Estimation of Anti-Obesity Potential of *Tribulus terrestris* in Wistar Rats (High Fat Diet Inducted Obesity)

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Abstract:

Background: An annual yellow colour flower bearing shrub Tribulus Terrestris (TT) (family-zygophyllaceae), a distinguished traditional shrub which was extensively involved in Ayurvedic and Chinese folklore medicinal therapy for various ailments. At present various in-vivo findings have suggested its beneficial role as anti-urolithic, antidiabetic, anti-inflammatory, antitumor, and antioxidant effects. **Objective**: Thus, the main aim of our study was to explore the role of *Tribulus Terrestris* in high fat diet fed obese rats. **Methodology**: Male Wistar rats (170-200 gm) were employed in the present study. To induce obesity high fat diet (30%) was administered for 90 days. To check the successful induction of obesity different anthropometric parameters, adiponectin, ghrelin and leptin levels were assessed and further correlated with glycemic, lipid profile and oxidative stress markers. **Results:** Significant impairment in anthropometric parameters, attenuation of ghrelin and adiponectin levels, and enhancement of leptin levels, impairment of glycemic and lipid profile and decreased antioxidant activity were observed in high fat diet rats. Treatment with *Tribulus Terrestris* showed dose dependent modulation of all the parameters. **Conclusion:** Thus, it can be concluded that *Tribulus terrestris* may proves to be a beneficial therapeutic strategy in obesity

Keywords: High Fat (30%lard fat) diet, Obesity, Wistar rats, *Tribulus terrestris*

INTRODUCTION

Obesity is a long-term medical condition characterized by an excess of body fat. It is instigated by an disproportion between calorie intake and calorie expenditure (1), and is typically measured using the body mass index (BMI), which categorizes individuals as overweight (BMI between 25 and 30 kg/m2) or obese (BMI greater than 30 kg/m2)(2). In addition to other health problems such as back pain, arthritis, and infertility, obesity can also

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increase the risk of developing serious diseases such as hypertension, heart disease, diabetes, gallbladder disease, and various types of cancer(3). Obesity has become a global epidemic, with the number of overweight and obese individuals tripling in the past 30 years and nearly 1/3rd of the world's populace now categorized as overweight or obese(4). It is important to address this issue and prevent obesity, as it affects people of all ages, regardless of geographic location, ethnicity, or socioeconomic status (5).

There are several approaches currently used to treat obesity, including lifestyle changes such as diet and exercise, medications that suppress appetite or block the absorption of fat, and bariatric surgery to reduce appetite and decrease nutrient absorption. These strategies aim to help individuals burn excess calories and achieve a healthier weight(6). However, the effectiveness of all these therapies in weight loss induction is quite low. Pharmacotherapy for obesity will be beneficial only, if the drugs are used continuously over an extended period of time. Orlistat, an anti-obesity drug that has been approved by the US FDA. However, longer duration use of orlistat can cause a range of side effects, including steatorrhea (excess fat in the faeces), flatulence, faecal incontinence, oily spotting, constipation, and others. It is important to discuss the potential risks and benefits of any treatment with a healthcare provider before starting therapy(7). Thus, there is an urgent need for alternative therapies to treat and treatment methods for obesity(8).

A plant known as *Tribulus Terrestris* (TT) belongs to family Zygophyllaceae is mostly found in South Africa, Australia, India, and Europe. It has historically been used to treat a number of illnesses in both Indian and Chinese traditional medicine systems. Among these, *T. Terrestris* (TT) is a popular therapeutic herb used by both modern and Ayurvedic herbalists(9). It is a perennial plant with yellow flowers (fig.1) that is cultivated around the world in hot climates, including India, Pakistan, China, Africa, France, Australia, southern and Western Europe, Bulgaria, Mexico, and North America. It has received certification for its pharmaceutical uses during the past few years, including enhancement of sexual function, heart protection, and anti-urolithic, antidiabetic, anti-inflammatory, anticancer, antioxidant benefits. The most significant metabolites with a variety of bioactivities are thought to be the steroidal saponins and flavonoids of TT(10).

Bearing in mind the extensive range of activities of the plant(11), the under discussion study was designed to evaluate the attenuating potential of hydroalcoholic and ethylacetate extracts of fruits of *T. Terrestris*

Figure 1: Aerial parts of Tribulus Terrestris

MATERIAL AND METHODOLOGY

Collection and Identification of Plant material: During their ideal season, the fruits of Tribulus terrestris Linn were acquired from a neighbourhood market. Plant authentication was completed at Sri Vankateshwara University in Tirupati, India and the voucher specimen 0540 is deposited in university's herbarium. The fruits of Tribulus terrestris was further processed by washing under running tap water, shade drying and then pulverising into coarse powder. The coarse powder was stored in airtight glass containers for further processes.

Preparation of hydroalcoholic and Ethylacetate extracts: The 100gm of powdered *T. terrestris* fruit was successively extracted with ethanol (60%) and ethylacetate by soxhlation for up to 72 hours. Following extraction, the extracts were filtered and concentrated using a rotary evaporator set at 40°C to produce semisolid or dry mass.

High-Performance Liquid Chromatography (HPLC) analysis

Instrument and Chromatographic conditions: Waters HPLC was used for carrying out the quantification of bioactive compounds present in the extracts of both plants as per specifications given by Naveen P, Lingaraju H, Prasad KS et.al 2017 (12)

Experimental design

Animals: For this study, male Wistar rats with an average weight of 170-200 grams were

divided into nine sets, with six-eight rats in each set of rats. These animals were housed in a controlled environment with a temperature of 24-28°C and a RH of 60-70%. They were subjected to a 12-hour light/dark cycle and had access to water ad libitum. The experiment was approved by the Institute Animal Ethics Committee (MMCP/IAEC/79) on 22/06/2020, and all procedures were conducted in accordance with the guidelines given by CPSCEA.

Animals were fed with normal pellet diet (normal group), (orlistat treated group+ HFD), High fat diet (HFD) induced obesity group treated with extracts.

Preparation of Dosage: Suspension of extracts (with CMC and tween 80) prepared in three different doses 150mg/kg, 300mg/kg, and 600mg/kg.

High fat diet induced Obesity model: Animals were fed with high fat diet containing 30% fat (lard), coconut oil, sugar content and gram flour content, vitamins and minerals as compared to normal pellet diet. To evaluate successful induction of obesity various anthropometric parameters (body weight, adiposity index, obesity index, and waist hip ratio) were measured.

Experimental Groups: The animals were arbitrarily dispersed into 9 groups comprising 6 male albino Wistar rats in each group.

Group I	Normal control
Group II	Positive control -HIGH FAT DIET
Group III	Obese rats given orlistat (30 mg/ kg) orally for Ninety days
Group IV	Obese rats given *Tribulus terrestris* Ethylacetate extract TT-EA (150 mg/ kg) orally for Ninety days
Group V	Obese rats given *Tribulus terrestris* Ethylacetate extract TT-EA (300 mg/ kg) orally for Ninety days
Group VI	Obese rats given *Tribulus terrestris* Ethylacetate extract TT-EA (600 mg/ kg) orally for Ninety days
Group VII	Obese rats given *Tribulus terrestris* hydroalcoholic extract TT-HA (150 mg/ kg) orally for Ninety days
Group VIII	Obese rats given *Tribulus terrestris* hydroalcoholic extract TT-HA (300 mg/ kg) orally for Ninety days
Group IX	Obese rats given *Tribulus terrestris* hydroalcoholic extract TT-HA (600 mg/ kg) orally for Ninety days

Blood sample collection and serum separation: Blood was isolated via retro-orbital puncture method on basal and 90 th day of the study to assess various biochemical and molecular markers. To separate serum, blood was centrifuged at 4000 rpm (4˚C) for 15 minutes.

Anthropometric Parameters(13):

Body weight and waist Hip Ratio- Body weight (gm) and waist hip ratio of animals in each group was measured periodically on basal and 90th day of study.

Obesity Index(14): This anthropometric parameter was assessed in all animal groups on basal and 90th day of study. The Body weight in gm and nasoanal length in mm was recorded for estimation of Obesity index as per the formula given below

$$\text{Obesity Index} = \frac{\text{Body weight of rat(gm)}}{\text{Nasoanal lenght(mm)}} \times 10000$$

Adiposity Index (14): After completion of the study protocol, on 90th day, animals were sacrificed by cervical dislocation under high dose of ketamine and white adipose tissue was collected and weighed for calculating Adiposity index as per formula given below:

$$Adiposity\ Index = \frac{Total\ Body\ fat(gm)}{Body\ weight(gm)} \times 100$$

Total body fat= Visceral fat+ retroperitoneal fat+ Epididymal Fat

Assessment of glycemic and lipid profile (15-18): The collected blood samples on basal and 90th day of study were analyzed for serum glucose (GOD-POD principle), total glyceride (Phospho-tungstic acid principle), total cholesterol (CHOD-PAP), LDL (Friedewald equation), HDL (Phospho-tungstic acid principle) and VLDL serum concentrations. Serum LDL levels were calculated by the equation given by Friedewald. Serum LDL = Total Cholesterol-(HDL+VLDL)

Liver Function test (19): This analysis (SGOT, SGPT) was done on basal, 90th day of study by using colorimetric method given by Reitman S, 1957 using Erba diagnostic kit.

Assessment of oxidative stress in liver:

Tissue homogenate Preparation- At the end of 90th day of the study animals were sacrifices, livers were isolated to assess oxidative stress parameters such as Thiobarbituric acid reactive substance (TBARS) levels, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase activity. Reduced glutathione estimations were performed via method as reported earlier by Beutler ((16), Superoxide Dismutase Estimation was performed according to kakkar P, 1984(20), method by Ohkawa et al. (21) was employed for the estimation of lipid peroxidation levels and method by Aebi(22, 23) was employed for the estimation of CAT activity.

Biomarker Estimation: The biomarkers for obesity (Leptin, Ghrelin, Adiponectin, and Insulin) were estimated by ELISA kits of Bioassay Technology Laboratory, Shanghai, Japan.

Statistical analysis: The collected data was analysed using PRISM statistics software, which included a one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. The results were expressed as the mean ± standard error of the mean (S.E.M.) and a p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Characterization of Bioactive compound

HPLC chromatographic method was employed for identification and quantification of bioactive compound present in both extracts *Tribulus terrestris* hydroalcoholic extract (TT-HA) and *Tribulus terrestris* Ethylacetate extract (TT-EA). The chromatogram confirmed the presence of Rutin trihydrate and quercetin as depicted in (fig 2-4).

The percentage of bioactive constituent present in extracts is given in table 1

Anthropometric parameters:

Effect of TT-HA &TT-EA on Body weight, waist hip ratio and obesity Index- All the animals were divided into 9 groups with body weight 170-200gm and we observe no significant difference observed in all the groups on day zero (basal reading). The findings of these parameters found in High fat diet (HFD) treated rats ($p<0.001$) as equated with control group rats are given in Table 2.

Effect of TT-HA &TT-EA on Adiposity index- At 90th day of study adiposity index was calculated among all 9 groups of animals and recorded observations are exhibited in fig 5.

Effect of TT-HA &TT-EA on Glycemic and lipid Profile- Significant impairment in glycemic and lipid profile was observed in HFD treated rats as compared to control group rats as indicated by increase in plasma glucose, insulin, total cholesterol, TG, LDL, VLDL levels and decr-ease in HDL levels as given in table 3 and 4.

Effect of TT-HA &TT-EA on liver function parameters- There is significant increase in levels of SGOT, SGPT ($p<0.001$) in HFD fed groups as compared to normal groups. Treatment with TT-HA (150,300,600 mg/kg) and TT-EA (150,300,600 mg/kg) significantly and dose dependently modulated SGOT, SGPT levels as shown in table 5.

Effect of TT-HA &TT-EA on oxidative stress enzymes and TBARS- There is significant decrease in levels of SOD, GSH and CATALASE enzyme ($p<0.001$) in liver of HFD fed animals as compared to normal group animals and levels of TBARS increased significantly in HFD group as compared to normal group, however, treatment with TT-HA and TT-EA significantly and dose dependently modulated SOD, CAT, GSH and TBARS levels towards normal as depicted in fig 6.

Effect of TT-HA &TT-EA on Ghrelin, Leptin and adiponectin- Results has exhibited low levels of Ghrelin and adiponectin and high levels of Leptin ($p<0.001$) in HFD group compared to normal group. The level of these biomarkers were modulated towards normal by TT-HA (150,300,600 mg/kg) and TT-EA (150,300, 600 mg/kg) in dose dependent manner in animals fed with high fat diet as given in fig 7.

DISCUSSION:

As per earlier reported studies, it's evident that**,** *Tribulus terrestris* is an abundant source of flavonoids and phenolic compounds (24-26) and we have also validated this in our extracts via HPLC and found Rutin and quercetin in extracts. The presence of phenolic compounds is an indicator of good anti-oxidant as suggested by previous studies (25, 27-29).

Data from large number of *in-vivo* studies have reported that intake of high fat diet in rats led to rise in body weight(30), obesity index(31), adiposity index(32, 33) along with impairment of glycemic(34) and lipid levels(33) . In concurrence with this we also observed significant increase in body weight and other anthropometric parameters and impaired glycemic and lipid profile in HFD treated rats as compared to control group rats, which indicates successful induction of animal model of obesity (35-38). *Tribulus terrestris* was found to be beneficial in maintaining glycemic and lipid profile as supported by earlier studies (39-42). Our present study also goes parallel with previous findings as the hydroalcoholic (TT-HA) and ethylacetate (TT-EA) extracts of *Tribulus terrestris* has regulated lipid and glycemic profile in dose dependent manner in animals fed with high fat diet as comparable to normal fed diet group animals. However, to

the best of our knowledge there is no earlier study reported on effect of *Tribulus terrestris* on anthropometric parameters.

Over accumulation of adiposity (white adipose tissue) generates Oxidative stress which may also affect food intake(43). Studies have demonstrated that oxidative stress can lead to an increase in preadipocyte proliferation, adipocyte differentiation, and mature adipocyte size in both in vitro cell culture and in vivo animal experiments. This suggests that oxidative stress may play a role in the development and growth of fat cells (44). Reported studies have indicated that oxidative stress leads to hindered glycemic profile which causes elevated levels of glucose in blood leading to diabetes and liver damage(45). The attenuation of oxidative stress by *Tribulus terrestris* found to beneficial as hepatoprotective and also acts as antioxidant (46). In concurrence with these studies, our present study has also supported that TT-HA and TT-EA, dose dependently maintained SGOT and SGPT levels in high fat diet fed animals as compared to untreated animal group fed on HFD. Both the extracts have also maintained the levels of SOD, CAT, TBARS and GSH levels in animals fed with high fat diet as compared to animals untreated with extracts.

Studies have reported that ghrelin (anabolic hormone) levels are attenuated in obese individual as compared to lean individuals (47). The level of ghrelin is significantly less in visceral tissues than subcutaneous tissues. The high fat diet tends to exhibit ghrelin resistance by decreasing NPY/AgRP response to plasma ghrelin levels thereby suppressing the neuroendocrine ghrelin axis and under these conditions individuals tends to have more tendency of increasing good intake (48). So, it is evident that ghrelin resistance which occurred by high fat diet-induces hyperphagia leading to obesity induction (49). In accordance with this we have also observed that the levels of ghrelin were significantly low in HFD group as compared to normal group. However, ghrelin levels were significantly modulated by in TT-HA, TT-EA dose dependently in HFD fed rats.

Adiponectin reduces endothelial cells capacity to get activated in response to various inflammatory stimuli by blocking the generation of proinflammatory cytokines and chemokine's (50)**.** Recent studies has indicated the protective role of a fat derived hormone, adiponectin in insulin resistance, diabetes and atherosclerosis and its role in obesity was also evident as its levels are negatively related to BM(51)I. The levels of Adiponectin and its subsequent receptors were found to be low in current plasma adiponectin studies(52). In concurrence with this, we have also observed low levels (8 fold low) of Adiponectin in HFD group as compared to normal group, however the levels of Adiponectin found towards normal levels in TT-HA, TT-EA treated in dose dependent manner.

Studies has proved that leptin, a hormone secreted by body's fat cells controls the activity of NPY/AgRP and POMC neurons in the ARC nucleus to regulate energy balance and regulating food intake and so is also named as satiety hormone(36, 53). It is found from various studies that an obese person has high levels of leptin resulting in Leptin resistance thereby disturbing the channel with hypothalamus to regulate food intake resulting in more weight gain(54, 55). In accordance with this, we have also found that the levels of leptin are 9-fold more in HFD group as compared to normal group. The animals treated with TT-HA, TT-EA found to have lower levels of leptin in dose dependent manner as compared to HFD group.

CONCLUSION:

From present study, it can be concluded that Tribulus terrestris may proves to be a beneficial therapeutic strategy in obesity especially at the dose of 600 mg/kg due to modulation of anthropometric parameters, enhancement of ghrelin and adiponectin levels, attenuation of leptin levels, glycemic and lipid profile improvement and potent antioxidant activity. However, further studies are required to study the detailed mechanism of action.

Table 1: Quantification of bioactive constituent in extracts

Sr. No	Sample	RUTIN TRHYDRATE	QUERCETIN
1.	STANDARD	95%	95%
2.	TT-EA	7.64%	0.042
3.	TT-HA	1.27%	--

Table 2: Effect of *Tribulus terrestris* hydroalcoholic extract (TT-HA) and *Tribulus terrestris* Ethylacetate extract (TT-EA) on Lipid profile in high fat diet inducted obesity

GROUPS	LIPID PROFILE									
	TOTAL CHOLESTEROL		HDL		LDL		TG		VLDL	
	0 DAY	90 DAYS	0 DAY	90 DAYS	0 DAY	90 DAYS	0 DAY	90 DAYS	0 DAY	90 DAYS
CONTROL	148.88 ±0.02	149.27±0.05	29.71±1.16	30.79±0.95	18.163 ±0.45	19.818±1.34 4	74.05±0.0 1	76.88±0.01	14.81±0	15.38±0
HFD	152.58 ±3.07	460.88± 0.11@***	28.4±0.82	9.51±1.46@** *	21.577 ±1.848	43.742±1.26 3@***	72.5±0.6	136.8±0.45@ ***	14.5±0.12	27.36±0.09@ ***
ORL+HFD	148.71 ±0.15	168.82±0.09 @***	28.71±1.14	26.09±1.28@ **$***	19.04± 0.801	22.13±1.169 $***	76.07±0.2 8***	81.07±0.67@ $***	15.21±0.06***	16.21±0.13@ $***
HFD+TT-EA 150mg/kg	149.51 ±1.73	352.67±1.86 @#$***	29.42±0.3	13.35±0.49@ ***#$***	23.408 ±1.71	56.617±1.12 @#$***	74.51±0.7	174.69±0.45 @#$***	14.9±0.14	34.94±0.09@ #$***
HFD+TT-EA300mg/kg	150.36 ±1.59	292.52±0.9@ #$***	29.9±0.22	18.33±0.71@ #$***	22.444 ±1.694	46.768±1.18 5@#$***	74.86±0.3 2	156.97±0.51 @#$***	14.97±0.06	31.39±0.1@#$ ***
HFD+TT-EA600mg/kg	148.15 ±0.47	166.5±0.91@ $***	30.23±0.46	20.83±0.54@ #$***	21.974 ±1.681	23.193±0.46 2****	73.5±0.33	131.29±0.38 @#***	14.7±0.07	26.26±0.08@ #$***
HFD+TT-HA150mg/kg	144.32 ±0.62	351.58±1.02 @#$***	29.64±0.21	15.67±0.35@ #$***	23.357 ±1.688	56.618±1.07 9@#$***	73.86±0.7 7	167±0.46@#$ ***	14.77±0.15	33.4±0.09@#$ ***
HFD+TT-HA300mg/kg	140.77 ±0.57***	290.97±0.64 @#$***	30.01±0.22	19.87±0.33@ #$***	22.662 ±1.733	46.913±1.24 3@#$***	72.58±0.6 8$**	150.63±0.59 @#$***	14.52±0.14$**	30.13±0.12@ #$***
HFD+TT-HA600mg/kg	142.48 ±1.03#*	166.66±0.48 @#***	30.29±0.47	26.51±0.25@ ***	21.933 ±1.668	26.088±0.69 9@*#***	73.88±0.7 3	123.17±0.6@ #$***	14.78±0.15	24.63±0.12@ #$***

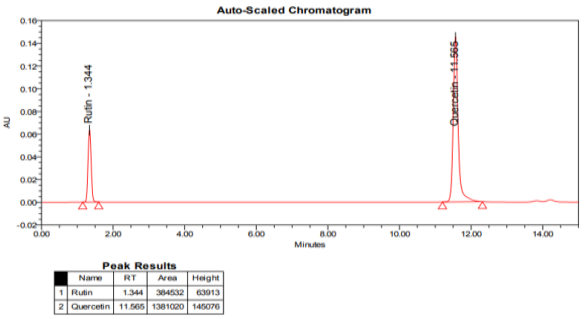

Figure 2: HPLC chromatogram for standard rutin and quercetin

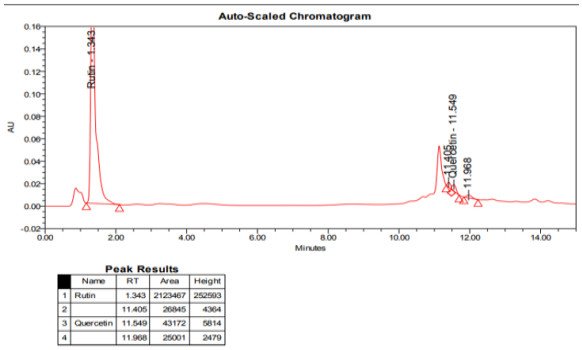

Figure 3: HPLC chromatogram for TT-EA

Table 3: Effect of Tribulus terrestris hydroalcoholic extract (TT-HA) and Tribulus terrestris Ethylacetate extract (TT-EA) on Anthropometric parameters high fat diet inducted obesity in Wistar rats

GROUPS	ANTHROPOMETRIC PARAMETERS					
	BODY WEIGHT		WAIST HIP RATIO		OBESITY INDEX	
	0 DAY	90 DAYS	0 DAY	90 DAYS	0 DAY	90 DAYS
CONTROL	181.76±2.29	213.97±3.01	0.42±0.03	0.53±0.01	215.968±0.058	217.007±0.054
HFD	184.33±1.2	372.82±2.63@***	0.4±0.02	1.18±0.06@***	219.795±0.074@***	257.625±1.103@***
ORL+HFD	187.83±0.31@*	226.55±2.35@***$***	0.38±0.02	0.74±0.01@***	214.694±0.073@*$***	225.582±0.212@$***
HFD+TT-EA 150mg/kg	183±0.45	358.88±2.52@***$*$***	0.36±0.03	0.67±0.02@**$***	214.622±0.041@*$***	213.394±0.071@#$***
HFD+TT-EA300mg/kg	183.67±0.95	344±4.13@#$***	0.4±0.02	0.54±0.02#$***	213.948±0.005@#$***	210.356±0.063@#$***
HFD+TT-EA600mg/kg	182.67±0.99	311.31±4.24@#$***	0.34±0.03	0.45±0.01#$***	212.68±0.065@#$***	206.663±0.049@#$***
HFD+TT-HA150mg/kg	185±0.86	343.56±3.01@#$***	0.39±0.05	0.63±0.01@*$***$*	214.526±0.522@**$***	155.465±0.053@#$***
HFD+TT-HA300mg/kg	185.17±1.01	311.84±3.22@#$***	0.4±0.03	0.52±0.01#$***	213.684±0.62@#$***	114.38±0.045@#$***
HFD+TT-HA600mg/kg	184.67±1.15	274.33±3.61@#$***	0.37±0.02	0.41±0.01@**#$***$***	214.236±0.007@#$***	101.554±0.1@#$***

Table 4: Effect of *Tribulus terrestris* hydroalcoholic extract (TT-HA) and *Tribulus terrestris* Ethylacetate extract (TT-EA) on Glycemic profile high fat diet inducted obesity in Wistar rats

GROUPS	GLYCEMIC INDEX			
	GLUCOSE		INSULIN	
	0 DAY	90 DAYS	0 DAY	90 DAYS
CONTROL	61.33±2.95	65.61±1.85	5.2±0.02	6.99±0.25
HFD	61.22±3.48	133.9±1.98@***	5.54±0.12	10.36±0.75@**
ORL+HFD	62.82±2.48	68.08±1.9#***	5.39±0.09	5.47±0.36#***
HFD+TT-EA 150mg/kg	64.17±2.56	112.89±1.38@#$***	5.74±0.26	5.84±0.39#***
HFD+TT-EA300mg/kg	59±3.46	89.45±1.47@#$***	5.5±0.14	5.63±0.44#***
HFD+TT-EA600mg/kg	63.54±2.81	74.38±2.7#***	5.28±0.07	5.53±0.37#***
HFD+TT-HA150mg/kg	61.67±3.07	111.48±2.5@#$***	5.53±0.13	6.33±0.79#***
HFD+TT-HA300mg/kg	59.1±3.42	94.11±2.74@#$***	5.48±0.16	6.15±0.66#***
HFD+TT-HA600mg/kg	64.32±2.59	76.28±2.41@*#***	5.49±0.16	5.85±0.45#***

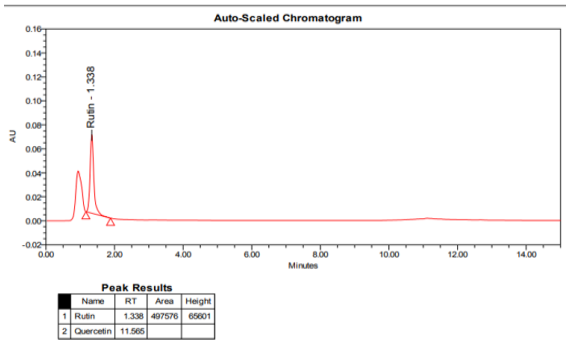

Figure 4: HPLC chromatogram for TT-HA

Table 5: Effect of Tribulus terrestris hydroalcoholic extract (TT-HA) and Tribulus terrestris Ethylacetate extract (TT-EA) on liver function parameters high fat diet inducted obesity in Wistar rats

GROUPS	LIVER FUNCTION TEST			
	SGOT		SGPT	
	0 DAY	90 DAYS	0 DAY	90 DAYS
CONTROL	38.07±0.01	36.53±0.05	43.7±0.19	42.06±1.11
HFD	38.21±0.56	68.64±0.86@***	44.21±1.02	76.91±2.54@***
ORL+HFD	41.72±0.11@**#**	30.55±0.29@***#***	41.87±0.14	46.94±3.49#***
HFD+TT-EA 150mg/kg	39.88±0.29	57.9±0.61@#$***	46.3±0.29$**	80.7±0.35@$***
HFD+TT-EA300mg/kg	37.15±0.17$***	49.99±0.42@#$***	44.98±0.04	59.57±0.36@#$***
HFD+TT-EA600mg/kg	38.26±0.78$*	38.51±0.89@*#***	43.53±0.09	45.8±0.73#***
HFD+TT-HA150mg/kg	38.96±1.1	57.83±0.55@#$***	45.37±0.75	67.72±0.54@#$***
HFD+TT-HA300mg/kg	36.48±0.51$***	51.16±0.5@#$***	44.57±0.75	60.88±0.58@#$***
HFD+TT-HA600mg/kg	37.96±0.28$**	36.61±1.6@*#***	42.88±0.56	45.98±0.69#***

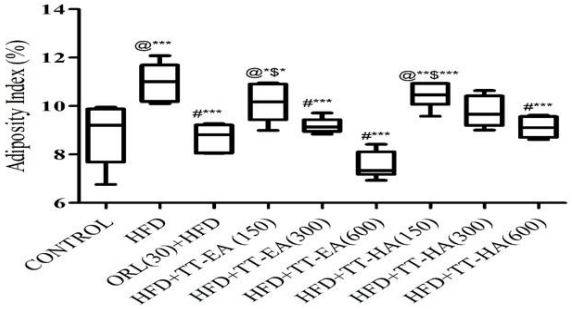

Figure 5: Effect of *Tribulus terrestris* hydroalcoholic extract(TT-HA) and Tribulus

terrestris Ethylacetate extract(TT-EA) on Adiposity index high fat diet inducted obesity in Wistar rats. Data are expressed as mean± S.E.M. Data are expressed as p<0.05 denoted by *, p<0.01 denoted by **, p<0.001

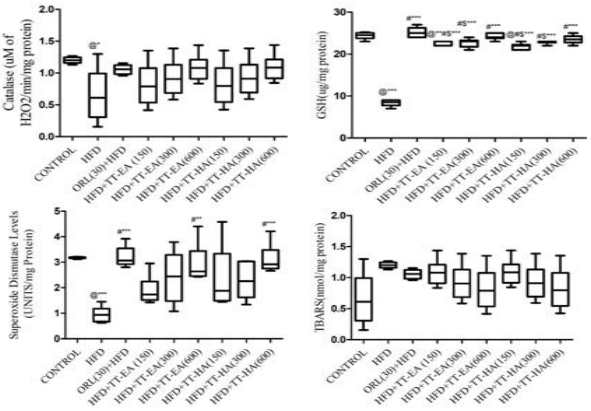

Figure 6: Effect of Tribulus terrestris hydroalcoholic extract (TT-HA) and *Tribulus terrestris* Ethylacetate extract (TT-EA) on oxidative stress enzymes and TBARS high fat diet inducted obesity in Wistar rats. Data are expressed as mean± S.E.M. Data are expressed as p<0.05 denoted by *, p<0.01 denoted by **, p<0.001 denoted by ***.@ denotes vs. Control; # denotes vs HFD, $ denotes vs ORL-HFD. HFD=High fat diet, ORL=Orlistat, TT-HA=*Tribulus terrestris* hydroalcoholic TT-EA= *Tribulus terrestris* ethylacetate

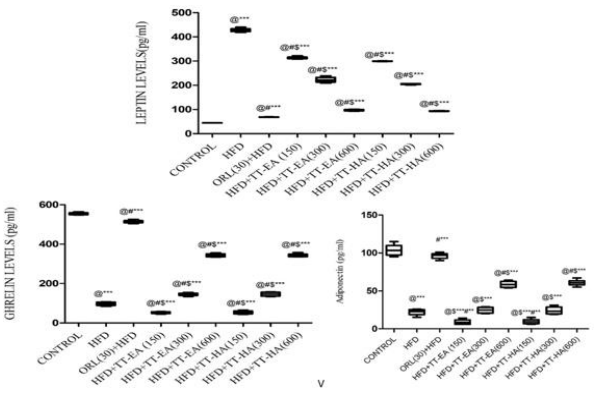

Figure 7: Effect of *Tribulus terrestris* hydroalcoholic extract (TT-HA) and *Tribulus terrestris* Ethylacetate extract (TT-EA) on Ghrelin, Leptin and adiponectin TBARS high fat diet inducted obesity in Wistar rats. Data are expressed as mean ± S.E.M. Data are expressed as p<0.05 denoted by *, p<0.01 denoted by **, p<0.001 denoted by ***. @ denotes vs. Control; # denotes vs HFD, $ denotes vs ORL-HFD. HFD=High fat diet, ORL=Orlistat, TT-HA=*Tribulus terrestris* hydroalcoholic TT-EA= *Tribulus terrestris* ethylacetate

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