



ETHANOL EXTRACT OF *Passiflora edulis f. Flavicarpa* Degener LEAVES SUPPRESSES ADIPOGENESIS BY DOWN REGULATION OF PPAR- γ IN *IN VIVO* AND *EX VIVO* MODELS

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ABSTRACT

The anti-adipogenic as well as antihyperlipidemic effects *in vivo* of the ethanolic extract of *Passiflora edulis f. Flavicarpa* Degener leaves (EEPE) were investigated. The pharmacological effects were assessed by measuring bodyweight (every 5th day), serum total lipid profile (30th day) using High fat diet (HFD) induced model. After cervical dislocation, the *in vivo* antioxidant property was analysed by lipid peroxidation assay using rat liver slices. The liver slices were also subjected to reverse transcriptase PCR to analyse expression of PPAR- γ gene in obesity and adipogenesis. Significant decrease in weight was observed along with marked decrease in lipid profile levels as well as atherogenic index too, demonstrated the antihyperlipidemic activity of the extract. The percentage of lipid peroxidation of extract treated was substantially reduced which implies the *in vivo* antioxidant property of the extract. The expression of PPAR- γ in extract treated was found to be down regulated. Significant hypolipidemic effects produced in all the *in vivo* parameters indicate for the first time that leaves of this plant effectively attenuates HFD- induced obesity, atherosclerosis as well as hepatic steatosis by inhibiting adipocyte formation.

KEY WORDS

Hyperlipidemia, atherosclerosis, Peroxisome Proliferator Activating Receptor (PPAR- γ), Ethanolic Extract of *Passiflora edulis f. Flavicarpa* Degener (EEPE)

INTRODUCTION:

Animal models remain indispensable for discovering, validating and optimizing novel therapeutics for their safe use in humans. In recent decades, the popularity of mouse models has surged to the point that the physiology of mice and rats is closer to that of humans than non-mammalian species and that currently ~60% of all preclinical animal research is conducted in *Mus musculus*. Diet-induced obesity has considerable face validity with human obesity and consequently is a widely used paradigm to study the interaction of diet and genes in manifest obesity and insulin resistance. Determination of physical parameters like body weight and biochemical parameters (1) like blood serum analysis helps us to determine the effect of plant extract

and our standard drug (Simvastatin -10 mg) on diet induced hyperlipidemic rats. The *in vivo* antioxidant property was analysed by lipid peroxidation assay using rat liver slices. Then the liver slices were also subjected for Reverse transcriptase PCR to analyse expression of PPAR- γ gene in obesity and adipogenesis.

Herbal drugs constitute a major part in all traditional systems of medicines. Herbal medicine is a triumph of popular therapeutic diversity (2). Passion (*Passiflora edulis f. flavicarpa* Degener) fruit plant possesses several bioactivities (3). The juice, seed and peel of passion fruit showed antioxidant properties, the passion fruit peel extract and pulp could reduce blood pressure folklorically found to be used for lowering the blood glucose level (3) and has antidiabetic properties too .

These are less explored plants on account of its antihyperlipidemic activity, since it possess phytoconstituents such as phenols and flavonoids (5) which are purported to exhibit wide pharmacological activities (4). However, there were no scientific, systematic investigations carried out with these plants for antihyperlipidemic activity. Out of my quest for knowledge and keeping in view of therapeutic efficacy of herbal medicines, the plant *Passiflora edulis f. Flavicarpa Degener* has been selected to evaluate antihyperlipidemic activity using *in vivo* models and *ex vivo* gene expression methods.

2. MATERIALS

2.1 DRUGS AND CHEMICALS:

Simvastatin (10mg), Indian Vanaspati ghee, coconut oil, Span diagnostic kits, Carboxy methyl cellulose (CMC), Phosphate buffer, Tris – HCl buffer, Ethidium bromide, Ethanol (Merck), RNA Isolation Kit (Invitrogen – Product code 10296010), Gel casting apparatus along with gel comb, Gel documentation system (Invitrogen), Cooling centrifuge (RemiCM12),

2.2 STATISTICAL ANALYSIS

Data are represented as Mean $\pm$ Standard error (SEM). The *in vivo* antioxidant experiments were done as three replicates independent experiments, while the rest *in vivo* experiments were done as six replicates. Statistical significance was determined by one-way analysis of variance (ANOVA) and the Tukey post hoc test (Graph Pad Prism® version 6.01, Graph Pad Software, La Jolla, USA). Two Way ANOVA followed by Tukey's multiple comparison *post hoc* test was used for measurement of body weight. A p value of less than 0.05 ($p < 0.05$) was considered to be statistically significant.

3. METHODS:

a. Selection of Animals

Male Wistar albino rats obtained from Animal house, Department of Pharmaceutical Sciences, Cheruvandoor, were acclimatized to the experimental room having temperature $25 \pm 2^\circ\text{C}$, controlled humidity conditions and 12 h light-dark cycle. The rats were fed with commercially available rat standard pelleted diet collected from Hindustan Lever Limited, Bangalore and water ad libitum. Study for antihyperlipidemic activity was conducted after obtaining clearance from the Institutional Animal Ethical Committee IAEC /M. PHARM

/ DPS / 2018-05. Studies were performed in accordance with the CPCSEA guidelines.

b. ACUTE TOXICITY STUDY

Acute toxicity study was conducted in Wistar Albino rats as per OECD guidelines 423. (Organisation of Economic Cooperation and development) (Acute Toxic class method). The test extracts were administered orally to overnight fasted animals at the dose of 2000 mg/kg. Animals were observed continuously and showed neither any sign of death or clinical abnormality. Therefore 400mg/kg and 200mg/kg were selected as the higher and lower dose for the experiment.

c. PREPARATION OF DOSES

The extract was prepared by soxhletation (5) and suspended in Carboxy methyl cellulose (CMC, 0.5% w/w) and administered orally by gavage in volume not greater than 1 ml/100 g body weight daily for 30 days. The suspensions were freshly prepared for the experimental purpose each day.

d. COMPOSITION OF HIGH FAT DIET

The high fat diet was prepared by mixing Indian vanaspati ghee and coconut oil in the ratio of 3:1. It was given to the rat at a dose of 1.5 ml per 100g body weight of the rat using oral gavage tube (6)

4. IN VIVO ANIMAL STUDY:

For screening of hypolipidemic activity, 30 Swiss albino rats procured from the Department of Pharmaceutical Sciences, Cheruvandoor, weighing initially 100–120 g were used in this study. The experimental protocol for animal experiments was approved by the Institutional Animal Ethics Committee IAEC/M.PHARM/DPS/2018-05.

After a 1-month period of acclimation, rats were randomly divided into five groups, with six animals in each group:

NORMAL CONTROL GROUP- 0.5% w/v of CMC - 30 days

POSITIVE CONTROL GROUP - Vanaspati ghee in coconut oil (3:1) (HFD) - 30 days

STANDARD DRUG TREATED GROUP –

HFD (0-15 days) + Simvastatin- 10mg/kg (15-30 day)

HIGH DOSE EXTRACT TREATED GROUP –

HFD (0- 15 days) + 400mg/kg of EEPE (15-30 day)

LOW DOSE EXTRACT TREATED GROUP –

HFD (0- 15 days) + 200mg/ kg of EEPE (15-30 day)

4.1 PHYSIOLOGICAL PARAMETERS

4.1.1 WEIGHT MEASUREMENT

The body weight of each rat was recorded every 5th day during the 30 days study using standard weight machine with the net weight gain and calculated as below:

Net Weight = New Weight – Initial Weight

4.2 BIOCHEMICAL INVESTIGATIONS

At the end of treatment day, the animals were fasted overnight and before sacrificing the animal, the blood was collected by retro-orbital puncture. Serum total cholesterol (TC), triglycerides (TG) was estimated by method of CHOD-PAP and high-density lipoprotein-cholesterol (HDL-c) by the method of GPO-PAP using span diagnostic kits. Serum LDL-c (11), VLDL-c level were estimated from equations,

LDL= Total cholesterol – HDL- (Triglycerides/5)

VLDL= (Triglycerides/5)

4.3 ATHEROGENIC INDEX

The Atherogenic Index is a measure of the atherosclerotic lesion extent based on serum lipids (7), was determined in all groups. The Atherosclerotic Index and percentage (%) of protection was calculated from the following equation: (8).

$$\text{Atherogenic Index} = \frac{\text{TC} - \text{HDL}}{\text{HDL}}$$

$$\% \text{ Protection} = \frac{\text{AI (Control)} - \text{AI (Treated)}}{\text{AI (Control)}} * 100$$

4.4 IN VIVO ANTIOXIDANT ACTIVITY

Lipid peroxidation (LPO) in liver tissue

After the blood collection for biochemical analysis was done, the animals were subjected for cervical dislocation. For the estimation of non-enzymatic and enzymatic antioxidant activity, liver tissues were taken out, cleaned, minced and homogenized (10% w/v) in 0.1 M phosphate buffer (pH 7.0) and centrifuged at 5000 rpm for 10 min and the resulting supernatant was used for enzyme assays.

The tissue was homogenized in 0.1 M Tris – HCl buffer, pH 7.5 and allowed to stand for 5 minutes. 1 ml of tissue homogenate was combined with 2 ml of TBA-TCA-HCl reagent and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling the flocculant, precipitate was removed by centrifugation at 1000 rpm for 10 minutes. Absorbance of the sample was read at 535 nm against the blank that contain no tissue homogenate. The lipid peroxidation was calculated on the basis of the molar extinction coefficient of

Malondialdehyde (MDA) and expressed as nm μ g/MDA units.

4.5 EVALUATION OF mRNA EXPRESSION LEVELS

The gene selected to know the expression level during adipogenesis was PPAR- γ and the preferred housekeeping gene was GAPDH.

4.5.1 ISOLATION OF TOTAL RNA (TRIZOL METHOD)

Total RNA was isolated using the total RNA isolation kit. 1ml of trizol reagent was added to the 100mg tissue sample and homogenized until it formed a fine paste. The contents were then transferred to a fresh sterile eppendorf tube. 200 μ l of chloroform was added and shaking was done vigorously for 15 seconds and incubated for 2-3minutes at room temperature, followed by centrifugation at 14000 rpm for 15 minutes at 4°C. The aqueous layer was collected and 500 μ l of 100% isopropanol was added. It was incubated for 10 minutes at room temperature and then centrifuged at 14000 rpm for 15 minutes at 4°C. Supernatant was discarded and pellet thus obtained was washed with 200 μ l of 75% of ethanol (Merck). It was then centrifuged at 14000 rpm for 5 minutes at 4°C in a cooling centrifuge (RemiCM12). The RNA pellet was dried and suspended in TE buffer.

4.5.2 REVERSE TRANSCRIPTASE PCR ANALYSIS

Reverse transcription polymerase chain reaction (RT-PCR) is a variant of polymerase chain reaction commonly used in molecular biology to generate many copies of a DNA sequence, a process termed “amplification”. In RT-PCR, however an RNA strand is first reverse transcribed into its DNA complement (Complementary DNA or cDNA) using the enzyme reverse transcriptase and the resulting cDNA is amplified using PCR or real time PCR. RT-PCR technique was performed using primer designed specifically for amplified gene.

The cDNA synthesis was performed using Thermo scientific verso cDNA Synthesis kit Product code AB-1453/A. About 4 μ l of 5X cDNA synthesis buffer, 2 μ l of dNTP mix, 1 μ l of anchored oligo dT, 1 μ l of RT Enhancer, 1 μ l of Verso Enzyme Mix and 5 μ l of RNA template (1ng of total RNA) were added to an RNase free tube. Then the total reaction volume was made up to 20 μ l with the addition of sterile distilled water. The solution was mixed by pipetting gently up and down. The thermal cycler (Eppendorf Master Cycler) was programmed to undergo cDNA synthesis. The following cycling

conditions were employed, 30minutes at 42°C and 2 minutes at 95°C

When boiled in an aqueous buffer, agar dissolve and upon cooling solidifies to a gel. 1.5% agarose gel was prepared in 1x TE buffer and melted in hot water bath at 90°C. Then the melted agarose was cooled down to 45°C. 6µl of 10 mg/ml of ethidium bromide was added and poured in to gel casting apparatus with the gel

comb. After setting, the comb was removed from the gel. The electrophoresis buffer was poured in the gel tank and the platform with the gel was placed in it so as to immerse the gel. The gel was loaded with the samples and run at 50 V for 30 minutes. The stained gel was visualized using a gel documentation system (E gel imager, Invitrogen).

OLIGO NAME	FORWARD	REVERSE
	SEQUENCE (5' ->3')	SEQUENCE (5' ->3')
R-GAPDH	AATGCATCCTGCACCACCAACTGC (24)	GGAGGCCATGTAGGCCATGAGGTC (24)
R-PPAR γ	ATGAGCTCATGGGTGAAACT	ATCTCGAGCTAATACAAGTC

5. RESULTS AND DISCUSSION:

5.1 WEIGHT MEASUREMENT

The body weight of each rat was recorded every 5th day during the 30 days study using standard weight machine and the Mean $\pm$ SEM on 0th, 5th, 10th, 15th, 20th, 25th, 30th days recorded are shown in the table 1 and graphically plotted on Figure 1.

Table 1: Body Weight (Mean $\pm$ Sem) Of Treatment Groups

	BODY WEIGHTS (MEAN $\pm$ SEM)						
	Day 0 th	Day 5 th	Day 10 th	Day 15 th	Day 20 th	Day 25 th	Day 30 th
NORMAL CONTROL GROUP	207.0 $\pm$ 5.342	207.0 $\pm$ 5.342	208.5 $\pm$ 4.972	209.0 $\pm$ 5.428	207.5 $\pm$ 4.801	206.2 $\pm$ 5.049	206.2 $\pm$ 5.043
POSITIVE CONTROL GROUP	208.0 $\pm$ 5.939	^a 211.0 $\pm$ 5.715	^a 218.3 $\pm$ 5.970	^a 223.3 $\pm$ 6.349	^a 226.8 $\pm$ 6.290	^a 230.3 $\pm$ 6.458	^a 235.0 $\pm$ 6.377
LOW DOSE GROUP	214.8 $\pm$ 7.490	221.0 $\pm$ 7.294	^a 229.0 $\pm$ 7.690	^a 236.7 $\pm$ 7.601	^b 228.0 $\pm$ 7.750	^b 225.3 $\pm$ 8.147	221.8 $\pm$ 7.943
HIGH DOSE GROUP	238.0 $\pm$ 9.048	^a 244.7 $\pm$ 9.002	^a 252.7 $\pm$ 8.333	^a 258.8 $\pm$ 7.644	^b 249.7 $\pm$ 7.957	^b 247.2 $\pm$ 7.931	^b 243.8 $\pm$ 7.666
STANDARD GROUP	214.5 $\pm$ 6.965	^a 221.0 $\pm$ 6.990	^a 232.0 $\pm$ 7.280	^a 237.3 $\pm$ 7.205	^b 230.8 $\pm$ 7.134	^b 227.5 $\pm$ 6.999	^b 223.7 $\pm$ 7.084

Data are expressed as Means $\pm$ SEM; (^a) $p < 0.001$, when compared with normal group; (^b) $p < 0.001$, when compared with positive control group; On performing repeated measures TWO WAY ANOVA with Tukey's multiple comparison *post hoc* test.

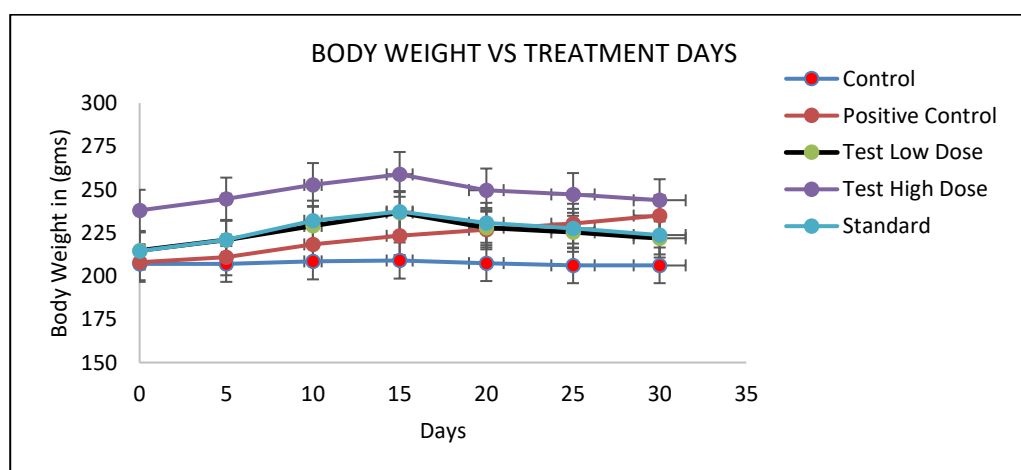
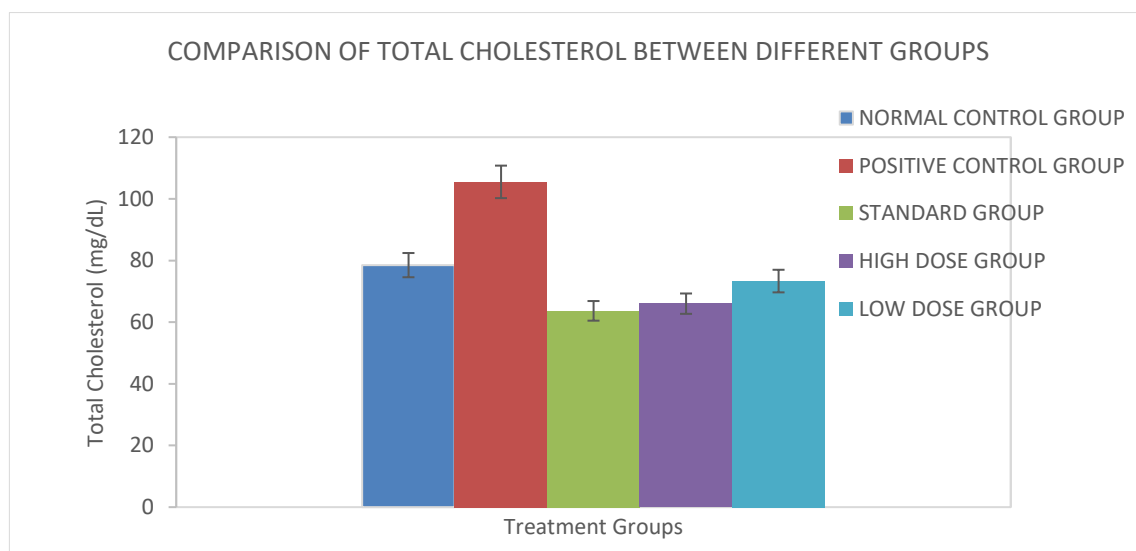
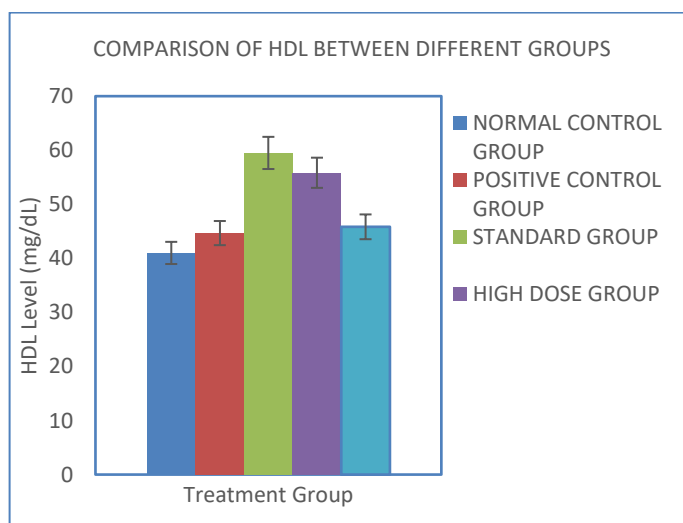


Figure 1. Graphical representation of body weight of animal and treatment days.

TABLE 2: EFFECT OF EEPE ON SERUM LIPID PARAMETERS ON HFD FED HYPERLIPIDAEMIC RATS

GROUPS	TC	TGL	HDL	LDL	VLDL
NORMAL CONTROL GROUP	78.50± 0.4282	61.17± 1.7780	41.00± 1.5250	25.27± 1.652	^(b) 12.23± 0.3556
POSITIVE CONTROL GROUP	105.5± 1.727	101.7± 2.348	44.67± 0.8819	40.50± 2.361	20.33± 0.4695
STANDARD GROUP – Simvastatin(10mg/kg)	^(a) 63.67± 1.174	^(a) 37.00± 1.155	^(a) 59.50± 0.6191	^(a) 3.567± 0.5667	^(a) 7.400± 0.2309
HIGH DOSE GROUP (400mg/kg)	^(a) 66.00± 0.7303	^(a) 39.50± 1.708	^(a) 55.83± 1.922	^(a) 18.60± 1.501	^(a) 7.900± 0.3416
LOW DOSE GROUP (200mg/kg)	^(a) 73.33± 0.3146	^(a) 51.00± 1.317	45.83± 1.869	^(a) 19.07± 1.334	^(a) 9.967± 0.4014

Data are expressed as Means ± SEM where n=6; ^(a) p< 0.001, When compared with positive control group; On performing repeated measures ONE WAY ANOVA with Tukey's multiple comparison *post hoc* test.


Figure 2: Plot of total cholesterol levels among the treatment groups

Figure 3: Plot of HDL levels among the treatment groups

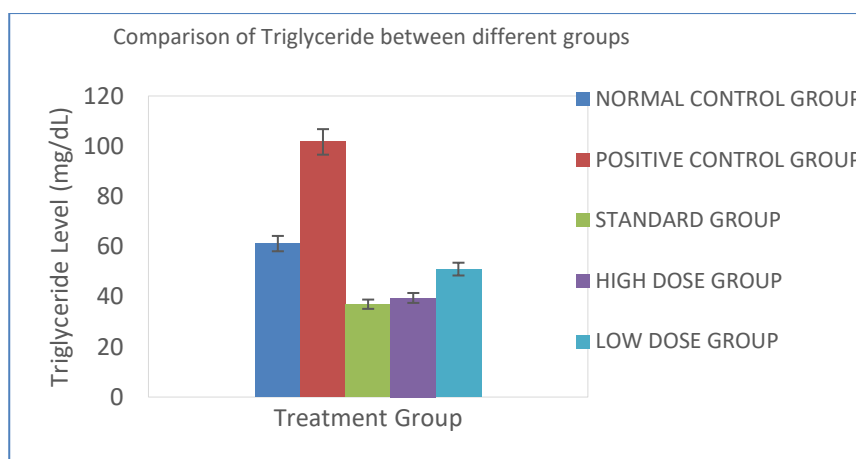


Figure 4: Plot of triglycerides levels among the treatment groups

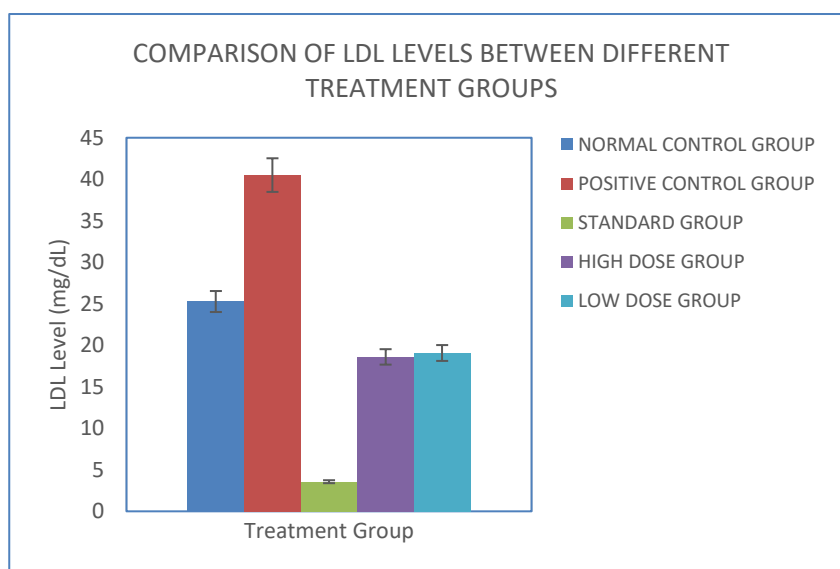


Figure 5: Plot of LDL levels among the treatment groups

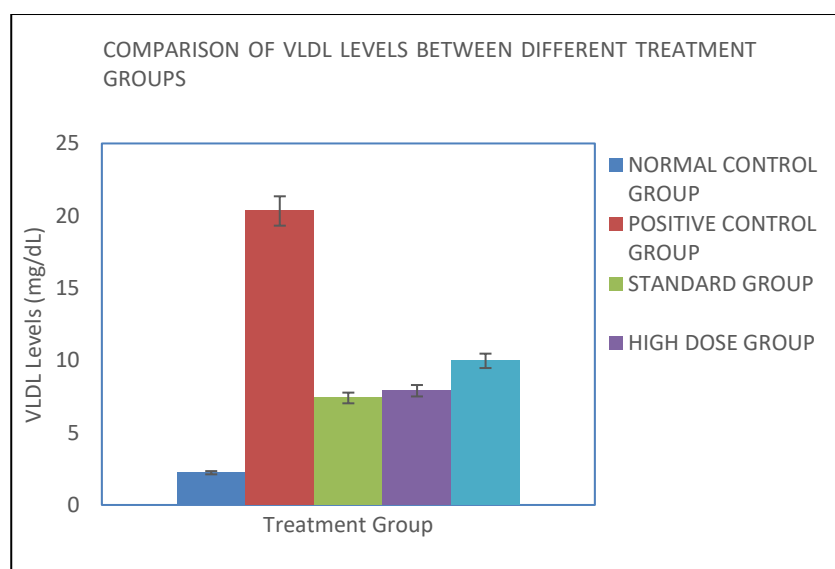
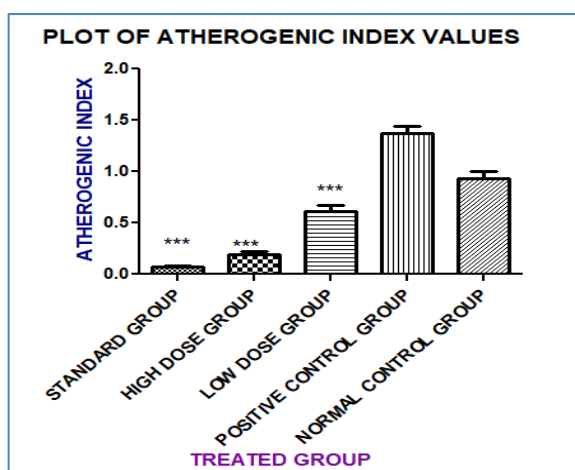


Figure 6: Plot of VLDL levels among the treatment groups

TABLE 3: ATHEROGENIC INDEX AND PERCENTAGE PROTECTION VALUES PRODUCED BY HFD RATS UPON TREATMENT

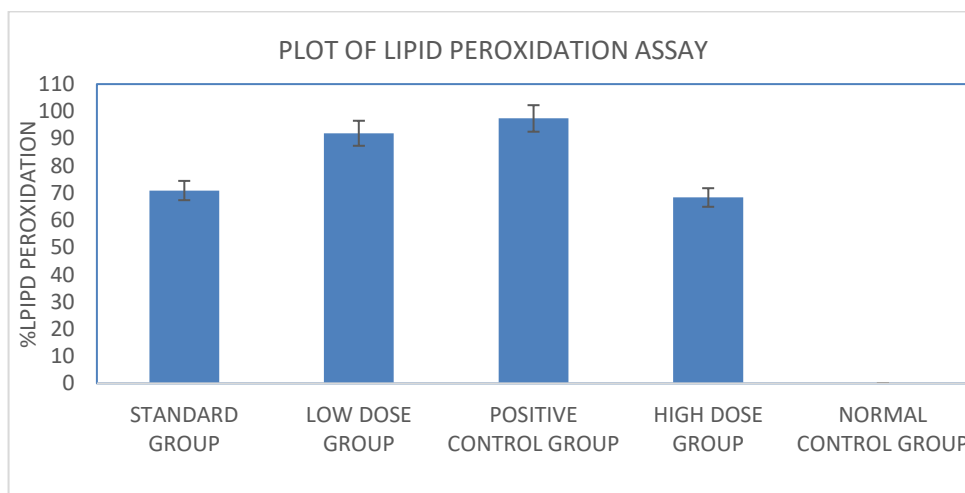
	STANDARD GROUP	HIGH DOSE GROUP	LOW DOSE GROUP	POSITIVE CONTROL	NORMAL CONTROL
MEAN $\pm$ SEM	***0.07057 $\pm$ 0.01451	***0.1879 $\pm$ 0.03591	***0.6122 $\pm$ 0.06141	***1.369 $\pm$ 0.07452	***0.9287 $\pm$ 0.07657
%PROTECTION	94.40%	86.27%	55.28%	-----	-----

Data are expressed as Means $\pm$ SEM; *** $p < 0.001$, when compared with positive control group, on performing repeated measures ONE WAY ANOVA with Tukey's multiple comparison *post hoc* test.


Figure 7: Graphical plot of effect of EEPE on Atherogenic index of the treatment groups
TABLE 4: IN VIVO LIPID PEROXIDATION ASSAY VALUES

	STANDARD GROUP	HIGH DOSE GROUP	LOW DOSE GROUP	POSITIVE CONTROL	NORMAL CONTROL
Mean $\pm$ SEM (μ g/MDA units)	0.8006 $\pm$ 0.00105	0.7358 $\pm$ 0.0242	2.889 $\pm$ 0.099	8.900 $\pm$ 0.0652	0.2331 $\pm$ 0.001004
Lipids Peroxidation (%)	^a 70.88 %	^a 68.32 %	^a 91.93 %	97.38 %	0%

Data are expressed as Means $\pm$ SEM where $n=3$; (^a) $p < 0.001$, When compared with positive control group; On performing repeated measures ONE WAY ANOVA with Tukey's multiple comparison *post hoc* test.


Figure 8: Graphical plot of lipid peroxidation values

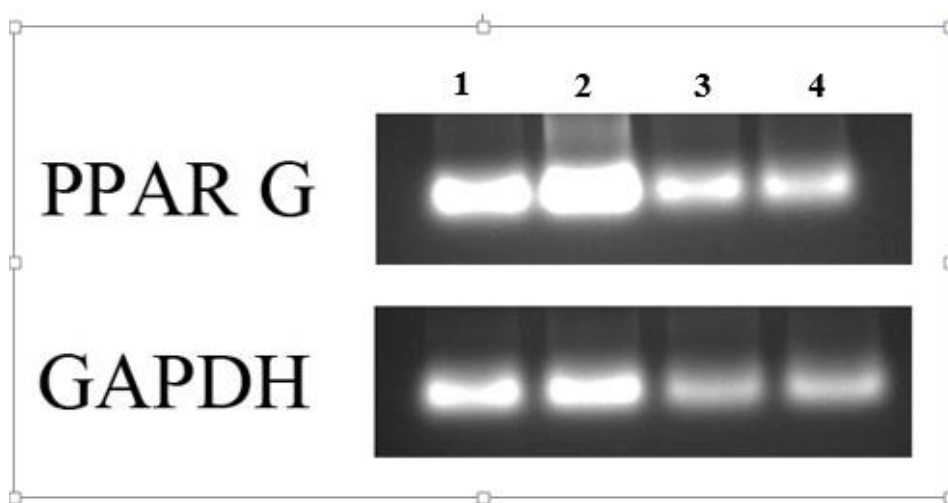


Figure 9: Expression Analysis of gene R-PPAR γ gene using Reverse Transcriptase PCR

Gel Loading Order: 1- GNC (Group Normal Control) 2- GPC (Group Positive Control) 3- GHD (Group High Dose) 4- GSD (Group Standard Dose)

TABLE 5: THE RELATIVE EXPRESSION VALUES OF PPAR γ AND GAPDH

SAMPLE	PPAR GAMMA	GAPDH	Relative expression
GNC	378.716	241.706	1.566846
GPCI	518.309	314.299	1.649095
GHDI	227.576	176.383	1.290238
GSDI	176.662	196.952	0.89698

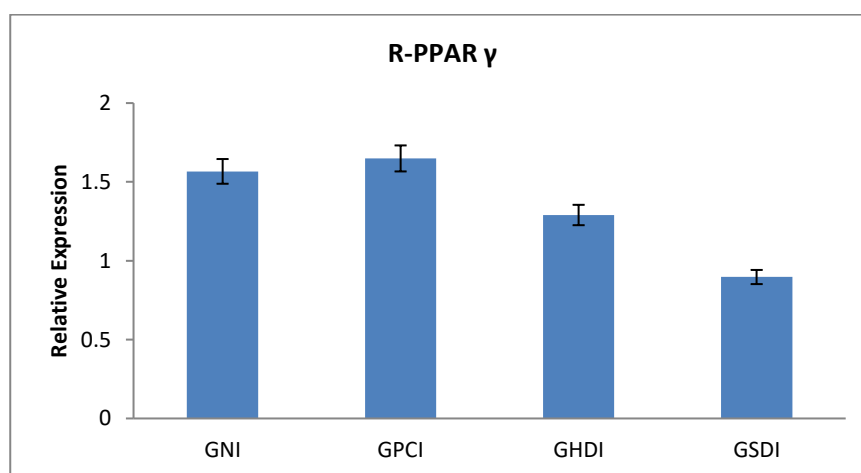


Figure 10: Graphical Plot of Relative expression of R-PPAR γ in various treated samples

An increase in body weight was observed in all groups except normal control group since cholesterol was fed in all the other groups for all the 30 days. As per statistics, in case of the positive control the weight was increasing significantly all the 30 days till the end of the experiment. Were as in case of standard group treated (Simvastatin- 10mg); low dose treated (200mg/kg) as well as high dose treated group (400mg/kg), a decrease

in weight was observed from 15th day onwards after treatment of simvastatin, low dose extract and high dose extract treatment respectively. The two-way ANOVA showed a statistical significance of $p < 0.001$. Therefore, our extract produces a comparable action for reduction of body weight (anti-obesity) as of the standard drug when compared with the cholesterol fed

group that confirms the antiobesity activity of the extract.

5.2 EFFECT OF EEPE ON SERUM LIPID PARAMETERS ON HFD FED HYPERLIPIDAEMIC RATS

In cholesterol induced model during the first fifteen days of HFD treatment, the total lipid profile was found to increase compared to the normal control group. During the following 15 days (15-30 days) of schedule, oral administration of **EEPE (200mg/Kg, 400mg/Kg p.o)** significantly reduced the elevated Total cholesterol, Triglycerides, Low Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL) levels but increased High Density Lipoprotein (HDL) levels compared to normal and positive control group. Comparison of total cholesterol, HDL, Triglycerides, LDL, VLDL levels between different groups are graphically plotted in figures 2, 3, 4, 5, 6 respectively.

5.3 ATHEROGENIC INDEX:

The Atherogenic Index for positive control compared to normal group was found to be increased by 34.16%. While the AI for standard was found to be decreased by 94.84% compared to positive control group; decreased for the high dose treated group by 86.27% compared to positive control group and showed a statistical significance of $p < 0.001$. The Atherogenic index and percentage protection values are shown in Table 3 and graphically shown in figure 7.

The percentage protection of heart is the ability of drug or extract to prevent atherosclerotic plaques and lesions. The high dose of extract showed ~80% heart protection compared to the heart protection produced by simvastatin.

Data are expressed as Means $\pm$ SEM where $n=6$; ^(a) $p < 0.001$, When compared with positive control group; On performing repeated measures ONE WAY ANOVA with Tukey's multiple comparison *post hoc* test.

5.4 IN VIVO LIPID PEROXIDATION ANTI OXIDANT ASSAY:

Liver tissue homogenate was prepared and used for the estimation of lipid peroxidation (expressed as malondialdehyde, MDA levels) in the rats. The Standard group treated with Simvastatin (10mg/kg) showed 70.88%; Low dose group treated with 200mg/kg extract showed 91.93% lipid peroxidation scavenging; High dose group treated with 400 mg/kg extract showed 68.32%. Lipid peroxidation of the Positive control group treated with Cholesterol showed 97.38%. The scavenging activity is inversely related to the amount of

MDA (TBARS) concentration (lipid peroxidation) in the sample (7). According to the results, it is confirmed that high dose treated group shows most scavenging value followed by the standard drug treated group. The Simvastatin treated group showed a lesser scavenging value than the high dose extract treated group. Since the cholesterol treated positive control group has more free radicals in it, group shows a higher percentage (%) of TBARS value than others and consequently low scavenging activity. All the groups were compared with the normal control group to calculate the lipid peroxidation scavenging percentage. TBARS concentration in plasma was found to be 91.93% and 68.32% with supplementation of Ethanolic extract of *Passiflora edulis f* Flavicarpa leaves at the dose of 200 mg/kg and 400 mg/kg/day respectively. As shown in the study, Ethanolic extract of *Passiflora edulis f* Flavicarpa extract (400mg/kg) decreases elevated levels of TBARS to about normal control. The MDA levels were seen to be decreased significantly on administration of EEPE at dose levels of 200mg/kg ($p < 0.001$) and 400mg/kg ($p < 0.001$) in comparison with positive control. The effect of EEPE on MDA levels is tabulated in the Table 4 and graphically depicted in the Figure 8.

Data are expressed as Means $\pm$ SEM where $n=3$; ^(a) $p < 0.001$, When compared with positive control group; On performing repeated measures ONE WAY ANOVA with Tukey's multiple comparison *post hoc* test.

5.5 EXPRESSION OF mRNA USING REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION

Adipocyte requires transcriptional factors for adipogenic differentiation using adipogenic markers including Peroxisome proliferator activated receptor γ (10)(PPAR γ). Adipocyte differentiation which was activated by Peroxisome proliferative activated receptor (11).

Here the liver tissues were used to study the mRNA expression studies. Relative expression studies were conducted with reference to a house keeping gene, GAPDH. HFD induced diet causes the cholesterol rise in all groups. Therefore, the relative expression of PPAR γ was found to increase to 1.649 compared to normal treated group. While the relative expression for standard treated group as well as extract treated was found to be decreased to 0.8969 and 1.2909 respectively. The standard drug simvastatin reduced the expression more, while the extract also decreased the expression considerably. Treatment with extracts and

standard, decreased the cholesterol and lipid level by down regulation of the PPAR γ gene; our extract also inhibited the differentiation of adipocytes in the rat liver.

The expression analysis of PPAR γ gene and GAPDH gene are tabulated in Table 5 and shown in Figure 9 followed by the graphical representation in Figure 10 respectively.

6. CONCLUSION:

- The *in vivo* antihyperlipidemic activity was evaluated using High Fat diet induced rat model. The physiological parameters like body weight assessment, biochemical parameters (lipid profile) was assessed for hypolipidemic activity. The high dose and low dose of EEPE was effective in normalising total cholesterol, triglycerides, LDL and VLDL.
- The Atherogenic index of extract was found to be lowered and percentage of heart protection offered by the extract was also found to be increased and the extract proved to be anti-atherosclerotic and as well as cardio protective.
- The *in vivo* antioxidant properties were assessed by estimating lipid peroxidation levels in the liver tissue homogenates. The high dose of EEPE was effective in lowering the lipid peroxidation level and this action due to its antioxidant, membrane restorative effects and hypolipidemic potential of the extract.
- The gene expression studies were evaluated by RT-PCR method and the PPAR γ gene selected for studying the adipogenic differentiation and maturation; GAPDH was selected as housekeeping gene. The EEPE was very much effective in down regulating the expression of PPAR γ . Thus, the extract was anti-adipogenic in nature and proved to be hypolipidemic and effective in anti-obesity treatment.

From the above results we can conclude that the ethanolic extract of *Passiflora edulis f. Flavicarpa Degener* leaves possess antiobesity as well as antihyperlipidemic activities. The activity can be confirmed due to the presence of flavonoids and phenolics (5) (after phytochemical analysis) which might be due to its anti-oxidant and membrane restorative properties.

The results of *in vivo* as well as *ex vivo* gene expression studies prove and emphasize the hypolipidemic as well as anti-obesity activity of ethanolic extract of *Passiflora edulis f. Flavicarpa Degener* leaves.

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