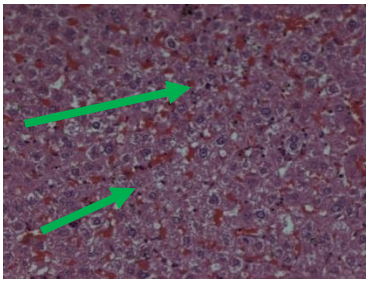

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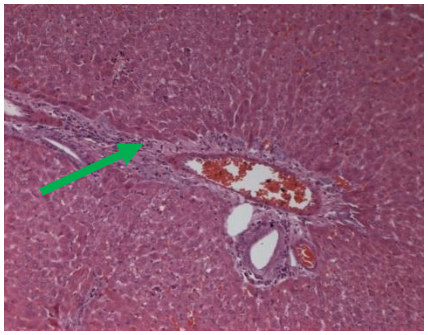
- **Photomicrograph of STD Liver (Fenofibrate):**

Hepatocytes are appeared normal, but mild sinusoidal hemorrhages [Red arrow] and peri portal connective tissue proliferation noticed – green arrow



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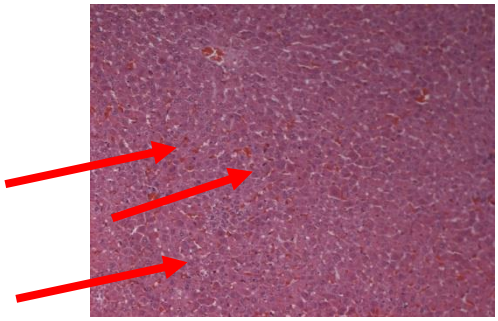

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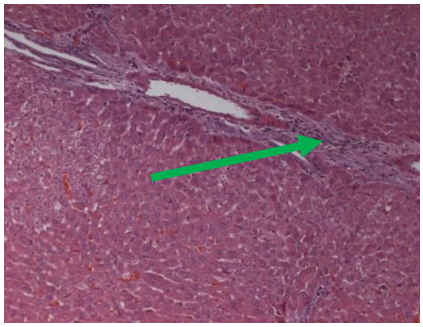

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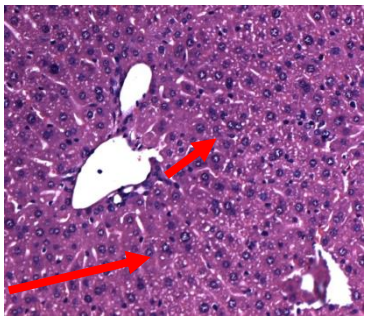
- **Photomicrograph of High Dose Liver:**

Hepatocytes appeared normal, Most of maro vesicular/ micro vesicular fatty changes revert back to normal hepatocytes in portal, peri portal and centri lobular region of liver – Red arrow



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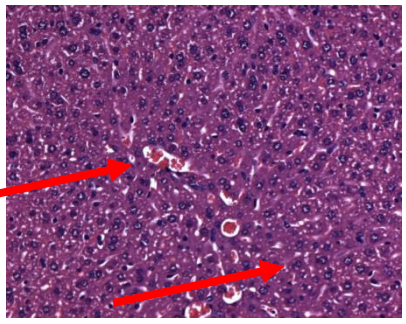

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4.CONCLUSION

This study showed that oral administration of NC30 at a dose of 400 mg/kg produced significant antihyperlipidemic effects in HFD-induced hyperlipidemic rats. NC30 200 mg/kg p.o. reduces lipid levels and body weight less than 400 mg/kg p.o. An acute toxicity study showed that the extracts have no significant toxic effects. The effect of EECE in normal rats, HFD rats, and Triton-challenged rats also showed that it has superior hypolipidemic effects compared to normal control animals. In addition, the drug administered to HFD-induced hyperlipidemic rats showed a significant reduction in serum lipids (eg, TC, TG, and LDL) as well as an increase in HDL levels. NC30 also showed a decrease in triglyceride levels in

Triton-challenged rats. These findings concluded that ethanolic extract of Nutraceutical plant has antihyperlipidemic activity. The purpose of the mechanism is to prevent the synthesis of cholesterol and triglycerides.

5.ACKNOWLEDGEMENTS

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