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In-Vivo Evaluation of Antihyperlipidemic Activity of Ethanolic Extract of *Garcinia indica* Leaves in a Rat Model

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Keywords: *Garcinia indica*, Lipid Profile, Oxidative Stress, Antioxidant Activity, Guggul, Hyperlipidemia

ABSTRACT

The present study was carried out "To Evaluate Anti-hyperlipidemic activity of Ethanolic Extract of *Garcinia indica* (Leaves Powder) using high-fat diet-induced Hyperlipidemia". The high-fat diet increases levels of TC, TG, and LDL-C. Male Albino Wistar rats, divided into 5 groups: Vehicle control group; Disease control group; Treatment group supplemented with Guggul 81mg/kg (Standard mono-herbal drug); Treatment groups received EEGI Low dose(200mg/kg) and EEGI High dose(400mg/kg). A high-fat diet was administered throughout the study i.e. 57 days. On 28th and 57th day, levels of TC, TG, LDL-C, and HDL-C were estimated using an autoanalyzer. Except vehicle control group, all other groups showed a significant increase in the level of TC, TG, and LDL-C on 28th day whereas on 57th day, the EEGI High dose(400mg/kg) exhibits a significant decrease in TC, TG, and LDL-C with an increase in HDL-C level compared to EEGI Low dose (200mg/kg) and Disease control group. Based on the data, the results show that the EEGI High dose (400mg/kg) has significant ($p < 0.001$) antihyperlipidemic activity in High fat diet-induced rats. Hence, this study suggest that EEGI supplementation can be useful in preventing complications related with Hyperlipidemia.

1. INTRODUCTION

Hyperlipidemia is a metabolic disorder, specifically characterized by alterations in serum lipid and lipoprotein profile due to increased concentration of Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), Very Low-Density Lipoprotein Cholesterol (VLDL-C), and Triglycerides with a concomitant decrease in the concentration of High-Density Lipoprotein Cholesterol (HDL-C) in the serum.[1][2]

Hyperlipidemia has been considered as one of the most important risk factors contributing to the increased prevalence of coronary heart disease, atherosclerosis, and ischemic heart disease. According to WHO reports, high blood cholesterol contributes to approximately 56% of cardiovascular diseases worldwide and causes 4.4 million death every year. By the year 2030, it is estimated that almost 23.6 million people will die from cardiovascular diseases mainly heart disease and stroke. A 50% reduction in heart diseases was observed when the levels of serum cholesterol were reduced by 10% in men aged 40, similarly, a 20% reduction in heart diseases can be observed in men ages 70 by thesame serum cholesterol reduction. Epidemiological studies have shown that there is a direct relationship between serum cholesterol and coronary artery disease (CAD).[3][4][5]

Hyperlipidemia is the underlying cause of cardiovascular diseases, CHDs, and atherosclerosis. Antihyperlipidemic agents have significant potential to retard the process of atherosclerosis therefore they have been increasingly used as prophylactic in the above disorders associated with hyperlipidemia.

Traditional systems of medicine based on medicinal plants are significant in providing health care to a wide segment of the population, particularly in underdeveloped nations. To get the most out of these systems and to understand how they work, one must have a basic understanding of their many elements. The Indian Systems of Medicine is one of the most well-known traditional medical systems in the world.[6] Traditional Indian medicine is based on several different systems, including Ayurveda, Siddha, and Unani. The evaluation of these medications is generally based on phytochemical, pharmacological, and allied methodologies, which include chromatography, microscopy, and other instrumental procedures. Although the underlying concepts and practices of each of these traditional Indian medical systems are distinct, they have a similar thread. The evaluation of traditional medicine's rich past is

crucial, given the growing global interest in adopting and researching traditional systems, as well as utilizing their potential based on various healthcare systems.[7]

Currently available drugs to treat people with detrimental lipid levels include statins, fabric acid derivatives, bile acid-binding resins, and cholesterol absorption inhibitors.

GARCINIA INDICA

In the present study, the leaves of *Garcinia indica* commonly called kokum belongs to the family Clusiaceae. It is found in Maharashtra, Ratnagiri District. The useful parts of the plant are leaves, fruits, and seeds. Traditionally, kokum has been used to cure acidity, bloating and flatulence and some ayurvedic benefits of kokum are anti-viral, anti-bacterial, immune system enhancer, anti-inflammatory, etc. The leaves were reported to have anthelmintics activity.[8] Antioxidants in kokum fruits bind with free radicals, preventing oxidative damage to body cells. They also help cells regenerate and repair themselves. Hydroxycitric acid, garcinol, and isogarcinol are the main ingredients in kokum with antioxidant effects. Other compounds with potential antioxidant properties in kokum are citric acid, malic acid, polyphenols, carbohydrates, anthocyanin pigments, and ascorbic acid. *Garcinia indica* is a plant commonly called kokum (Marathi), or Ratambi (Hindi).

Fig. No. 1 a) *Garcinia Indica* (kokum) Leaves

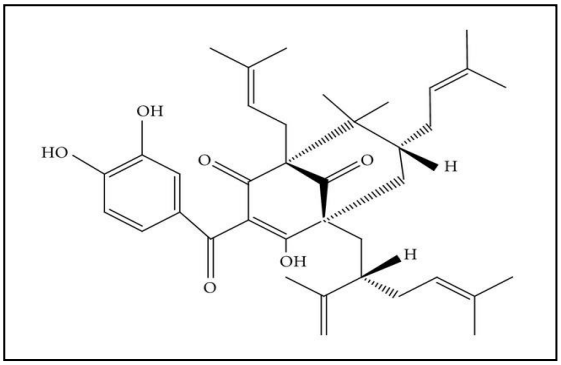


Fig. No. 1 b) Structure of Garcinol

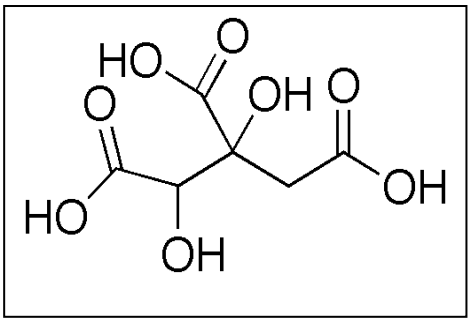


Fig. No. 1 c) Structure of Hydroxy citric acid

2. MATERIAL AND METHODS

2.1 Material

2.1.1 Collection and Authentication of Plant Material

The *Garcinia indica* leaves were procured from Guhagar, Ratnagiri district, Maharashtra.

In December 2020, the leaves were identified and authenticated by the Alarsin organization, Andheri(E), Mumbai.

2.1.2 Experimental Animals

The experiment was carried out using adult Albino Wistar male rats (n=30) weighed between 120-150gm. The animals were placed in an institutional animal house facility under a standard conditions of temperature (24±2°C), Humidity (40-70%), and 12-hour light, 12-hour dark cycle. The rats were housed in polypropylene cages with corn cob bedding and access to animals' laboratory feed with the *ad. Libitium* Water. The experiment was conducted according to the ethical guidelines approved by IAEC. The experiment was carried in an animal house, Bharati Vidyapeeth's College of Pharmacy, C.B.D. Belapur, Navi Mumbai. 762/PO/C/03/CPCSEA,10th May 2003 [Approval Protocol No: BVCP/IAEC/03/2020].

2.1.3 Biochemical Estimation

The lipid profile parameters like Total Cholesterol (TG),[9] High-density Lipoprotein Cholesterol (HDL-C),[10] Low-density Lipoprotein Cholesterol (LDL-C)[10] and

Triglycerides (TG)[11] were estimated using Erba kit and evaluated by using Erba autoanalyzer. [Erba Mannheim CHEM-7]

2.2 Methods

2.2.1 Extract Preparation of Leaves

The authenticated leaves of *Garcinia indica* were cleaned by using fresh tap water. Then leaves were placed to shade dry for 12 days. The dried leaves were subsequently ground into a fine coarse powder using the sharp blender. The extraction process is carried out by the Soxhlet apparatus, 60gm of fine coarse powder of *Garcinia Indica* leaves was treated with 500ml of 95% ethanol for 6-7 cycles.[12] The solvent evaporated using the distillation method and yield was calculated as grams of extract divided by grams of original powder taken. The extract was stored in a desiccator and used for further evaluation.

2.2.2 Determination of body weight

The body weight of individual rat of each group was measured twice a week throughout the study.

2.3 Animal grouping, feeding, and administration of the herbal drug

2.3.1 Experimental design

After one week of acclimatization period, the animals were divided into Five groups of six rats in each group. Each group of animals was treated as follows:

Group 1: Vehicle control group

Animals received standard animal laboratory feed with *ad Libitium* water for 24 hours over 8 weeks, and 1% Carboxymethylcellulose daily by oral route.

Group 2: Disease control Group

Animals received standard animal laboratory feed with *ad Libitium* water for 24 hours over 8 weeks, and a high-fat diet of 3ml/kg/day (Vanaspati ghee: Coconut oil 3:1 ratio) by oral route for 8 weeks.[13]

Group 3: Standard Treatment Group

Animals received standard animal laboratory feed with *ad Libitium* water for 24 hours over 8 weeks, and a high-fat diet of 3ml/kg/day (Vanaspati ghee: Coconut oil 3:1 ratio) by oral route for 8 weeks. Shuddha Guggul monoherbal drug formulation 81mg/kg was given by oral route for the last 4 weeks.[14]

Group 4: Low Dose Group EEGI

Animals received standard animal laboratory feed with *ad Libitium* water for 24 hours over 8 weeks, and a high-fat diet of 3ml/kg/day (Vanaspati ghee: Coconut oil 3:1 ratio) by oral route for 8 weeks. Ethanolic extract of *Garcinia Indica* leaves powder 200mg/kg was administered orally for the last 4 weeks.

Group 5: High Dose EEGI

Animals received standard animal laboratory feed with *ad Libitium* water for 24 hours over 8 weeks, and a high-fat diet of 3ml/kg/day (Vanaspati ghee: Coconut oil 3:1 ratio) by oral route for 8 weeks. Ethanolic extract of *Garcinia Indica* leaves powder 400mg/kg was administered orally for the last 4 weeks.

Throughout the experimental period, the body weight of rats was recorded and the doses were modulated accordingly. The Low and High dose of 200mg/kg/day and 400mg/kg/day of EEGI respectively, was selected based on previous performed Acute Toxicity Studies (BVCP/IAEC/01/2019).

2.3.2 High-fat Diet Composition

The high-fat diet was prepared by mixing Indian Vanaspati ghee and coconut oil in the ratio of 3:1(v/v) and induced orally. It was given at a dose of 3ml/kg/day according to the body weight of rats.[13]

2.3.3 Collection of serum

The blood samples were collected on the 28 and 57 day of the study, and the blood withdrawn from the retro-orbital plexus using glass capillary and collected in Eppendorf tubes. The blood was allowed to clot for 30 min at room temperature. The blood was separated by centrifugation at 2500 rpm for 15 min (REMI C-24 BL), and serum was

collected for determination of lipid profile parameters such as Serum Total Cholesterol, Triglycerides, High-density lipoprotein-cholesterol using Erba kit autoanalyzer [Erba Mannheim CHEM-7].[15]

3. Measurement of Oxidative stress

MDA, Reduced Glutathione, and catalase levels from liver homogenate were evaluated to assess the oxidative stress developed in the model. Catalase activity was measured by the method of Aebi,[16] MDA levels were assessed using Reba acid reactive substance method,[17] Reduced glutathione content assay was carried out according to the method of Beutler *et*.[18]

4. Histopathology

The liver was isolated and preserved in 10% formalin and sent for histopathological evaluation.[19] For the *In-vivo* liver enzyme assay, the liver was separated immediately after sacrification, washed with pH 7.4 buffer, and weight of liver was recorded. A part of the liver was minced and then homogenized (REMI IKA®T25) in pH 7.4 buffer and was used for the evaluation of tissue parameters.[20]

5. Statistical Analysis

Data obtained were subjected to a computerized graph pad (version 9.1.0). Results were expressed as mean ± SEM. The data obtained from the antihyperlipidemic study were subjected to one-way ANOVA, followed by Dunnett's test for statistical significance $P<0.05$ is considered to be statistically significant.

6. RESULTS AND DISCUSSION

6.1 Effect on Body Weight

	Body Weight (gm)				
Groups	Vehicle Control	Disease Control	Standard Treatment (Guggul 81mg/kg)	Low Dose 200mg/kg EEGI	High Dose 400mg/kg EEGI
Week 1	147.5±0.88	140.0±1.12	146.5±1.83	136.±5.36	137.2±2.12
Week 2	168.5±2.50	166.2±2.21	155.7±2.18	148.1±2.71	151.7±4.36
Week 3	178.5±1.52	174.7±4.21	160.5±3.34	157.8±2.10	161.5±7.61
Week 4	199.7±2.90	203.5±3.18	204.0±4.70	198.8±2.33	201.5±5.89
Week 5	224.3±2.34	247.2±4.63	220.8±6.63	215.0±5.01	216.7±5.57
Week 6	234.0±2.19	301.2±5.12	236.5±6.69	230.5±2.56	237.8±1.01
Week 7	249.8±3.55	316.0±2.43	239.2±6.69	220.8±2.68	233.5±3.17
Week 8	262.3±3.07	324.6±3.97	230.8±5.40	215.8±2.57	222.5±3.35

An increase in body weight was observed at the end of 8 weeks, i.e, day 56, in the high-fat diet-induced group compared to that of a vehicle control group. Treatment with the standard mono-herbal formulation Shuddha Guggul reduced body weight compared to the vehicle control group. Treatment with ethanolic extract of *Garcinia Indica* leaves, however, showed a decreased body weight. (Table no.1)

Table No. 1: The effect of EEGI on Body weight in the different groups

Data represented as the means ± SEM, (n=6).

EEGI – Ehanolic extract of *Garcinia indica*

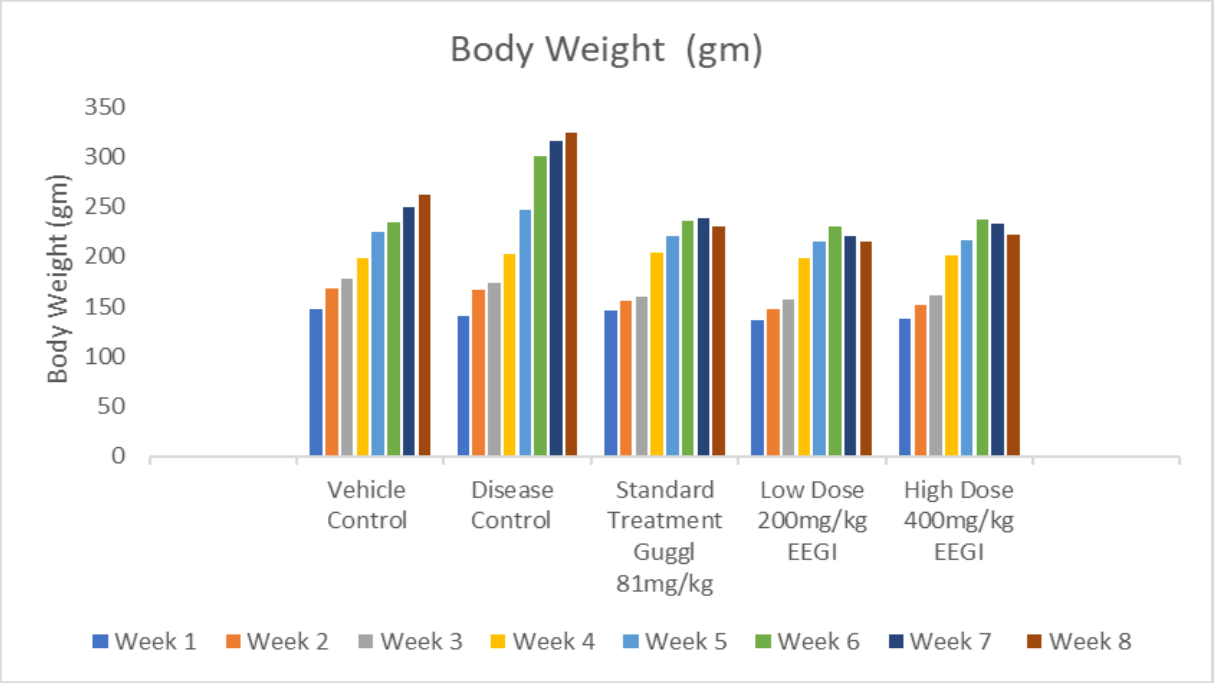


Fig. No. 2 The effect of EEGI on Body weight in the different groups

EEGI – Ethanolic extract of *Garcinia indica*

6.2 Effect of EEGI on Serum Total Cholesterol

A significant increase in the serum cholesterol levels was observed in rats fed with a high-fat diet when compared with that of the Vehicle control group. Treatment with EEMO 200 mg/kg showed a significant ($p<0.01$) decrease in the total cholesterol levels. Whereas, treatment with EEMO 400 mg/kg had shown a statistically significant ($p<0.001$) decrease in total cholesterol levels when compared with the disease group. (Fig. no.3)

6.3 Effect of EEGI on Serum Triglycerides

Rats fed with a high-fat diet had shown a statistically significant ($p<0.001$) increase in the serum TG levels. Treatment with EEMO 200 mg/kg had shown a marked ($p<0.01$) decrease in the serum TG levels. Whereas, treatment with EEMO 400 mg/kg had shown a statistically significant ($p<0.001$) decrease in TG levels. A statistically significant ($p<0.001$) decrease in the TG levels was observed in rats treated with Monoherbal formulation Shuddha Guggul 81mg/kg. (Fig. no. 4)

6.4 Effect of EEGI on Serum HDL-C levels

Rats of the Disease control group had shown a statistically significant ($p<0.01$) decrease in the HDL-C levels when compared with that of the vehicle control group. Whereas, rats treated with EEGI 200 and 400 mg/ kg had shown a marked increase ($p<0.001$) in the HDL-C levels when compared with that of the vehicle and standard Monoherbal formulation Shuddha Guggul treated animals. (Fig. no. 5)

6.5 Effect EEGI on Serum LDL-C

A significant increase in the LDL-C levels was observed in the Disease control group. Whereas, treatment with EEGI 200 and 400 mg/kg/day had shown a statistically significant ($p<0.001$) decrease in the serum LDL-C levels. Moreover, treatment with monoherbal formulation Shuddha Guggul had shown a statistically significant ($p<0.001$) decrease in the LDL-C levels. (Fig. no. 6)

Table No. 2: The effect of EEGI on Serum Total Cholesterol, Serum Triglycerides, Serum HDL- C levels and Serum LDL-C levels in different groups

Groups	Serum Total Cholesterol		Serum Triglycerides		Serum HDL-C		Serum LDL-C	
	Day 28	**Day 57**	**Day 28**	**Day 57**	**Day 28**	**Day 57**	**Day 28**	**Day 57**
Vehicle Control	53.66± 0.76	54.33± 0.84	87.5± 2.02	87.83± 2.68	25.0± 1.03	25.8± 0.64	11.13± 0.71	11.18± 1.35
Disease Control	119.6± 2.38	126.3± 1.69	239.3± 1.08	241.1± 1.39	14.16± 0.60	10.16± 0.47	53.08± 2.93	75.43± 3.11
Standard Treatment	116.5± 1.57	68.5± 1.57***	236.0± 0.96	154.6± 1.26***	13.5± 0.61	22.5± 0.40***	56.8± 1.93	15.06± 1.67***
Low Dose 200mg/kg EEGI	118.0± 1.44	89.0± 2.26**	234.6± 0.88	173.1± 0.48***	14.33± 0.66	16.3± 0.76**	58.5± 2.79	37.86± 1.92**
High Dose 400mg/kg EEGI	123.0± 1.34	69.0± 0.66***	236.33± 0.88	160.6± 1.91***	14.33± 1.61	19.6± 0.47***	61.4± 1.63	17.53± 0.66***

Data represented as the means ± SEM, (n=6). EEGI – Ehanolic extract of *Garcinia indica*

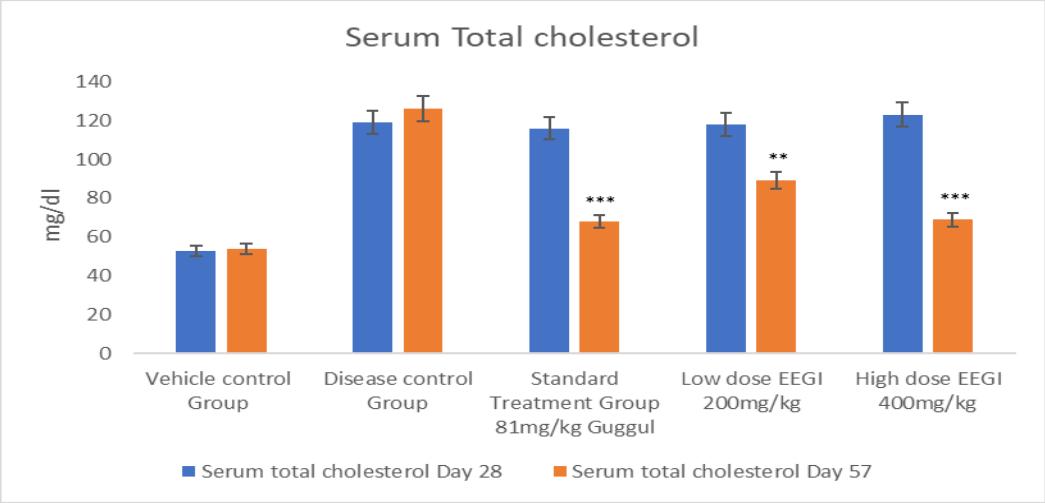


Fig. No. 3: Effect of administration of ethanolic extract of *Garcinia Indica* Leaves on serum total cholesterol of rats.

when compared with day 28.

All the values are expressed as Mean ± SEM for n=6 animals ** p<0.01and *** p<0.001 when compared using Dunnett's test.

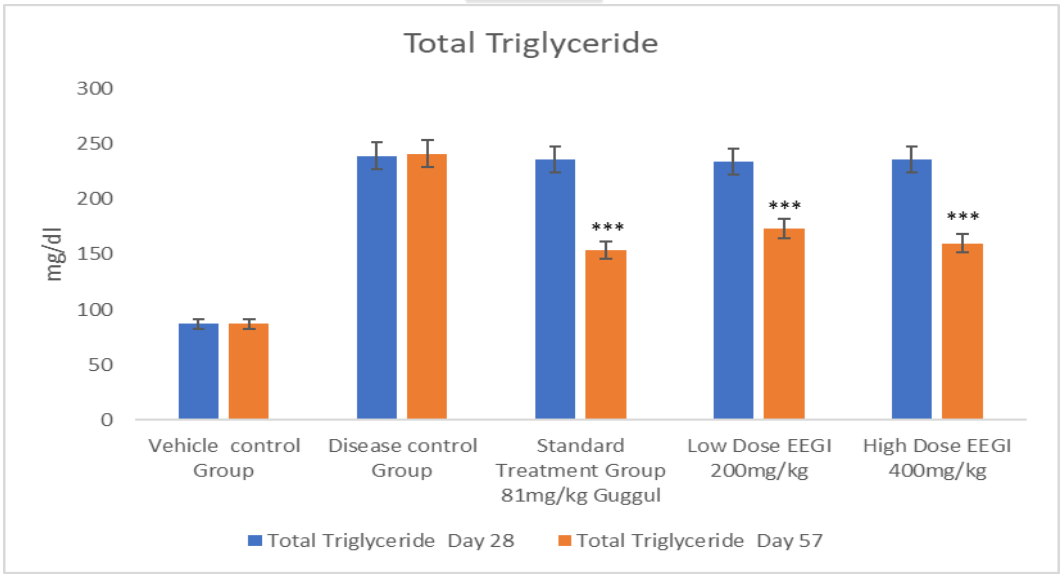


Fig. No. 4: Effect of administration of ethanolic extract of *Garcinia Indica* Leaves on serum Total Triglyceride of rats.

when compared with day 28.

All the values are expressed as Mean ± SEM for n=6 animals ** p<0.01and *** p<0.001 when compared using Dunnett's test.

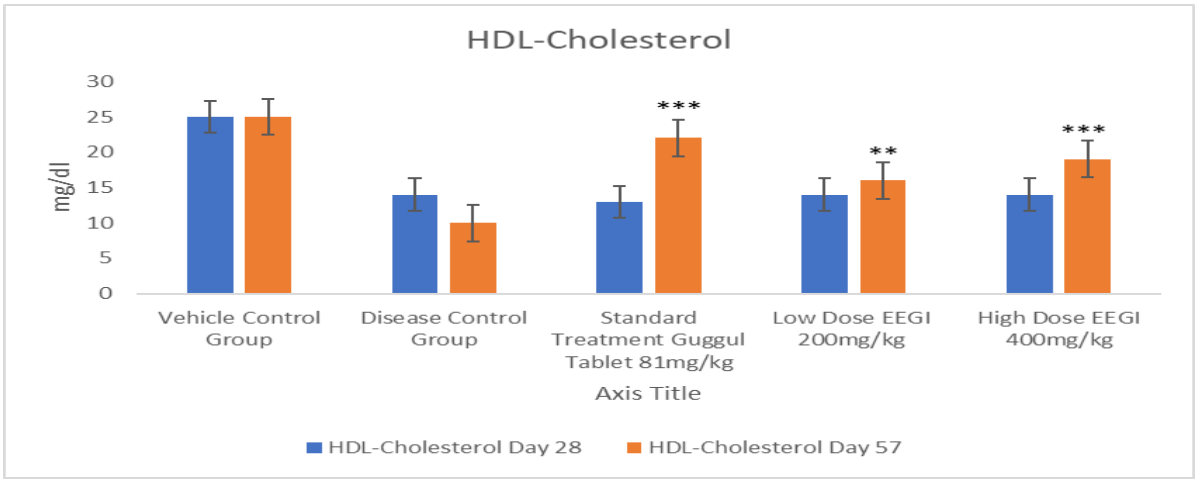


Fig. No. 5: Effect of administration of ethanolic extract of *Garcinia Indica* Leaves on serum HDL-cholesterol of rats.

when compared with day 28. All the values are expressed as Mean ± SEM for n=6 animals ** p<0.01and *** p<0.001 when compared using Dunnett's test.

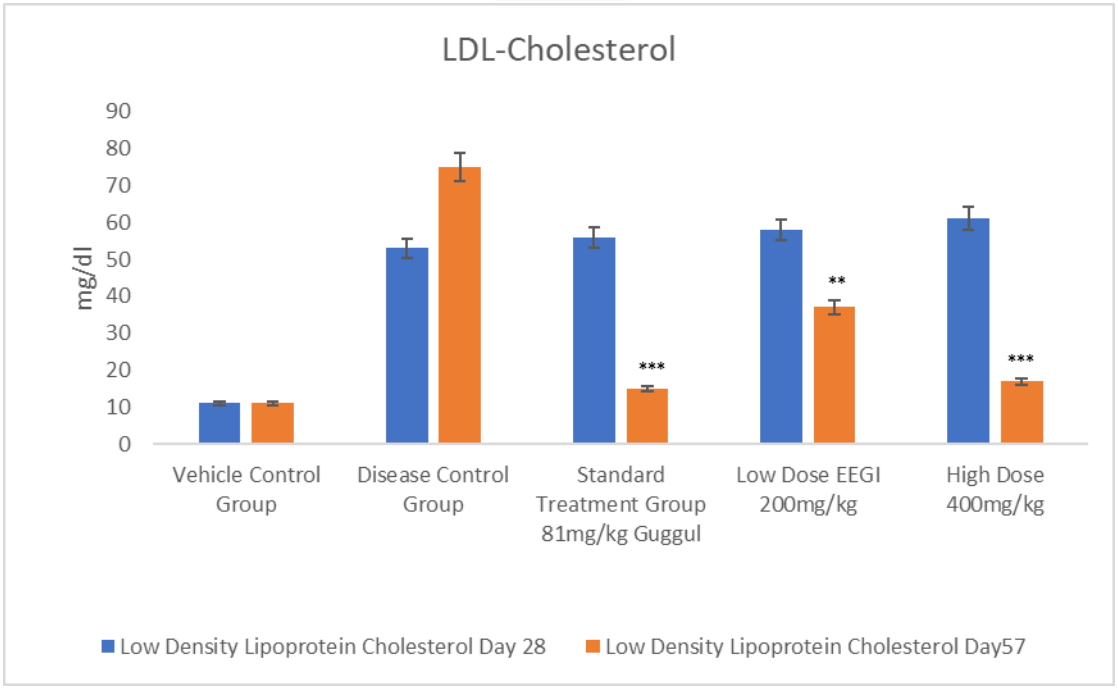


Fig. No. 6: Effect of administration of ethanolic extract of *Garcinia Indica* Leaves on serum LDL-cholesterol of rats.

when compared with day 28.

All the values are expressed as Mean ± SEM for n=6 animals ** p<0.01and *** p<0.001 when compared using paired t-test.

6.6 Effect on Oxidative Stress

Oral administration of ethanolic extract of *Garcinia Indica* Leaves powder (400 mg/kg) offered a significant p<0.001 dose-dependent protection against high-fat diet-induced oxidative stress in rat's liver, as reflected in the levels of MDA, catalase, and reduced glutathione (Table no. 3).

Effect of administration of ethanolic extract of *Garcinia Indica* Leaves on the levels of MDA, catalase, and reduced glutathione of rats

Groups	**Lipid peroxidation MDA µmol/gm weight of Liver Tissue**	**Catalase Enzyme Activity**	**Reduced Glutathione**
Vehicle Control Group	4.30±0.009	79.89±1.12	3.24±0.04
Disease Control Group	21.0±0.094	44.35±1.93	1.86±0.02
Standard Treatment Group Guggul 81mg/kg	4.58±0.004	63.25±1.93	3.02±0.02
Low Dose Group 200mg/kg EEGI	5.68±0.020	51.60±1.10	2.16±0.01
High Dose Group 400mg/kg EEGI	5.12±0.014	56.69±1.24	2.79±0.01

Data represented as the means ± SEM, (n=6).

EEGI – Ehanolic extract of *Garcinia indica*

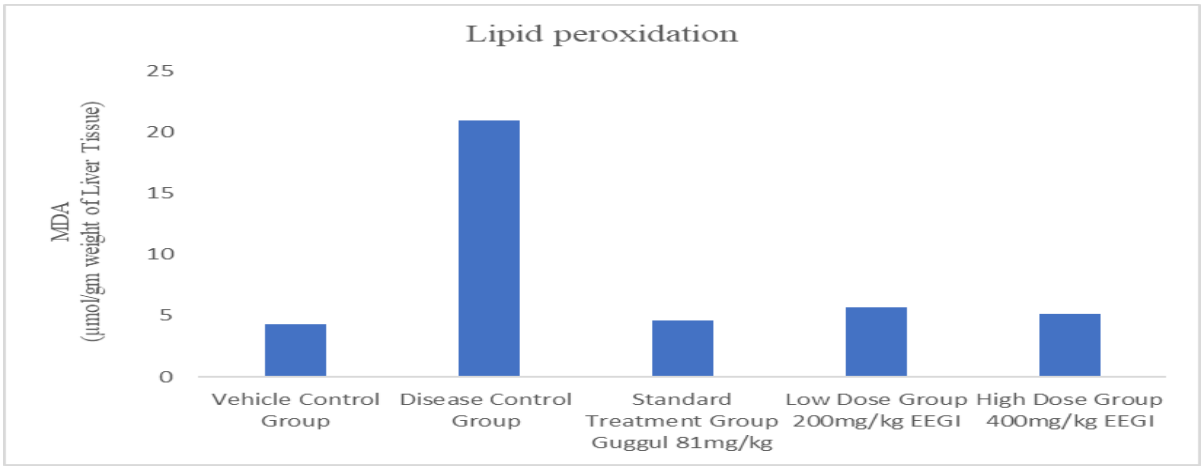


Fig. No. 7: Effect of administration of ethanolic extract of *Garcinia Indica* Leaves on Lipid Peroxidation in the liver.

MDA: Malondialdehyde, # Decrease in MDA with respect to Disease control group

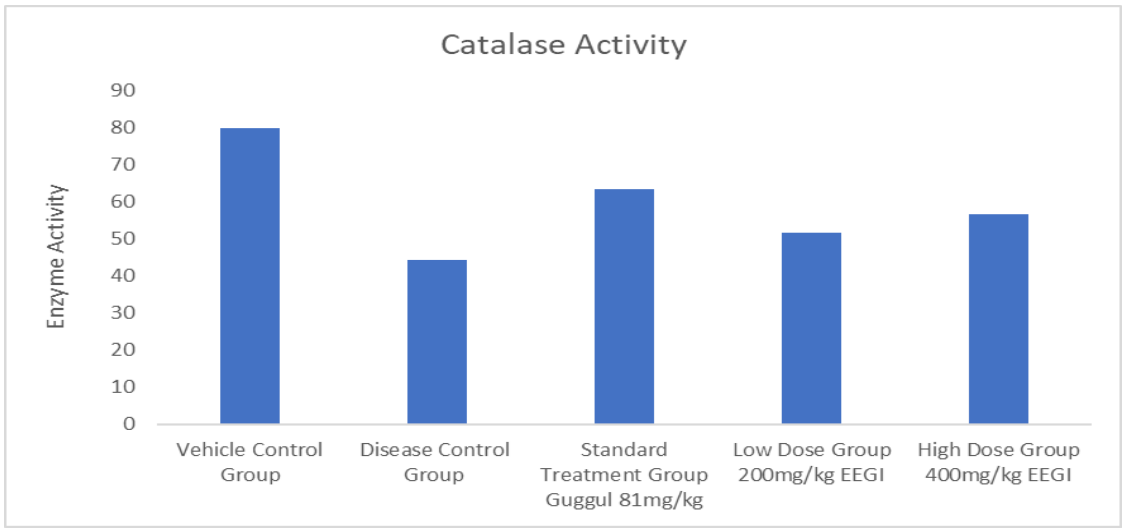


Fig. No. 8: Effect of administration of ethanolic extract of *Garcinia Indica* Leaves on Depletion of Catalase in Liver.

Increase in enzyme activity with respect to Disease control group

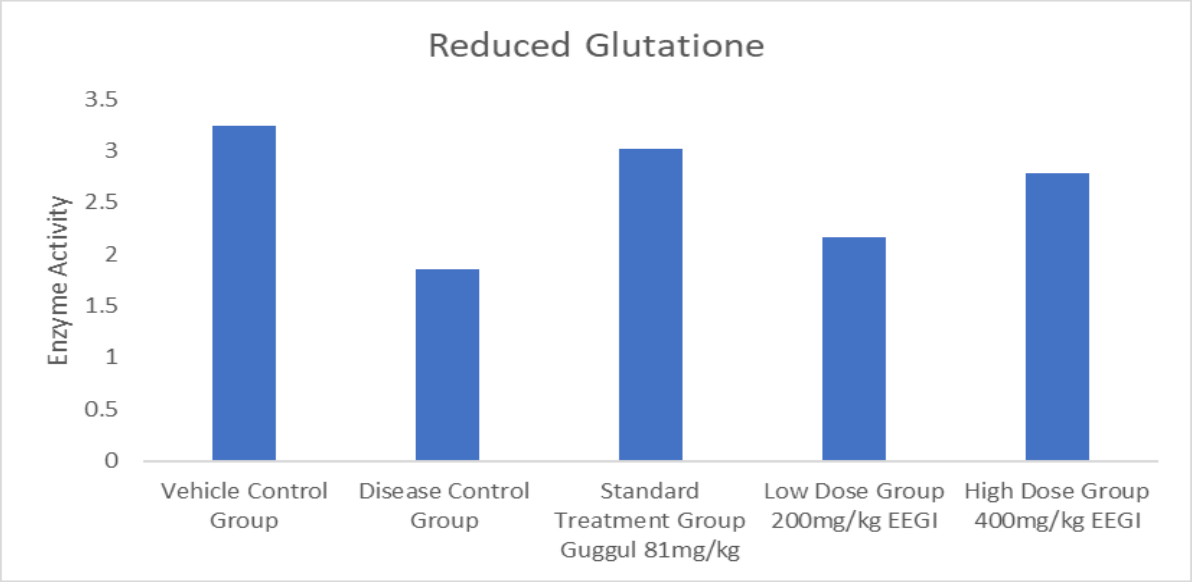


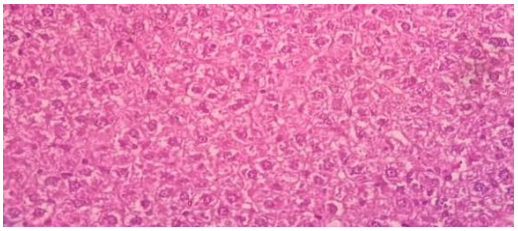
Fig. No. 9: Effect of administration of ethanolic extract of *Garcinia Indica* Leaves on Depletion of Reduced Glutathione Enzyme in Liver.

Increase in enzyme activity concerning Disease control group

Histopathological Results

High-fat diet-treated disease control group rats produced significant changes in hepatic tissue such as increase in Multifocal congestion (+++); multifocal haemorrhages (+++); multifocal hepatic degeneration (+++); Hepatic necrosis (+++), Fatty infiltration (+++) as compared to normal liver histology. Treatment group with Ethanolic Extract of *Garcinia Indica* Leaves 400 mg/kg significantly attenuated these effects of the high-fat diet, as compared to Standard group Shuddha guggul 81mg/kg (Figure 10).

NAD	:	No Abnormality Detected.	**MNC**	:	Mononuclear Cell Infiltration.
Grades of Severity of Lesions:					
+	:	**Minimal**: Very small amount of change < 10%	++	:	**Mild**: Lesion is easily identified but of limited severity 11-25%
+++	:	**Moderate**: Lesion is prominent 26 to 75%.	++++	:	**Severe:** the degree of changes is either as complete 76 – 100% as possible or great enough in intensity or extent to expect significant tissue or organ dysfunction.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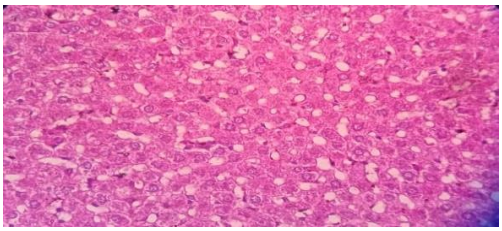
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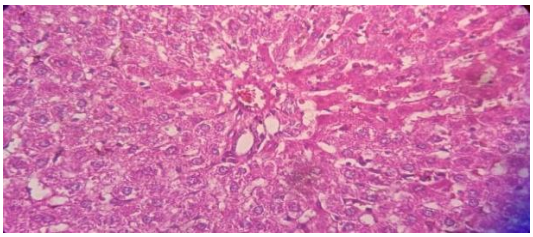
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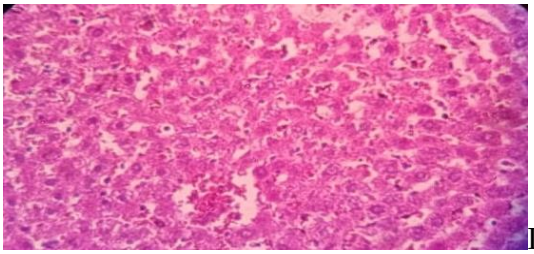
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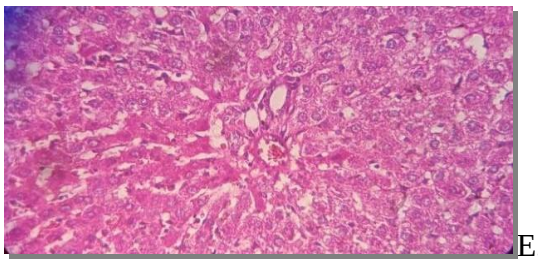
E.

Fig. No. 10 A: Vehicle Control Group – No abnormality detected; B: Disease Control Group with high-fat Diet - liver showing multifocal fatty infiltration; C: Standard treatment group of High fat Diet with monoherbal drug formulation Shuddha guggul 81mg/kg - liver showing minimal grade of Central vein and sinusoidal necrosis; D: Low Dose Group with Ethanolic Extract of *Garcinia Indica* Leaves 200mg/kg along with high-fat diet - liver showing multifocal degeneration and necrosis; E: High Dose Group with Ethanolic Extract of *garcinia Indica* leaves 400mg/kg along with high-fat diet - Microphotograph of liver showing multifocal hepatic degeneration.

7. CONCLUSION

This study concludes that the EEGI demonstrated significant antihyperlipidemic effects at the dose of 400mg/kg in a high-fat diet-induced hyperlipidemia model. The crude extract can be fractionated to identify and isolate the phytoconstituent responsible for the antihyperlipidemic activity. Further studies like Isolation, Fractionation, Molecular Docking, HPLC, HPTLC of *Garcinia Indica* extract can be carried out.

8. ACKNOWLEDGEMENTS:

I would like to Acknowledge Bharati Vidyapeeth's College of Pharmacy, C.B.D Belapur, Navi Mumbai for providing me all the necessary facilities for completing my project work successfully.

9. COMPLIANCE WITH ETHICAL STANDARDS:

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: "All applicable international, national, and/or institutional guidelines for the care and use of animals were followed." "This article does not contain any studies with human participants or animals performed by any of the authors."

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