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RESEARCH ARTICLE

"EVALUATION OF HYPOLIPIDEMIC ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF MORINGA CONCANENSIS NIMMO IN GUINEA PIGS"

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Key words:-
Moringa Concanensis Nimmo, Cholesterol Powder, Hypercholesterolemia, Lipid Profile, Liver Enzymes

Abstract

Objective & Scope: To evaluate the hypolipidemic activity of ethanolic extract of leaves of *Moringa concanensis*nimmo in guinea pigs

Methods: 48 Guinea pigs of either gender were randomly divided into 6 groups. Hyperlipidemia was produced by giving cholesterol powder (500 mg/kg) for 60 days. Rosuvastatin 1.5 mg/kg served as active control group. While *Moringa* was given as 200 mg/kg for 30 days as preventive and curative groups for 0 to 30 days and 31 to 60 days respectively. Distilled water with normal diet for 30 days was served as Normal control group. The preventive and curative effect of ethanolic extract of *Moringa* were assessed by serological and histopathological parameter.

Results: Cholesterol administration produced increased level of Lipids and liver enzymes along with histopathological alteration of liver and thoracic aorta. Preventive and therapeutic administration of 200 mg/kg ethanolic extract of *Moringa* resulted into a significant decrease of lipid profile and restoration of histopathological architecture of liver and thorasic aorta as compared to disease control group.

Conclusion: Because of antioxidant properties, curative and preventive administration of 200 mg/kg ethanolic extract of *Moringa* exerts lipid lowering effect and can be used to treat and prevent dyslipidemia.

Introduction:-

Hyperlipidemia is a medical condition characterized by an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids and or plasma lipoproteins including very low-density lipoprotein and low-density lipoprotein along with reduced high-density lipoprotein levels. This elevation of plasma lipids is among the leading risk factors associated with cardiovascular diseases (Phogat, P *et al.,* 2010). Hyperlipidemia is an important risk factor in development of atherosclerosis and heart disease. Risk factors for hyperlipidemia may be male > 45 years old, female > 55 old, family history of CHD (coronary heart disease), hypertension, low HDL-C (<40 mg/dl), Smoking genetic factors or by generalized metabolic disorders like diabetes mellitus, excessive alcohol intake, hypothyroidism, or primary biliary cirrhosis. (VERMA, N *et al.,* 2013).

Cardiovascular diseases, especially coronary heart disease (CHD), are epidemic in India. The Registrar General of India reported that CHD led to 17% of total deaths and 26% of adult deaths in 2001-2003, which increased to 23% of total and 32% of adult deaths in 2010-2013. The World Health Organization (WHO) and Global Burden of

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Disease Study also have highlighted increasing trends in years of life lost (YLLs) and disability-adjusted life years (DALYs) from CHD in India. (DBIDC, 2018). The association between CVD and higher low-density lipoprotein cholesterol (LDL-C) is well known and it plays a major role in the development of CVD, independently (Mizuno, 2012; Li M, 2013). Dyslipidaemia induces oxidative stress in liver, heart, and kidney by reactive oxygen species which plays a significant role in the etiology of atherosclerosis and CVD (Suanarunsawat, 2011).

Herbal products have many biological active phytochemical compounds that shown their valuable affects on CVD by hypolipidemic action. It is also cost effective, safe and therapeutically effective as compared to present allopathic drugs (Stephen Leeder et al., 2004). Moringa concanensis belongs to the family Moringaceae. It is abundantly seen in Perambalur district of Tamilnadu commonly known as Kattumurungai or Peyimurungai. This small tree with strong central trunk, thick bark, bipinnate leaves and with an intense horseradish odor has vast therapeutic uses in traditional medicine. The different parts of the plants are used in different types of ailments and various human diseases such as anti inflammatory, antifertility agent, analgesic, antimicrobial, reduce cholesterol, skin tumor, diabetes, and eye care etc.(Joy*et al.*, 2013).

Herbal products may be useful as a safest way to burn excess calories (Vidya T J *et al.*, 2002). In India, roots of Moringa concanensis are used as a folk medicine for obesity and hyperlipidemia. The studies on anti-inflammatory, analgesic, antimicrobial effect of Moringa concanensis already done but the study on hypolipidemic action of the leaves are lacking. On the basis of these, we have planned this study to evaluate hypolipidemic activity of Leaves of Moringa concanensis Nimmo in guinea pigs.

Material &Methodology:-

The study was conducted at Animal room, Department of Pharmacology, Government Medical College, Bhavnagar, Gujarat, after approval (IAEC No. 49/2016) from Institutional Animal Ethics Committee of same institute. CPCSEA guidelines were followed during animal experiments of our study. Guinea pigs were housed in a stainless steel cages and acclimatization for 15 days before experiment. Experimental animals were kept under normal light, temperature and humidity conditions (i.e. 24 ± 2°C; 12-hour light/dark cycle) throughout the study period. Standard laboratory feed and water *ad libitum* were provided throughout the experiments.

Animal groups:

64 healthy adult guinea pigs of 400- 800 grams, either sex were taken. Weighing and sex determination was done of each animal. After selecting the animals, they were kept in the animal room of the department of pharmacology, Government Medical College, Bhavnagar, Gujarat for 15 days for acclimatization. After that, the guinea pigs were divided in to eight groups as following:

1. Group 1 (control - Normal diet) were receive mixtures of cereals and pulses in the morning –total 50 grams/animal and evening-Green leafy vegetables-30 grams/animal.(Distilled Water for 60 days).
2. Group 2: (High fat diet) were receive cholesterol powder (500 mg/kg, 60 days) mixed in the wheat and green gram flour by 40 grams of the above mixtures of the normal diet /animal in morning and in evening green leafy vegetables-30 grams/animal.
3. Group 3: (Extract Control): will receive normal diet(for 60 days) + 400 mg/kg ethanolic extract of leaves of Moringa concanensis Nimmo (for 31 to 60 days).
4. Group 4: (Low dose): were receive high fat diet(for 60 days) + 200 mg/kg ethanolic extract of leaves of Moringa concanensis Nimmo(for 31 to 60 days).
5. Group 5: (Active Control): were receive high fat diet(for 60 days) + 1.5 mg/kg, Rosuvastatin (for 31 to 60 days).
6. Group 6: (Preventive low dose) : were receive high fat diet + 200 mg/kg ethanolic extract of leaves of Moringa concanensis Nimmo (Both for 30 days).

Diet composition:

Normal diet:

Mixtures of cereals and pulses (60% wheat plus 35% bengal gram plus 15% peanuts) in the morning- Total 50 grams/animal In evening- Green leafy vegetables -30 grams / animal.

High fat diet:
In morning:
Cholesterol powder (500 mg/kg) mixed in the wheat and bengal gram flour followed by 40 grams of the above mixtures of the normal diet/animal

In evening:
Green leafy vegetables – 30 gm/kg animal. During the acclimatization period all the animals were given normal diet and water ad libitum. In addition animals of the high fat diet groups (group- 2, 4, and 6), also given flour of the mixtures of wheat and bengal gram (70% wheat plus 30% bengal gram, 10 g/animal) to acclimatized the flour.

After overnight fasting, baseline blood sample and blood sample on day 30 was collected from lateral saphenous vein of hind paw of each animal. Blood samples were analyzed for Liver function test serum lipid profile and cardiac enzymes in the Clinical Biochemistry Laboratory of our institute which is accredited by National Accreditation Board for Testing and Calibration Laboratories (NABL). After baseline blood collection, the animals were divided as in above mentioned groups. Diet was given according torespective group diet throughout study period of 60 days in 1, 2, 3, 4, and 5 group and 30 days in group 8 as mentioned above. During the last 30 days of the experiment, distilled water was daily fed to animals of group 1. Animals of group 3, 4, 5and were given ethanolic extract of leaves of Moringa concanensis Nimmo in the dose of 200 mg/kg (low dose), respectively for 30 days. Group 5 animals were given rosuvastatin calcium in the dose of 1.5 mg/kg for last 30 days. Distilled water and above all the drugs were given orally by gavages feeding tube daily in the morning in the fasting state to ensure maximum absorption. Animals of all groups were sacrificed after blood collection from the saphenous vein in the overnight fasting stat at the end of 60 days. Blood was sent for the analysis of the Liver function test serum lipid profile and cardiac enzymes. We obtained the liver and thoracic aorta from each animal of above seven groups for histopathological analysis which was done by senior faculty from Pathology department of our institute.

- Serum lipid profile: Serum samples were analyzed for total cholesterol, HDL-C, triglycerides, LDL-C and VLDL-C.
- Biochemical Evaluation of Liver and Cardiac Injury: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels in serum were analyzed to evaluate any functionalabnormality of liver. Cardiac function was assessed by measuring serum level of lactate dehydrogenase (LDH).
- Weighing of the animals: Weight of each animal was recorded before and after study to confirm any effect of Ethanolic extract of leaves of Moringa concanensis Nimmo on the weight of the animals.

Statistical analysis
All parameters were expressed as Mean ± SEM. One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer Multiple comparison test was used to compare inter group differences of lipid profile, liver enzymes, cardiac enzymes and extent of body weight gain at the end of 60 days. Mean differences of lipid profile, liver enzymes, cardiac enzymes and extent of body weight gain at the end of 60 days in each group of animals were compared by paired t-test. The value of $P< 0.05$ was considered significant. The statistical calculations were done using GraphPad InStat, Demo version 3.06.

Result:-

Effect of Moringa concanensis Nimmo on Serum lipid profile:
0 day, 30 day and 60 days values of serum lipid profile in each diet treatment group were shown in table 1. In high fat diet fed group significant increase in the serum total cholesterol, serum triglyceride, LDL-C, VLDL-C level ($P< 0.05$) at 30 and 60 days as compared to 0 days, no significant changes in serum HDL-C level in disease control group (Table 1). In treatment low dose group, serum LDL-C level ($P<0.05$) significantly restored but level of serum total cholesterol ($P<0.05$), Triglyceride, HDL-C and VLDL-C, levels were restored but did not reach to statistical significant, as compared to Disease Control group at 60 days (Table 1). In treatment high dose group significantly restored the serum Total Cholesterol, Triglyceride, LDL-C and VLDL-C ($P<0.05$),but serum HDL-C level was restored but did not reach to statistical significant as compared to disease control group at 60 days (Table 1). In preventive low dose group levels of total cholesterol, Triglyceride, LDL-C, and VLDL-C were not significantly decreased and level of HDL-C was not significantly increased as compared to Disease Control group at 30 days (Table 1). Active Control group shown significantly restored in the serum total cholesterol, Triglyceride and LDL-C, VLDL-C, levels ($P< 0.05$) but serum HDL-C levels are restored but did not reach to statistical significant ($P< 0.05$) as compared to Disease Control group at 60 days (Table 1).

Effect of Moringa concanensis Nimmo on Liver Enzymes:
There was significant increased in AST, ALP, LDH and ALT ($P< 0.05$) in disease control group at 60 days compared to 30 days figure (Table 2). In treatment low dose group LDH level ($P<0.05$) significantly restored and levels of AST, ALP, and ALT were restored but did not reach to statistical significant, as compared to disease control group at 60 days (Table 2). In preventive low dose group, levels of AST, ALP, LDH and ALT were not significantly decreased, as compared to disease control group at 30 days (Table 2). Active Control group shown significantly restored in AST, ALP, LDH and ALT levels ($P<0.05$) as compared to disease control group at 60 days (Table 2).

Effect of Moringa concanensis Nimmo on Weight:
Increase in mean body weight of all groups at the end of 60 days was shown in table 3 but, extent of weight gain was not statistically significant among the groups.

Effect of Moringa concanensis Nimmo on Liver Enzymes histopathological studies:
Cholesterol Powder administration for 60 days in disease control group liver showed diffuse areas ballooning degeneration and macrovascular and microvascular fat deposition in hepatocytes of varying degrees (grade 3+, 4+), fatty changes (midzone and periportal) and congestion of central vein and hepatic sinusoids in all animals, histological study of aorta shows focal areas of foamy changes of varying degrees (grade 2+, 3+) in tunica media and tunica intima compared to normal control group. Restoration of macrovascular and microvascular fat deposition in liver and focal areas of foamy changes in aorta were noted in active control groups; Moringa concanensis Nimmo treatment group (Low dose and high dose) as well as in Moringa concanensis Nimmo preventive group (Low dose and high dose). as compared to disease control group.(Figure 1 A - G)

Discussion:-

In the present study, we evaluate the hypolipidemic action of Moringa concanensis Nimmo. in guinea pig because the metabolism of lipoproteins in guinea pig is closer to the human. Various research shows that guinea pigs are commendable models to evaluate hypolipidemic activity and lipoprotein metabolism (Fernandez, 2006). Result of present study showed that 60 day feeding of high fat diet resulted in increased serum lipid profile and histopathological changes like mid zone and peripheral diffuse ballooning degeneration and fatty changes like fat deposition (Figure 1 B) and liver cell degeneration in liver and aorta. This increase and change in the histology of aorta and liver were in accordance with the previous experimental studies with high fat diet (Wang Y, 1866; Suanarunsawat, 2011). We select study period sixty days which was sufficient to produce fatty changes in guinea pigs and it's also supported by previous studies (Ahmad, 2001).

Various pre-clinical, clinical and epidemiological studies had proved that high level of plasma cholesterol is a major risk factor for development of coronary heart disease. Atherosclerosis is a preliminary lipid disorder that affects the major arteries and many factors contributing to its etiology, among them diabetes, hypertension, smoking, glucocorticoid, diet and psychological factors (V.V. Pande et al., 2008) and sedentary life style are the major one (Stephanie E et al., 2006).

Foam cells and fatty streaks were formed in vessels by LDL-C oxidation which is trademark of early atherosclerosis (An S J et al., 2013). Several studies have shown that the improvement and incidence of CVD are associated with lowering the levels of total cholesterol and LDL-C (Nohara R et al., 2013). So, agents who have free radical scavenging and serum cholesterol lowering activity, they would be given importance in management of hyperlipidemia. Because of modern lifestyle, hypercholesterolemic patients could not be successfully managed in spite of much intervention. Thus, herbal medicines have got attention to treat hyperlipidemia, because of safe and cost-effective alternative (Suanarunsawat T et al., 2011).

In the present study, administrations of ethanolic extract of leaves of Moringa concanensis Nimmo (400 mg/kg) in guinea pigs restored serum total cholesterol, serum triglyceride, LDL-C and VLDL-C in treatment group as compared to disease control group at 60 days (Table 1) and reduced decreased in serum total cholesterol, Triglyceride levels, LDL-C, VLDL-C levels as compared to Disease Control group at 30 days. Ethanolic extract of leaves of Moringa concanensis Nimmo shown dose dependent action on lipid profile more restoring effect on serum total cholesterol, serum triglyceride, LDL-C, VLDL-C in 200 mg/kg dose of ethanolic extract of leaves of Moringa concanensis Nimmo in treatment group compared to disease control group at 60 days. 200 mg/kg of ethanolic

extract of leaves of Moringa concanensis Nimmo has decreased in serum total cholesterol, serum triglyceride, LDL-C, VLDL-C in preventive group.

Hypercholesterolemia endangers primary organs like liver and heart. High fat diet significantly increases retention of lipid in liver, followed by hepatic steatosis and reduce hepatic functions (T. Suanarunsawat*et al*., 2010). Our study results show that high fat diet suppressed hepatic functions which were expressed as augmentation of serum levels of AST, ALT, LDH, and ALP in disease control group as compared to normal control group significantly ethanolic extract of leavesof Moringa concanensis Nimmo (200 mg/kg) significantly reduce AST, ALT, LDH, and ALP level. where as compared to high fat diet group. Histology of liver show improvement in hepatocytes and decreased lipid content in high fat diet fed groups treated with ethanolic extract of leaves of Moringa concanensis Nimmo (Table 1). Medicinal plants with Ethanolic compound have the ability of scavenging hydroxyl radical, superoxide anion radicals and lipid peroxyradicals. compounds and flavanoid were present in our extract ethanolic extract of leaves of Moringa concanensis Nimmo. Saponins from plants have long been employed for their detergent properties. They are used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins The ethanolic leaves extract showed precipitation followed by emulsion formation. In medicine, they are used in hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory and weight loss etc. (Bhamadevi . D *et al*.,2015).

Rosuvastatin in the dose of 1.5 mg/kg for thirty days in high fat diet fed group animals also counteracts the rise in serum level of total cholesterol, triglycerides, LDL-C, VLDL-C and resist the fatty changes in the liver induced by high fat diet. It also increases level of HDL-C. High fat diet group treated with rosuvastatin has increased AST and ALP level at the end of sixty days as compared to baseline level.

Thus, the present study shows significant lipid lowering and antioxidant activities of ethanolic extract of leaves of Moringa concanensis Nimmo that might be due to their flavanoid and Saponins compounds. We did not know types of mechanism and ethanolic compounds responsible for lipid lowering actions which were the limitations of our study.

Conclusion:-

Treatment and preventive administration of 200 mg/kg ethanolic extract of leaves of Moringa concanensis Nimmo resulted in to a significant decreased of lipid profile and liver enzymes parameters and histopathological architecture of liver and thoracic aorta as compared to disease control group. the present study shows significant lipid lowering and antioxidant activities of ethanolic extract of leaves of Moringa concanensis Nimmo that might be due to their flavanoid and Saponins compounds and can be use to prevent and treat cholesterol powder induced hypercholesterolemia in guinea pigs.

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Table 1: Comparison of lipid parameters within (0 day vs. 30 day and 30 day vs. 60 day) and between study groups (at 60 day).

Groups	Time Period Day	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)
Group 1	0	46.6 ± 2.99	55.87 ± 3.74	4.5 ± 0.46	22.87±1.47	18.42 ± 0.95
	30	46.3 ± 3.00	56.5 ± 3.68	4.5 ± 0.53	22.5 ± 1.45	18.77 ± 1.00
	60	45.8 ± 2.95	57.00 ± 3.73	4.37 ± 0.32	22.62 ± 1.58	18.45 ± 0.94
Group 2	0	41.27 ± 2.09	58 ± 3.49	4.87 ± 0.61	22.62 ± 1.58	17.23 ± 0.84
	30	58.7 ± 0.89*	67 ± 4.07*	4.50± 0.56	43.00± 1.87*	24.13 ± 0.94*
	60	85.2 ± 2.85$^{\#}$	80.37± 3.59$^{\#}$	5.12 ± 0.54	61.37± 1.55$^{\#}$	27.63 ± 0.92$^{\#}$
Group 3	0	39.09 ± 1.61	59 ± 1.29	5.12 ± 0.44	22.12± 1.39	21.28 ± 1.30
	30	39.67 ± 1.62	58.87 ± 1.30	4.75 ± 0.36	21.5 ± 1.58	20.71 ± 1.34
	60	39.35 ± 1.55	57.87 ± 1.18	5.12 ± 0.47	20.87 ±1.48	20.38 ± 1.37
Group 4	0	38.17 ± 1.31	57.62 ± 2.17	4.87 ± 0.39	25.25± 2.00	18.46 ± 0.49
	30	51.00 ± 1.29*	71.12 ± 2.48*	5.12 ± 0.58	48.25± 2.16*	22.41 ± 0.93 *
	60	48.20 ± 1.60$^{\$}$	69.87 ± 2.36$^{\$}$	5.55 ± 0.70	46.25± 2.27$^{\#\$}$	21. 67 ± 0.75$^{\$}$
Group 5	0	40.97 ± 1.84	54.5 ± 1.95	5.25 ± 0.41	22.37 ± 1.94	17.80 ± 0.86
	30	40.72 ± 1.78$^{\$}$	53.75 ± 1.43$^{\$}$	5.62 ± 0.49	21.75 ± 1.64$^{\$}$	17.10± 0.85$^{\$}$
	60	39.63 ±1.71	55.0 ± 1.72	4.37± 0.49	26.12 ± 1.67	17.21 ± 0.30
Group 6	0	57.55 ± 1.09*	68.12 ± 1.68*	5.37 ±0.62*	51.87 ± 3.13*	22.53 ± 0.66*
	30	43.00± 1.06$^{\#\$}$	60.00 ± 1.73$^{\#\$}$	7.37 ±0.62$^{\#\$}$	43.5 ± 2.73$^{\#\$}$	18.02 ± 0.34$^{\#\$}$

Data are expressed as Mean ± SEM. *P< 0.05, paired *t*-test, intra group comparison between 0 and 30 days. $^{\#}P$< 0.05 paired *t*-test, intra group comparison between 30 and 60 days. $^{\$}P$< 0.05: as compared to disease control group using ANOVA followed by Tukey-Kramer Multiple comparison test (inter group comparison), LDL = low density lipoprotein, VLDL = very low-density lipoprotein, HDL = high density lipoprotein

Table 2: Comparison of Liver enzyme parameters within (0 day vs. 30 day and 30 days vs. 60 days) and between study groups (at 60day).

Groups	Time period	Groups	Time period	Groups	Time period
Group 1	0	60 ± 6.19	86.87 ± 4.02	86.25 ± 2.81	53.25 ± 1.98
	30	61.37 ± 6.27	86.88 ± 4.12	86.75 ± 2.61	53.87 ± 2.31
	60	61 ± 6.21	86.89 ± 3.92	87.25 ± 2.58	54.25 ± 2.28
Group 2	0	59.37 ± 4.41	86.37 ± 5.15	90 ± 3.74	56.12 ± 2.19
	30	97.37 ± 3.19*	125.62 ± 1.97*	134 ± 2.43*	92.37 ± 2.83*
	60	124.8 ± 2.76$^{\#}$	150.12 ± 4.81$^{\#}$	159 ± 2.40$^{\#}$	110 ± 3.52$^{\#}$
Group 3	0	57.50 ± 3.30	89.25 ± 6.13	89.25 ± 3.70	55.25 ± 1.55
	30	57 ± 2.24	88.87 ± 6.50	88.00 ± 3.80	56 ± 1.72
	60	58.75 ± 3.07	89.12 ± 6.26	88.87 ± 3.60	55.37± 1.47
Group 4	0	58.7 ± 2.64	83.50 ± 4.92	92.00 ± 3.16	56.62 ± 3.32
	30	92.87 ± 4.17*	134.50 ± 2.66*	145 ± 2.59*	93.87 ± 3.46*
	60	92.00 ± 4.32$^{\$}$	133.12 ± 2.51$^{\$}$	148 ± 2.73$^{\$}$	92.87± 3.61$^{\$}$
Group 5	0	57.7 ± 4.28	86.85 ± 5.70	91.62±4.50	56.37 ± 2.84
	30	93.25 ± 3.52*	133.12 ± 3.38*	137.12±4.40	95.87 ± 1.46
	60	79.25 ± 3.32$^{\#\$}$	107 ± 2.65$^{\#\$}$	121.12 ± 3.19$^{\#\$}$	79.81 ± 1.73$^{\#\$}$
Group 6	0	56.75 ± 3.63	86.75 ± 5.62	88.62 ± 3.60	52.25 ± 1.77
	30	56.25 ± 4.06$^{\$}$	86 ± 5.10$^{\$}$	87.75 ± 3.70$^{\$}$	51.37 ± 1.91$^{\$}$

Data are expressed as Mean ± SEM. *P< 0.05: paired *t*-test, intra group comparison between 0 and 30 days. $^{\#}P$< 0.05 paired *t*-test, intra group comparison between 30 and 60 days. $^{\$}P$< 0.05: ANOVA followed by Tukey-Kramer Multiple comparison test (inter group comparison) ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase.

Table 3: Comparison of Weight in grams parameters

Groups	**Weight of animal in grams**		
	0 Day	**30 Day**	**60 Day**
Group 1	566.3 ±11.6	567.5 ± 11.29	568 ± 11.20
Group 2	681.7 ± 15.06	692 ± 15.87	700.8 ± 16.02
Group 3	682.5 ± 17.87	679 ± 18.23	689.5 ± 18.04
Group 4	676.75 ± 20.2	682 ± 19.06	690.3 ± 19.28
Group 5	563.7 ± 20.26	580.3 ± 20.08	588.87 ± 19.44
Group 6	608.75 ± 21.58	611.82 ± 21.54	---

Data are expressed as Mean ± SEM.paired*t*-test for intra group comparison between 0 and 30 days. paired *t*-test for intra group comparison between 30 and 60 days. No statistically significant.

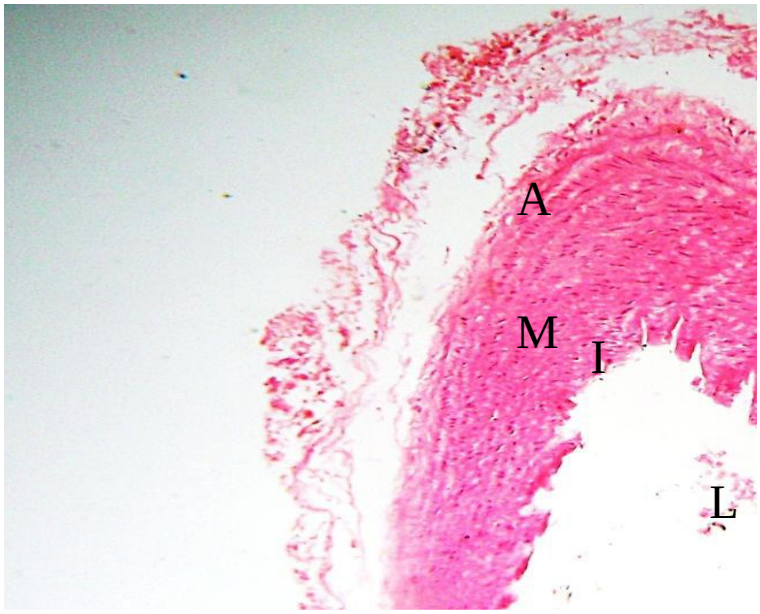
Table 4: Comparison of histopathological grading of Liver and Aorta between study groups at the end of experiment:

Study Group	Liver		Aorta
	Microvascular or Macrovascular fat disposition Grade	Degeneration Grade	Fat deposition in aorta
Group 1 (Normal control)	0	0	0
Group 2 (Disease Control)	4.06 ± 0.26$	3.37 ± 0.18$	3.25 ± 0.16$
Group 3 (Extrect Control)	0	0	0
Group 4 (Treatment Low Dose)	1.87 ± 0.22*	1.82 ± 0.22*	1.62 ± 0.18*
Group 5 (Active Control)	0.25 ± 0.16*	0.75 ± 0.25*	0.5 ± 0.18*
Group 6 (Preventive low dose)	1.86 ± 0.28*	1.26 ± 0.46*	1.37 ± 0.18*

Data are expressed as Mean ± SEM. $ $P<0.05$ as compared to normal control and * $P<0.05$ as compared to disease control group using Kruskal Wallis followed by Dunn's multiple comparison test.

Figure A to E: Histopathology evaluations:

Figure A: Group. 1 Normal Diet Group



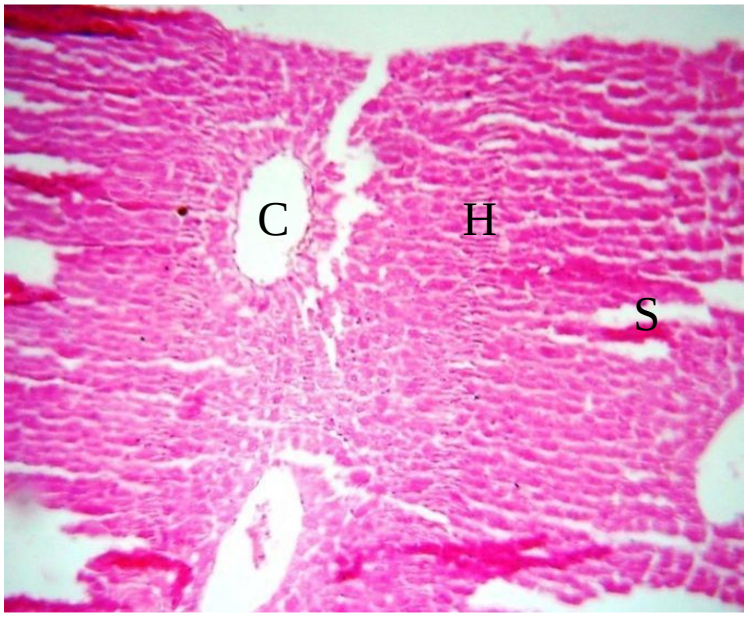
Guinea pig normal Aorta

A: Adventitia tunica

M: Media tunica

I : Intima tunica

L : Lumen



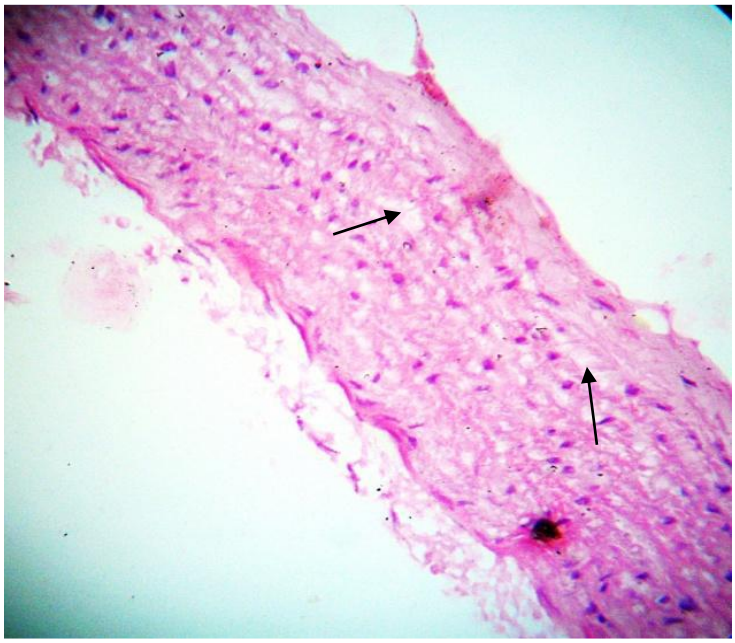
Guinea pig normal liver

C: Central vein

H: Hepatocyte

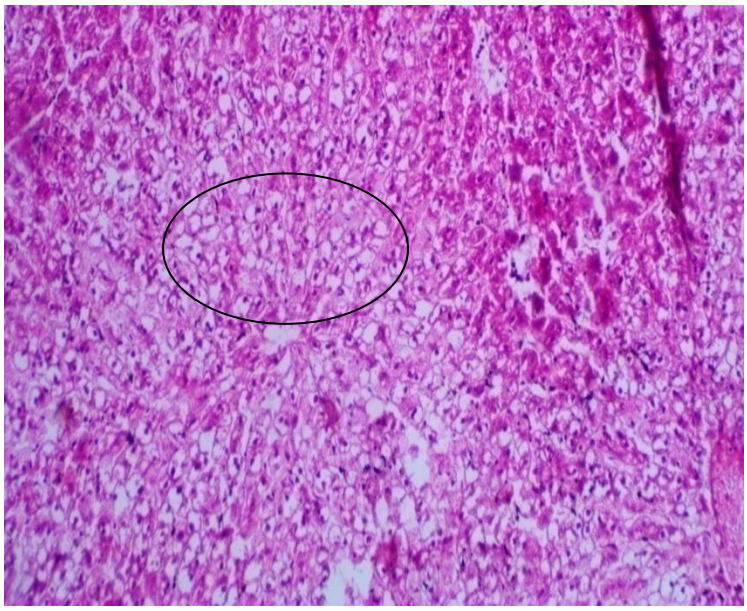
S : Sinusoid

Figure B: Group 2. High Fat diet group



Guinea pig Aorta

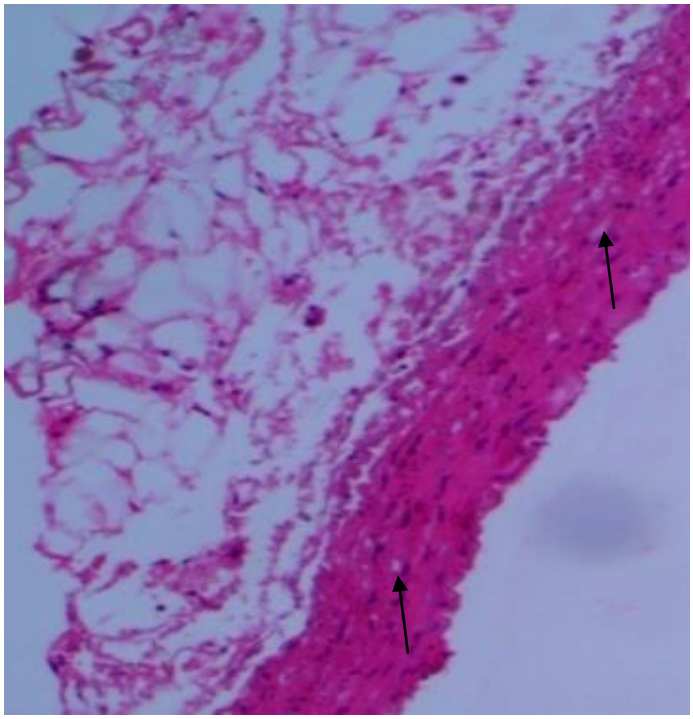
Black arrows shows foamy changes in intima and media (Grade 3+)



Guinea pig Liver

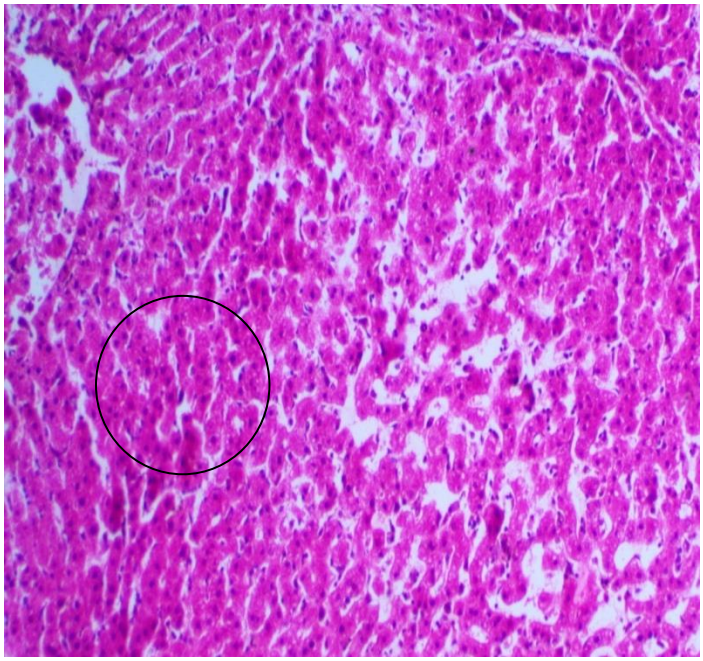
Round shows diffuse areas of ballooning degeneration (Grade 4+)

Figure C: Group 4. Treatment low dose



Guinea pig Aorta

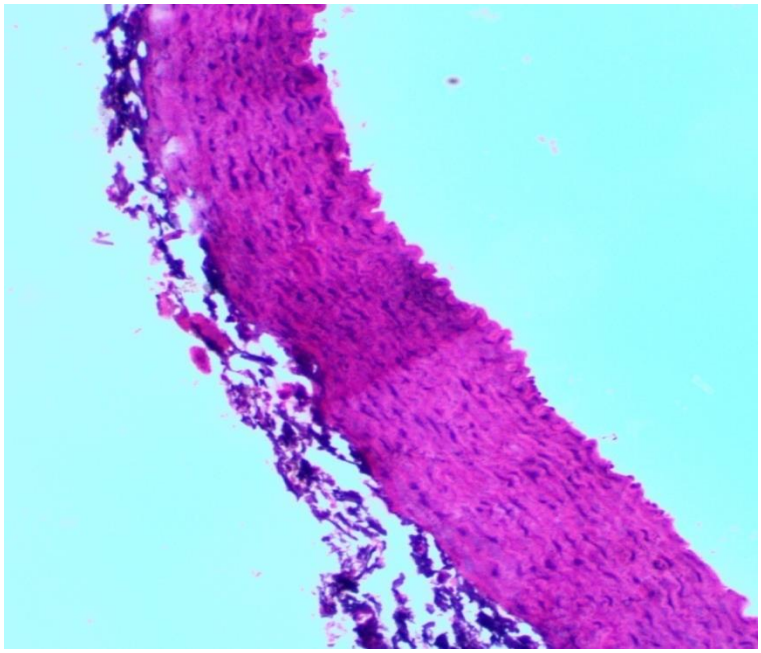
Black arrows shows foamy changes in intima and media (Grade 1+)



Guinea pig Liver

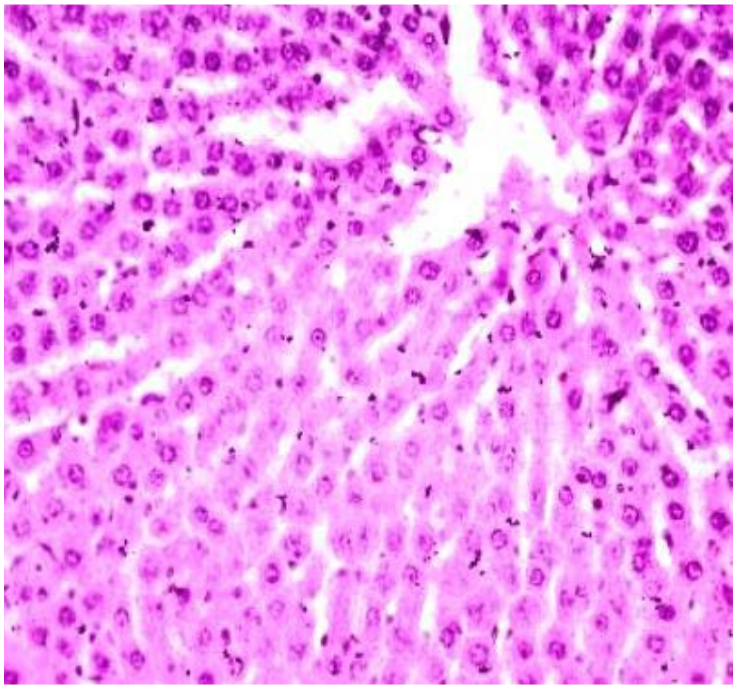
Black round shows fatty changes seen only Ballooning degeneration (Grade 2+)

Figure D: Group 5. Active control group



Guinea pig Aorta

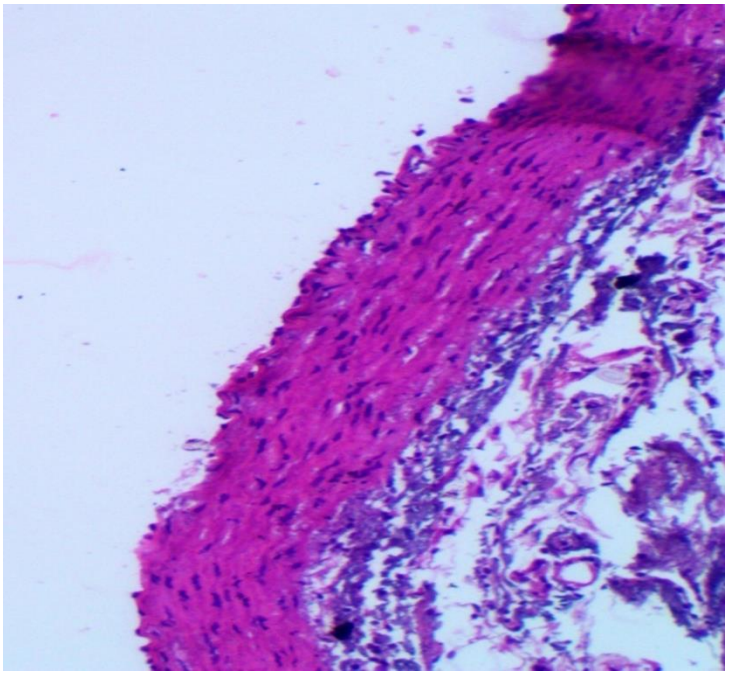
No histological Changes seen



Guinea pig liver

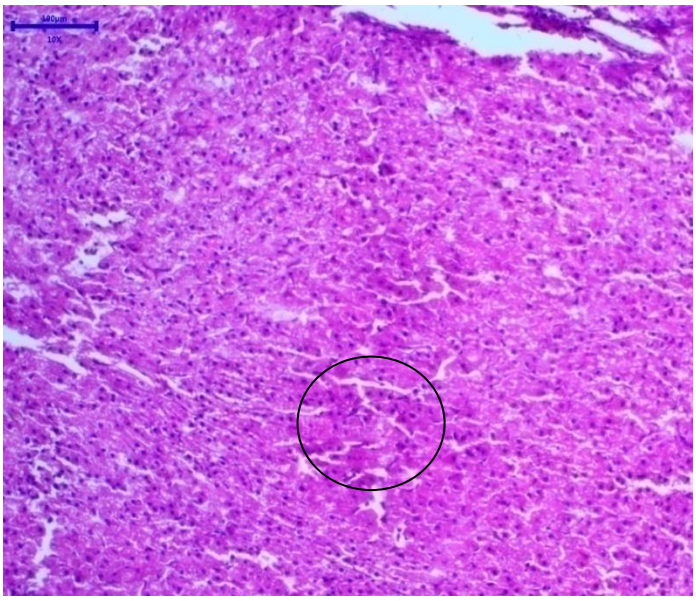
No histological changes seen

Figure E: Group 6. Preventive Low dose



Guinea pig Aorta

Black arrows shows foamy changes in intima and media (Grade 1+)



Guinea pig Liver

Black round no fatty changes seen only degeneration (Grade 2+) with repaired and regeneration process.