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PHARMACOLOGICAL EVALUATION FOR ANTI-OBESITY ACTIVITY OF THE CRUDE EXTRACTS OF *TAMARINDUS INDICA* (LEGUMINESAE) IN HIGH FAT DIET INDUCED OBESE RATS

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ABSTRACT

Normal and high fat diet induced obese rats were pre-treated with daily oral administration of 120 mg/kg of Orlistate, 250 mg/kg and 500 mg/kg of T. Indica fruit pulp extracts in 10 ml/kg of distilled water for 60 days. The effects of these drugs on % body weight change, waist hip ratio, adiposity index, obesity index serum glucose, triglyceride cholesterol and HDL-Cholesterol and oxidative stress were investigated. Treatment of Orlistat and fruit pulp of T. indica at dose of 250 mg/kg and 500 mg/kg significantly (P<0.05) attenuated the body weight, waist hip ratio, adiposity index, obesity index in comparison to high fat diet control group rats similarly serum glucose, triglyceride cholesterol and HDL-Cholesterol were found to be decreased as compared to HFD control group and also decrease oxidative stress.

Keywords: *Tamarindus indica*; Anti-obesity Plant; Obesity; Oxidative Stress; High Fat Diet

INTRODUCTION

Obesity is a multi-factorial, chronic disorder that has become a global epidemic [1].The etiology of obesity is multifactorial with genetic, environmental, socio-economical, behavioural and the psychological influences. It is a fundamental disorder of energy imbalance in which excessive energy stores accumulates with low energy expenditure[2].Obesity greatly increases the risk for heart disease, stroke, hypertension, hypercholesterolemia, Type-II diabetes, cancer, osteoarthritis, gallbladder disease, gallstones, fatty liver disease (also called nonalcoholic steatohepatitis or NASH), gastroesophageal reflux disease (GERD), gout and psychological and emotional effect[3,4].The complexity of obesity requires a multidisciplinary research approach that encompasses studies of behavioural, environmental, and biological perspectives. Use of herbal and alternative medicine has been increased since the last decade for the pharmacotherapy of obesity[5].

Tamarindus indica belongs to the family Leguminesae. It is a large tropical tree. It is cultivated and naturalized in the tropics throughout the world[6, 7].Tamarind fruit pulp has a sweet acidic taste due to a combination of high content of tartaric acid and reducing sugars [8].The pulp is widely consumed in many countries for seasoning, as a food component and in juices owing to its desirable taste. Its fruit is regarded traditionally as a digestive, carminative, laxative, expectorant and blood tonic[9].Oral administration of pulp extract of *T. indica* resulted in a dose dependent decrease in body weight of rats. Dose dependent decrease in body weight could also be attributed to the presence of anti-nutritional factors like saponins in the extract[10].The present study designed to investigate anti-obesity activity of fruit pulp extract of *T. indica* in high fat diet induced obesity in rats.

MATERIALS AND METHODS

(i) Collection of Plant Material

T. indica plant fruits were collected in the month of February 2012 from local area of Lucknow, Uttar Pradesh, India. These were identified and authenticated by Dr. Anand Prakash, Principal Scientist, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India. A voucher specimen No.216345has been deposited in the herbarium of NBRI. Fresh fruits were collected in bulk, washed under running tap water to remove adhering material, dried under shade and pulverized in a mechanical grinder. The coarse powder was passed through sieve no. 40 and used for further studies.

(ii) Preparation of extracts

Dried powders of fruits pulp (200gm) of *T. Indica* were placed in Soxhlet apparatus and extraction was carried out by using petroleum ether, chloroform and distilled water. The extracts were filtered, cooled and concentrated under reduced pressure at 40^0C.

(iii) Drug and chemicals

The standard drug Orlistat was purchased from Mankind Pharma. All others chemicals and diagnostic kits for glucose, cholesterol, triglycerides, and HDL cholesterol were purchased from Tranasia Bi-medical Ltd. All reagents used in this study were of analytical grade.

(iv) Experimental animal

Albino Wistar rats weight between 150 and 200 gm were used for experiments. The rats were obtained from animal house of M. M . College of Pharmacy, M. M. University Mullana, Ambalaand, Haryana, India in the month of March 2012. The experimental protocol used in the present study has been approved by Institutional Animal Ethical Committee (Registration: No. MMCP/IEC/11/03). The rats were maintained on commercially available rodent chow diet (Kisan Feeds Ltd., Chandigarh, India) and tap water ad libitum. The animals were housed in animal house at an ambient temperature between 22°C and 25 °C, humidity 55±5%, at group of five (n=5) per cage and were exposed to 12-h light and 12-h dark cycle. The animal care was as per guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India (Table No 1).

Table 1: Grouping of animal and drug treatment

Group of animal	
Group 1	Control (normal group)
Group 2	High fat diet (Positive control)
Group 3	High fat diet + pet.ether extract of *Tamarindus indica* (250 mg/kg/day/oral)
Group 4	High fat diet + pet.ether extract of *Tamarindus indica* (500 mg/kg/day/oral)
Group 5	High fat diet + chloroform extract of *Tamarindus indica* (250 mg/kg/day/oral)
Group 6	High fat diet + chloroform extract of *Tamarindus indica* (500 mg/kg/day/oral)
Group 7	High fat diet + aqueous extract of *Tamarindus indica* (250 mg/kg/day/oral)
Group 8	High fat diet + aqueous extract of *Tamarindus indica* (500 mg/kg/day/oral)
Group 9	High fat diet + standard drug Orlistat (120 mg/kg/day/oral)

(v) High fat diet (HFD) induced obesity

Obesity was induced by High-fat diet (30% fat by weight), which contained the same vitamin and mineral content as the control diet but was high in fat as well as refined sugar[11]. The HFD feed was prepared by mixing different constituents and was given to animals' everyday with water *ad libitum*. HFD was administered orally and weight gain was observed in rats by measuring their weight. Composition of the High fat diet (HFD) (g/kg) was according to the formula of Srinivasan[12]with some modifications as shown in (Table No. 2).

Table 2 : Composition of High Fat Diet (HFD)

Ingredients	Diet (g/kg)
Powdered NPD	375
Lard	290
Casein	265
Corn oil	10
Cholesterol	10
Vitamin and mineral mix	60
DI Methionine	03
Yeast Powder	01
Sodium Chloride	01

(vi) Measurement of Morphological Parameters

Body weight change was determined once a week up to 60 days with Mettler weighing balance (Mettler-Toledo model no XS200S, Mettler-Toledo Powai Mumbai, India).

$$\% \text{ Change in Body weight} = \frac{\text{Body weight on } 60^{th} \text{ day-body weight on } 1^{st} \text{ day X } 100}{\text{Body weight on } 1^{st} \text{ day}}$$

Waist Hip ratio was measured by the following formula

$$\text{Waist hip Ratio} = \frac{\text{Waist Circumference in inches}}{\text{Hip circumference in inches}}$$

Adiposity index was determined by total fat of body (Sum of the weights of peritoneal WAT, retroperitoneal WAT, and epididymal WAT) divided by body weight and multiplied by hundred[13,14].

Obesity index was determined, which is the cubic root of body weight in grams divided by naso-anal length in millimeters multiplied by 10^4[15].

$$\text{Obesity index} = \frac{\text{Cubic root of body weight in grams} \times 10^4}{\text{Naso - anal length in millimetres}}$$

(vii) Biochemical estimation

Blood samples were withdrawn in the morning after overnight fasting from eye. The blood samples were collected in centrifugal tube and centrifuged using Remi Laboratory (model RM-12C, REMI Sales & Engineering Ltd, New Delhi, India) at 4000 g for 15 min at 4^0C and serum was separated and analysed regularly for assessment of lipid profile including estimation of serum cholesterol level, serum HDL-cholesterol level, serum triglycerides level and serum glucose level and assayed using standard diagnostic test kits(Erba Diagnostics kit, Transasia Bio Medical Ltd. Daman, Gujarat, India) on U V Spectrophotometer (UV-1800f Shimadzu, Century instruments, Chandigarh, India).

(a) Estimation of total serum glucose level

Blood glucose level was estimated by using the Erba diagnostic kit based on Glucose Oxidase Peroxidase (GOD-POD) Method[16]using a commerically avilable kit

$$\text{Conc. of glucose (mg/dl)} = \frac{\text{Absorbance of test X Conc. of standard (mg/dl)}}{\text{Absorbance of standard}}$$

(b) Estimation of total serum cholesterol level

Total serum cholesterol was estimated by using the Erba diagnostic kit based on the enzymatic method described by modified Roeschlau's method[17].

$$\text{Serum cholesterol conc(mg\%)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{X Conc. of standard (mg/dl)}$$

(c) Estimation of serum high density lipoprotein cholesterol level

High density lipoprotein cholesterol was estimated by using the Erba diagnostic kit .This Phosphotungstate method was used as decribed by Burstein[18].

$$\text{Serum HDL conc. (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{X Conc. of standard } (\frac{\text{mg}}{\text{dl}}) \text{ X dilution factor}$$

(d) Estimation of total serum triglycerides level

Triglycerides level was estimated by using Erba Diagnostics kit. This GPO-Trinder method was described by Wako and modified by McGowan[19] and Fossati[20].

$$\text{Serum triglycerides conc.} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{X Conc. of standard } (\frac{\text{mg}}{\text{dl}})$$

(viii) Estimation of reduced glutathione

The reduced glutathione (GSH) content in serum was estimated using method of Beutler[21] and Singh[14,15].

(ix) Estimation of superoxide anion generation

The superoxide anion generation in serum was estimated in terms of measuring reduced nitroblue tetrazolium (NBT) [22,14,15].

$$\text{Amount of reduced NBT} = \frac{\text{A x V}}{\text{T x Wt x } \varepsilon \text{ x 1}}$$

(x) Estimation of lipid peroxidation

The total oxidative stress was assessed by measuring the lipid peroxidation product malondialdehyde in the serum using a colorimetric method based on that described in earlier studies [23, 14,15].

(xi) **Histopathological Analysis**

The Adipose tissue was excised and immediately immersed in 10% buffered formalin, dehydrated in graded concentrations of ethanol, immersed in xylene and then embedded in paraffin. The sections of 4 µm were cut and placed on slide using commercial Baker's mounting fluid. Paraffin wax was removed by warming the slide gently, until the wax melted and then was washed with xylene followed by washings with absolute alcohol and water to hydrate the sections and stained with haematoxylin-eosin and picrosirius red to determine Adipocyte diameter and collagen deposition, respectively stained slides were viewed using an optical photomicroscope (Model N—400ME, CEL-TECH Diagnostics, Hamburg, Germany) at 40× magnification.

(xii) Statistical Analysis

The results were expressed as mean ± S.E.M. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range test. The p-value < 0.05 was considered to be statistically significant.

RESULTS

(a)Effect on body weight, Waist Hip Ratio (WHR), obesity index and adiposity index

Table 3 shows that High fat diet (HFD) significantly increased body weight,Waist Hip Ratio (WHR), obesity index and adiposity index of HFD induced rats as compared to control group. Daily oral treatment with the graded oral dose of *T. Indica* fruit pulp extract (PE 250mg/kg, PE 500mg/kg, CE 250mg/kg, CE 500mg/kg, AE 250mg/kg, AE 500mg/kg) and 120 mg/kg Orlistat significantly ($p<0.05$) decreased body weight , Waist Hip Ratio (WHR),obesity index and adiposity index in dose dependent pattern as compared to high fat diet group (Figure 1-4).

Table 3: Effect of oral treatment with 250-500 mg/kg of *T. Indica* fruit pulp extract and 120 mg/kg Orlistat on % age Change in body Weight, WHR ratio, Obesity index and Adiposity index in rats

Group parameter	Control group	HFD control group	PEE 250 mg/kg	PEE 500 mg/kg	CE 250 mg/kg	CE 500 mg/kg	AE 250 mg/kg	AE 500 mg/kg	Orlistat 120 mg/kg
%age change in weight	16.432 ± 1.243	40.946 ± 2.020[a]	33.996 ± 0.827[b]	28.422 ± 0.853[b]	33.991 ± 0.394[b]	26.745 ± 0.962[b]	36.330 ± 2.312[b]	29.859 ± 2.813[b]	18.814 ± 1.570[b, c]
WHR ratio	0.894 ± 0.004	0.992 ± 0.005[a]	0.974 ± 0.004[b]	0.8986 ± 0.003[c]	0.951 ± 0.004[b]	0.892 ± 0.009[c]	0.940 ± 0.007[b]	0.903 ± 0.006[c]	0.855 ± 0.008[b]
Obesity index	120.159 ± 2.021	132.309 ± 1.594[a]	123.244 ± 1.427[b]	119.308 ± 2.030[b]	121.691 ± 1.088[b]	119.496 ± 1.221[b]	124.906 ± 2.228[b]	121.448 ± 1.199[b]	115.112 ± 0.932[b]
Adiposity index	2.476 ± 0.049	3.119 ± 0.086[a]	2.033 ± 0.056[b]	1.884 ± 0.070[b]	1.911 ± 0.029[b]	1.673 ± 0.025[c]	1.978 ± 0.016[b]	1.720 ± 0.037[c]	1.484 ± 0.037[b, c]

HFD=high fat diet, PEE= Petroleum Ether Extract, CE= Chloroform Extract, AE = Aqueous Extract. $p \leq 0.05$ is considered statistically significant.

[a] A significant increase at $p < 0.05$ when compared with Control group

[b] A significant decrease at $p < 0.05$ when compared with HFD Control group

[c] A significant decrease at $p < 0.05$ when compared with 250 mg/ kg

(b)Effect on cholesterol level, triglyceride level, glucose level and HDL-Cholesterol level

Table 4 shows that High fat diet (HFD) significantly increased cholesterol level, triglyceride level, glucose level of HFD induced rats as compared to control group. On treatment with the graded oral dose of T. *Indica* fruit pulp extracts and Orlistat significantly *(p<0.05)* decreased cholesterol level in dose dependent pattern as compared to high fat diet group. High fat diet (HFD) significantly decreased HDL-cholesterol level of HFD induced rats as compared to control group. On treatment with the fruit pulp extracts and Orlistat significantly *(p<0.05)* increased HDL-cholesterol level in dose dependent pattern as compared to high fat diet group. (Figure 5-8).

Table 2: Effect of oral treatment with 250-500 mg/kg of *T. Indica* fruit pulp extract and 120 mg/kg Orlistat on serum cholesterol, triglycerides HDL and glucose level in rats

Group parameters	Control group	HFD control group	PEE 250 mg/kg	PEE 500 mg/kg	CE 250 mg/kg	CE 500 mg/kg	AE 250 mg/kg	AE 500 mg /kg	Orlistat 120 mg/kg
Cholesterol (mg/dl)	99.2 ± 4.460	145.6 ± 5.657[a]	110.4 ±11.653[b]	105.6 ± 5.657[b]	94.4 ±10.886[b]	92.8 ±5.342[b]	108.8 ±6.771[b]	107.2 ±7.946[b]	81.6 ± 4.832[b]
Triglyceride (mg/dl)	88.013 ± 3.186	133.714 ± 4.356[a]	94.857 ± 7.804[b]	77.714 ± 4.836[b]	70.857 ± 7.516[b]	68.571 ±6.302[b]	84.571 ±5.637[b]	82.285 ±6.052[b]	66.285 ± 3.515[b]
HDL-C (mg/dl)	43.207 ± 0.692	38.743 ± 0.797[c]	39.680 ± 0.591[d]	39.36 ± 1.335[d]	40.284 ± 0.650[d]	41.347 ±0.620[d]	39.786 ±0.591[d]	39.884 ±0.904[d]	40.724 ± 1.108[d]
Glucose (mg/dl)	75.833 ± 5.669	117.5 ± 3.965[a]	62.5 ± 6.030[b]	65.07 ± 3.176[b]	58.333 ± 7.885[b]	59.166 ±3.657[b]	64.67 ± 2.323	61.66 ±1.993[b]	53.33 ± 2.945[b]

HFD=high fat diet, PEE= Petroleum Ether Extract, CE= Chloroform Extract, AE = Aqueous Extract. $p \leq 0.05$ is considered statistically significant.

[a] A significant increase at $p < 0.05$ when compared with Control group

[b] A significant decrease at $p < 0.05$ when compared with HFD Control group

[c] A significant decrease at $p < 0.05$ when compared with Control group

[d] A significant increase at $p < 0.05$ when compared with HFD Control group

(c) Effect on reduced lipid peroxidation oxidative stress (TBARS)

The lipid peroxidation was measured in terms of TBARS. High fat diet (HFD) significantly ($P<0.05$) increased level of TBARS HFD induced rats as compared to control group. Treatment with *T. Indica* fruit pulp extracts and Orlistat (120 mg/kg) significantly decreased level of TBARS as compared to high fat diet group (Figure 9).

(d)Effect on reduced glutathione oxidative stress

HFD significantly ($P<0.05$) increased level of reduced glutathione HFD induced rats as compared to control group. Treatment with *T. Indica* fruit pulp extracts and Orlistat (120 mg/kg) significantly decreased level of reduced glutathione as compared to high fat diet group (Figure 10).

(e)Effect on adipose tissue

Figures 11-19 are representative section of control, high fat diet, orlistate and 250-500 mg/kg*T. Indica* fruit pulp extracts treated rat adipose tissue respectively. Figure 12 has shown that adipocyte diameter is clearly reflected high in the high fat diet rats when compared with the control group. Oral Treatment with petroleum ether extract 250-500 mg/kg of *T. indica* shows decreased diameter of adipocyte index (Figure 13 and 14) when compared with the high fat diet group rats. Oral Treatment with chloroform extract 250 and 500 mg/kg of *T. indica* also decreased the diameter of adipocyte index (Figure 15 and 16) when compared with the high fat diet group. Treatment with aqueous extract 250mg/kg and 500mg/kg of *T. indica* produced more significant decrease in adipocyte diameter (Figure 17 and 18) when compared with the chloroform extract of *T. indica* and high fat diet group of rats. Treatment with Orlistat produced more significant decrease in diameter of adipocyte (Figure 19) when compared with petroleum ether extract, chloroform extract, aqueous extract of *T. indica* and high fat diet group.

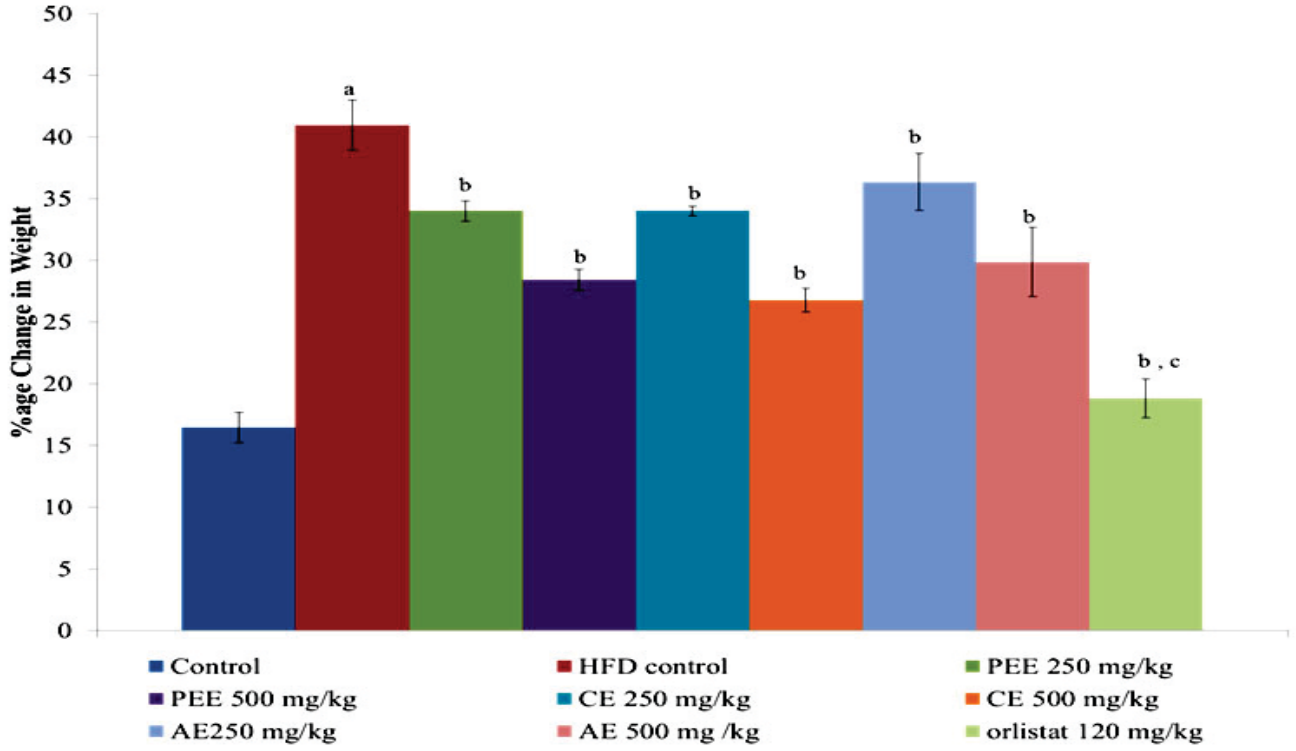

Figure 1: Effect of oral treatment with 250-500 mg/kg of *Tamarindus Indica* extract and 120 mg/kg Orlistat on % age Change in body Weight. HFD =high fat diet, PEE = Petroleum Ether Extract, CE = Chloroform Extract, AE =Aqueous Extract. p≤0.05 is considered statistically significant. a: vs. control: b: vs. HFD: c: vs. 250 mg/kg.

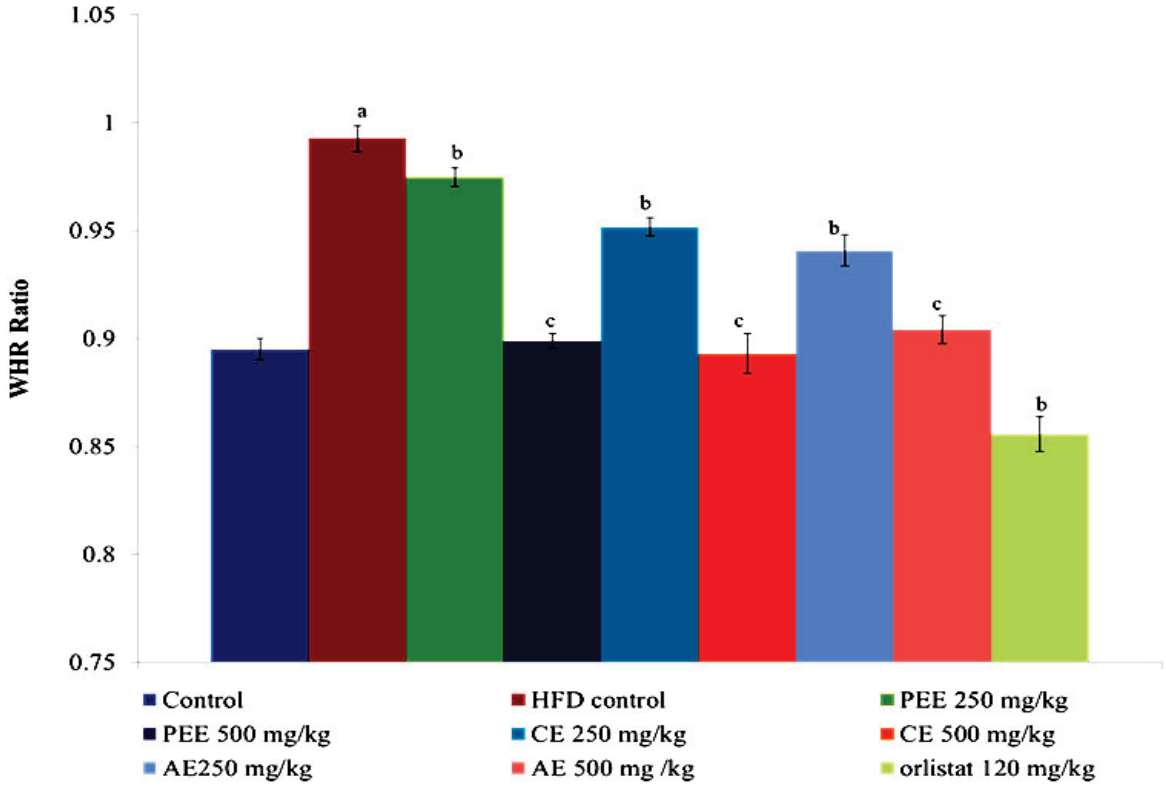

Figure 2: Effect of oral treatment with 250-500 mg/kg of *Tamarindus Indica* extract and 120 mg/kg Orlistat on WHR Ratio. HFD =high fat diet, PEE = Petroleum Ether Extract, CE = Chloroform Extract, AE =Aqueous Extract. p≤0.05 is considered statistically significant. a: vs. control: b: vs. HFD: c: vs. 250 mg/kg.

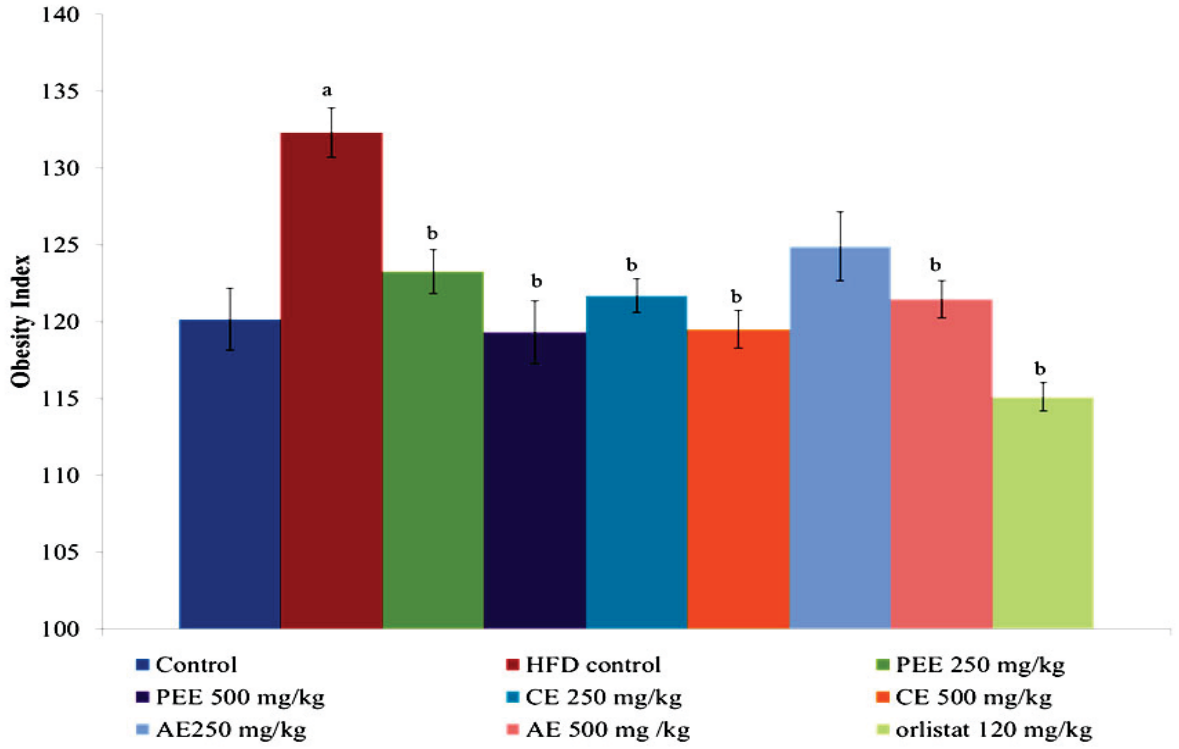

Figure 3: Effect of oral treatment with 250-500 mg/kg of *Tamarindus Indica* extract and 120 mg/kg Orlistat on obesity index Ratio. HFD =high fat diet, PEE = Petroleum Ether Extract, CE = Chloroform Extract, AE =Aqueous Extract. p≤ 0.05 is considered statistically significant. a: vs. control: b: vs. HFD: c: vs. 250 mg/kg.

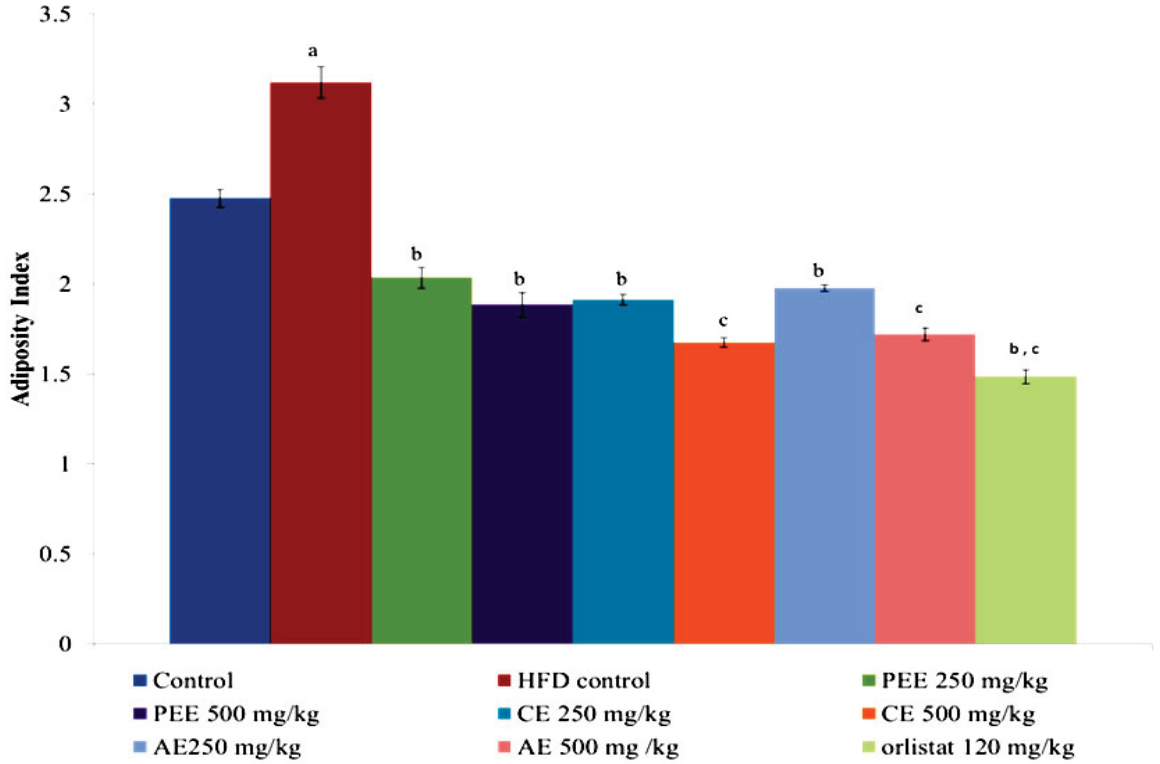

Figure 4 : Effect of oral treatment with 250-500 mg/kg of *Tamarindus Indica* extract and 120 mg/kg Orlistat on Adiposity index Ratio. HFD =high fat diet, PEE = Petroleum Ether Extract, CE = Chloroform Extract, AE =Aqueous Extract. p≤0.05 is considered statistically significant. a: vs. control: b: vs. HFD: c: vs. 250 mg/kg.

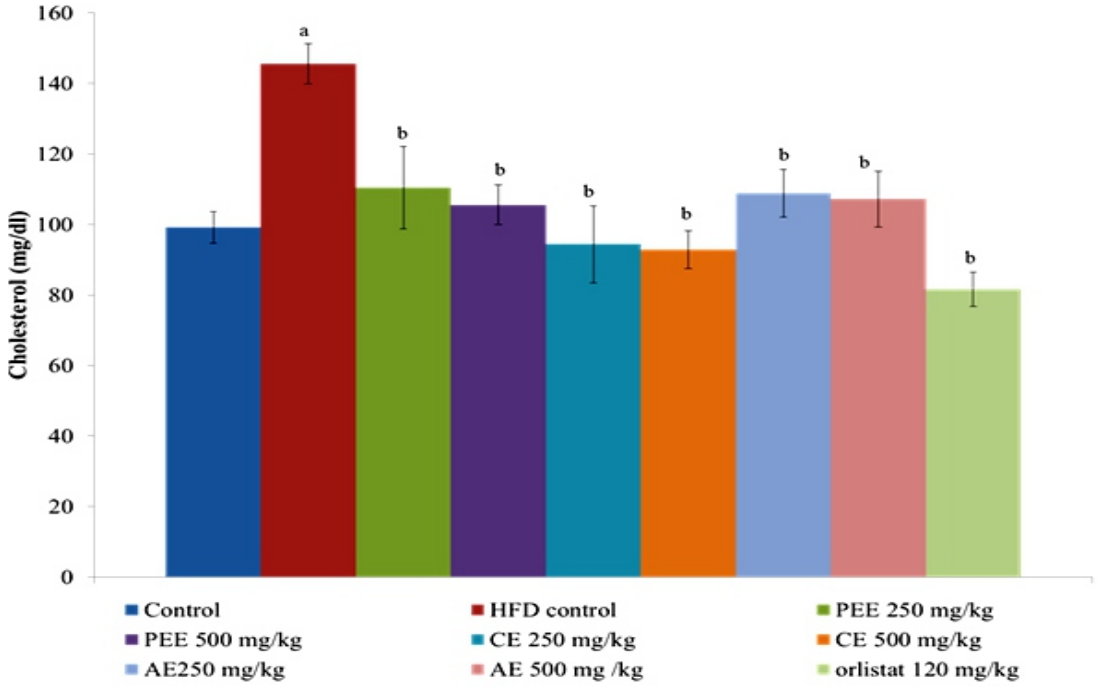

Figure 5 : Effect of oral treatment with 250-500 mg/kg of *Tamarindus Indica* extract and 120 mg/kg Orlistat on Cholesterol level. HFD =high fat diet, PEE = Petroleum Ether Extract, CE = Chloroform Extract, AE =Aqueous Extract. $p \leq 0.05$ is considered statistically significant. a: vs. control: b: vs. HFD: c: vs. 250 mg/kg.

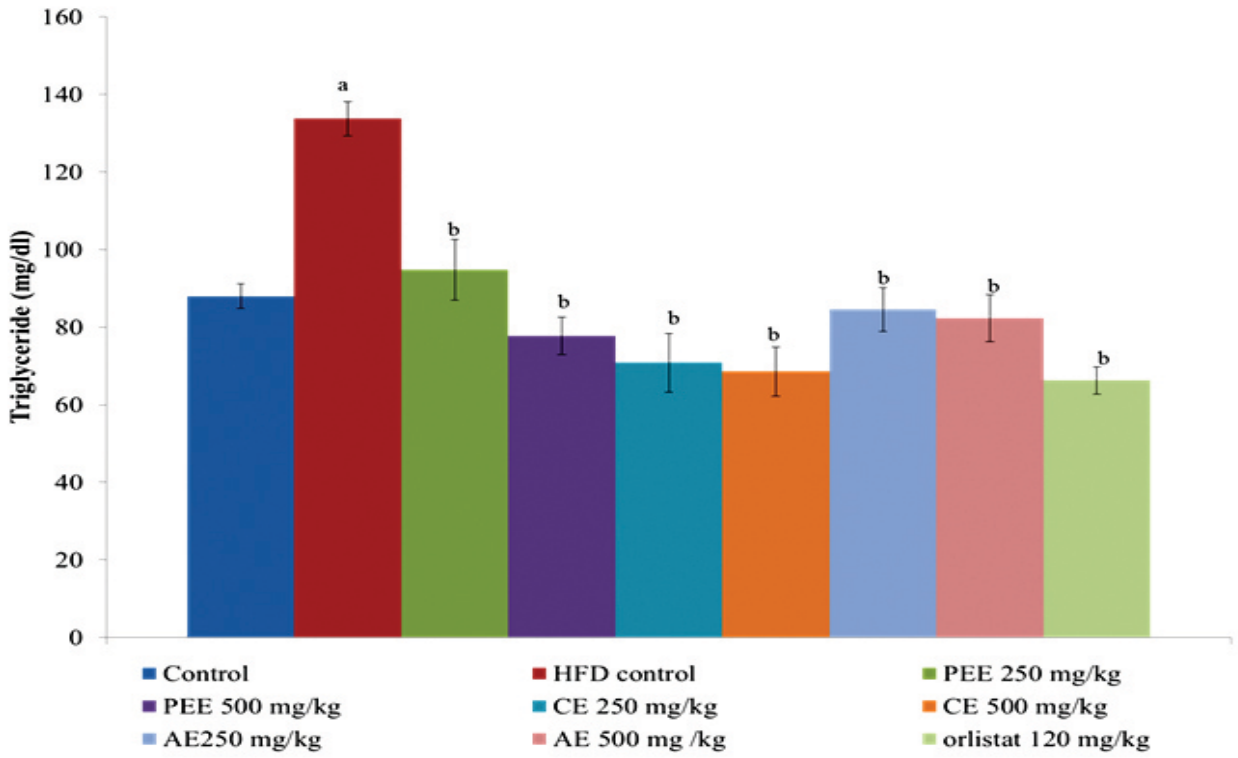

Figure 6: Effect of oral treatment with 250-500 mg/kg of *Tamarindus Indica* extract and 120 mg/kg Orlistat on Triglyceride level. HFD =high fat diet, PEE = Petroleum Ether Extract, CE = Chloroform Extract, AE =Aqueous Extract. $p \leq 0.05$ is considered statistically significant. a: vs. control: b: vs. HFD: c: vs. 250 mg/kg.

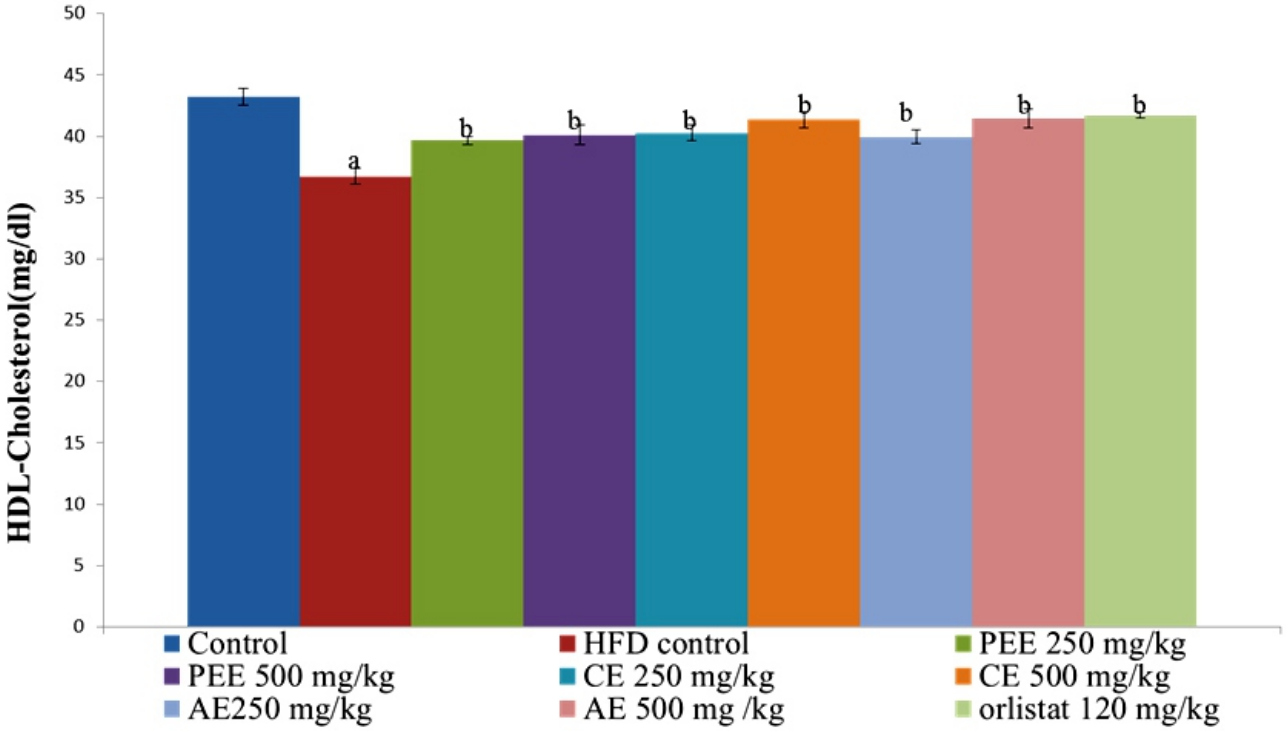

Figure 7 : Effect of oral treatment with 250-500 mg/kg of *Tamarindus Indica* extract and 120 mg/kg Orlistat on HDL-Cholesterol level. HFD =high fat diet, PEE = Petroleum Ether Extract, CE = Chloroform Extract, AE =Aqueous Extract. p≤0.05 is considered statistically significant. a: vs. control, b: vs. HFD

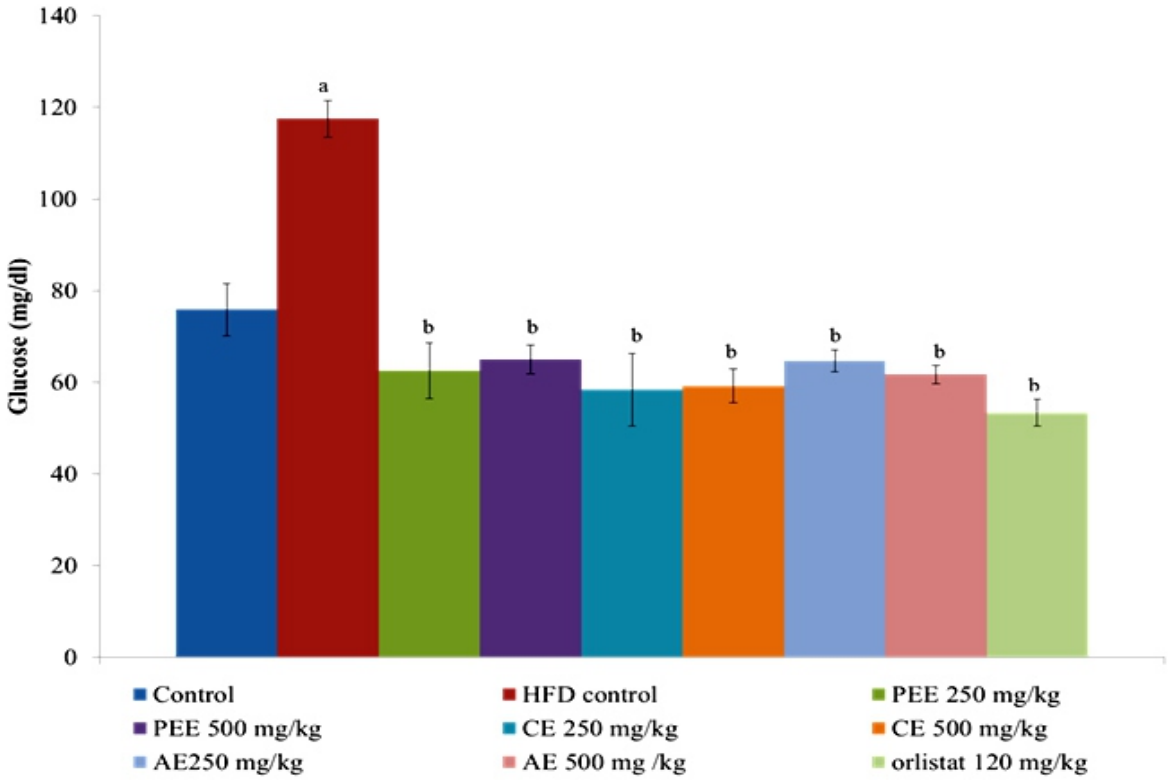

Figure 8 : Effect of oral treatment with 250-500 mg/kg of *Tamarindus Indica* extract and 120 mg/kg Orlistat on glucose level. HFD =high fat diet, PEE = Petroleum Ether Extract, CE = Chloroform Extract, AE =Aqueous Extract. p≤0.05 is considered statistically significant. a: vs. control: b: vs. HFD: c: vs. 250 mg/kg.

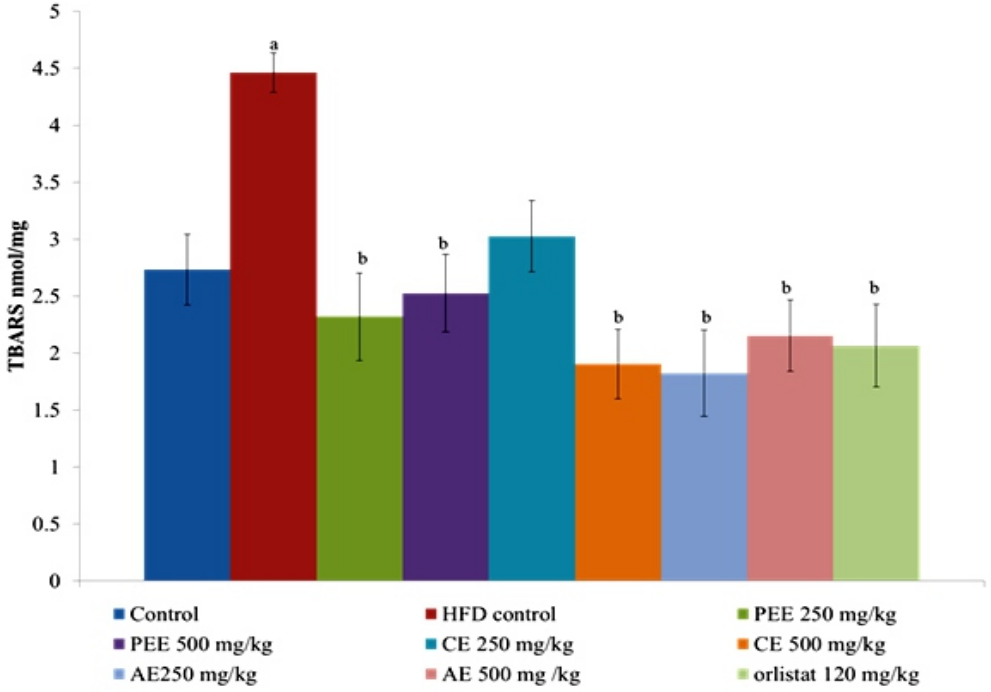

Figure 9 : Effect of oral treatment with 250-500 mg/kg of *Tamarindus Indica* extract and 120 mg/kg Orlistat on TBARS concentration. HFD =high fat diet, PEE = Petroleum Ether Extract, CE = Chloroform Extract, AE =Aqueous Extract. $p \leq 0.05$ is considered statistically significant. a: vs. control: b: vs. HFD: c: vs. 250 mg/kg.

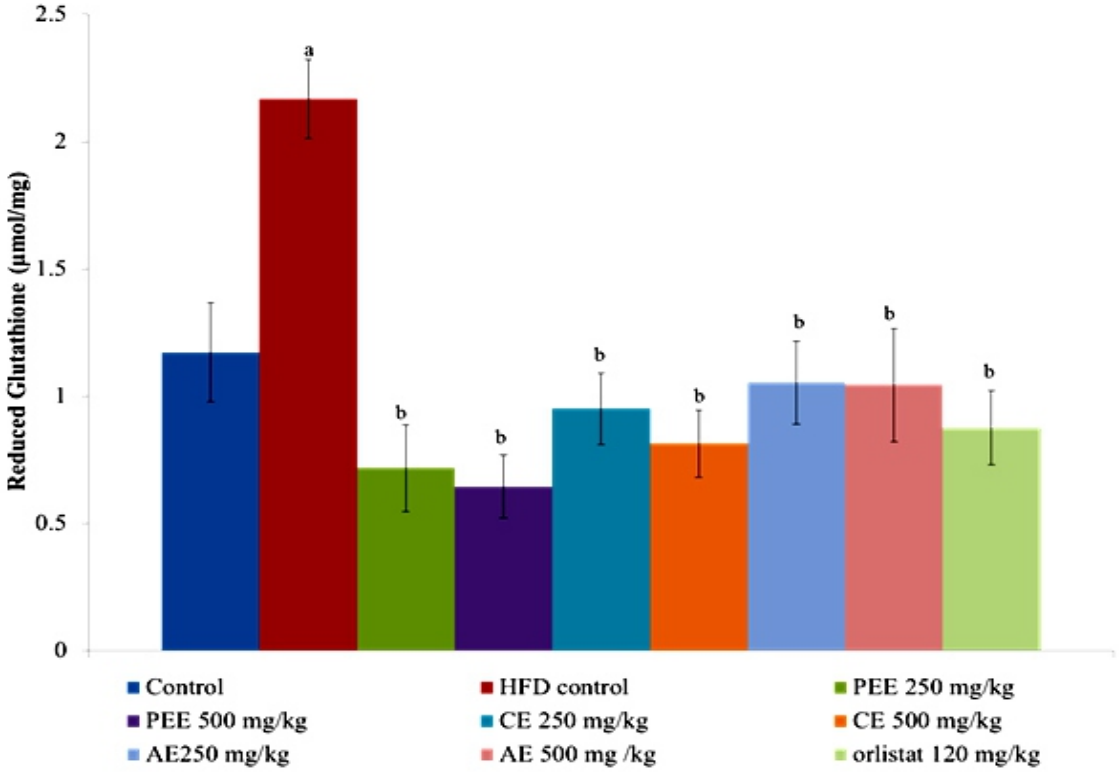

Figure 10: Effect of oral treatment with 250-500 mg/kg of *Tamarindus Indica* extract and 120 mg/kg Orlistat on reduced glutathione concentration. HFD =high fat diet, PEE = Petroleum Ether Extract, CE = Chloroform Extract, AE =Aqueous Extract. $p \leq 0.05$ is considered statistically significant. a: vs. control: b: vs. HFD: c: vs. 250 mg/kg.

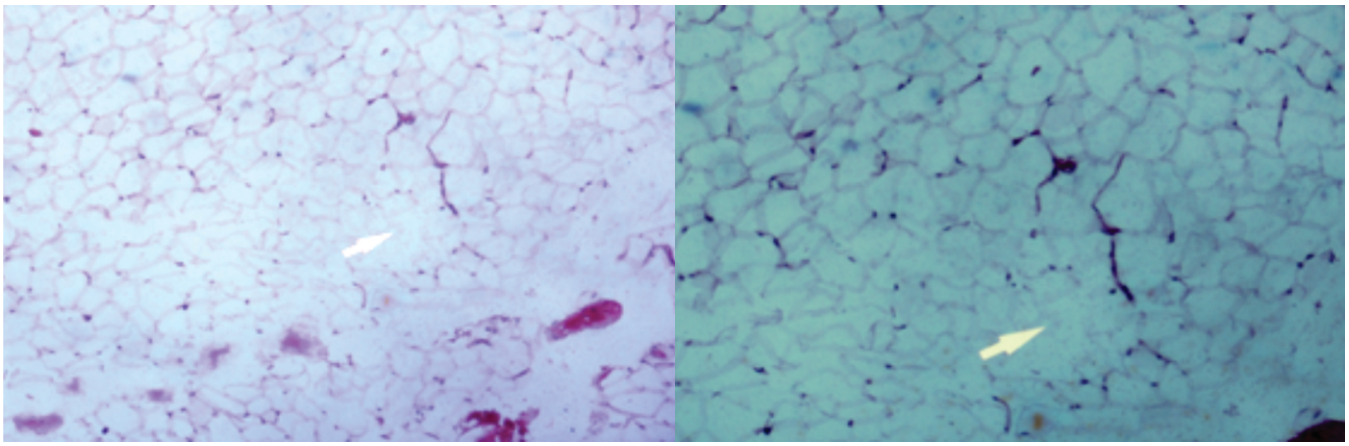

Figure 11 : Control Group

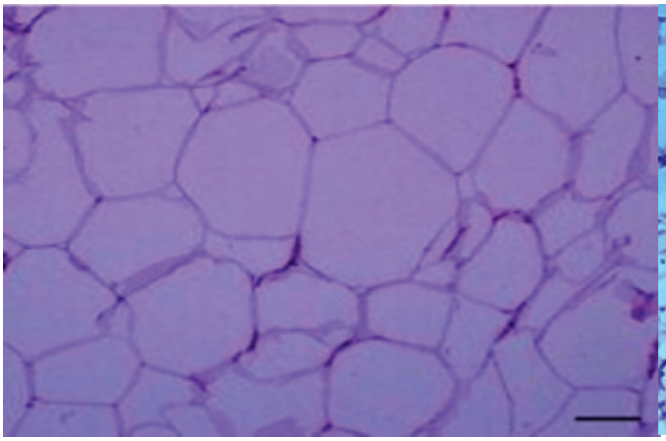

Figure 12 : HFD Group

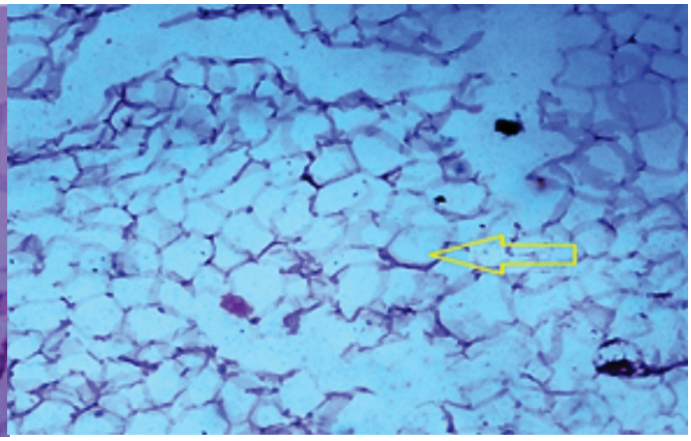

Figure 13: P. E.E. 250 mg/kg Group

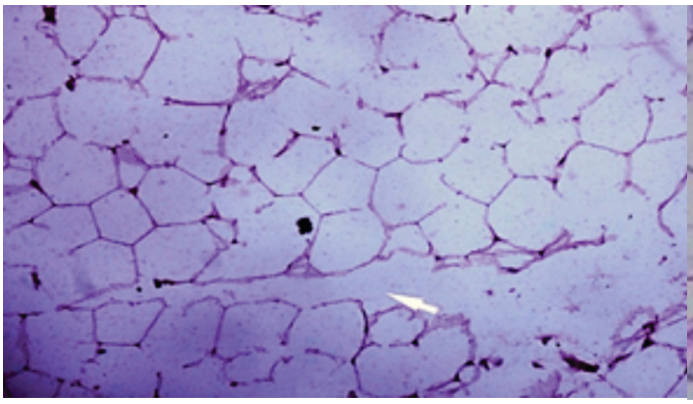

Figure 14 : P. E.E. 500 mg/kg Group

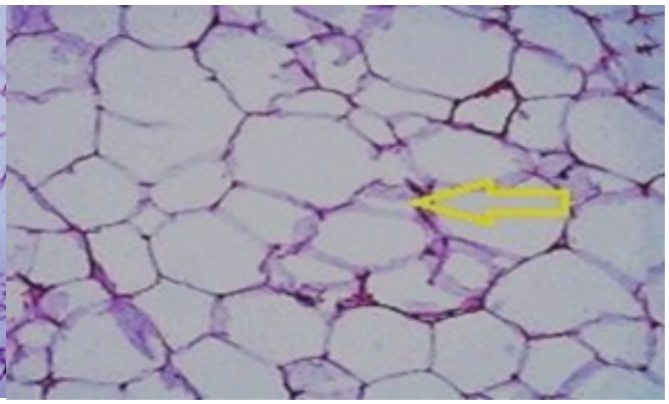

Figure 15: C.E. 250 Mg/Kg Group

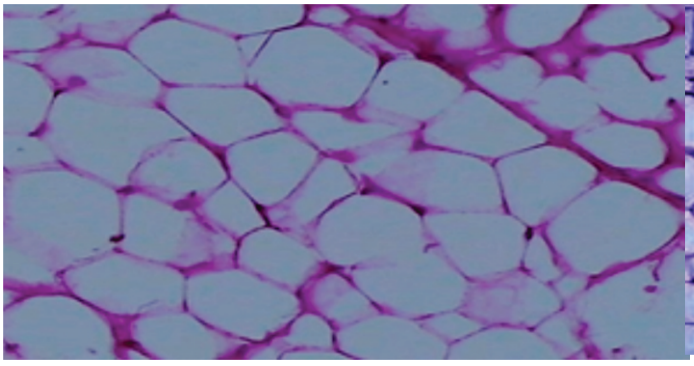

Figure 16 : C.E.500 Mg/Kg Group

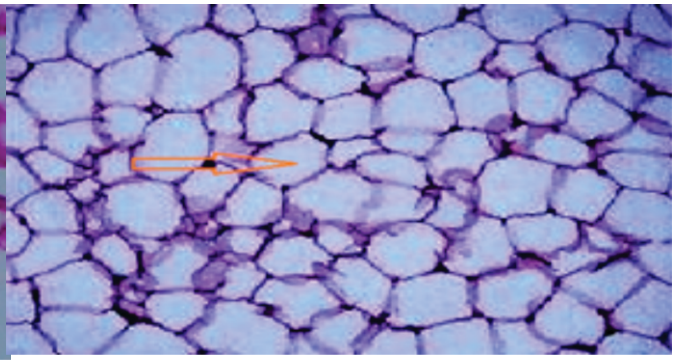

Figure 17 : Aqueous 250 Mg/Kg Group

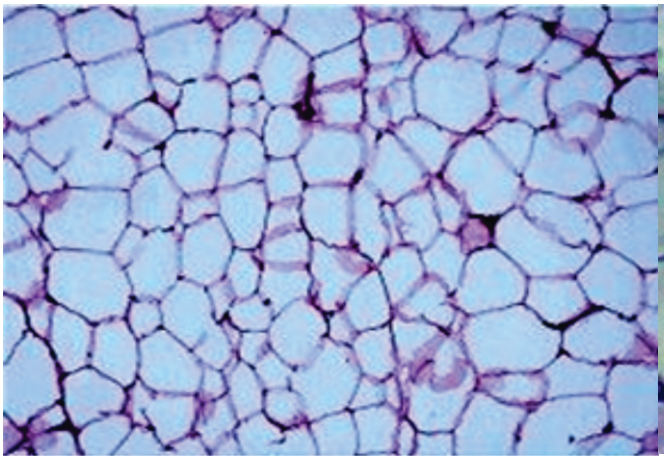

Figure18 : Aqueous 250 Mg/Kg Group

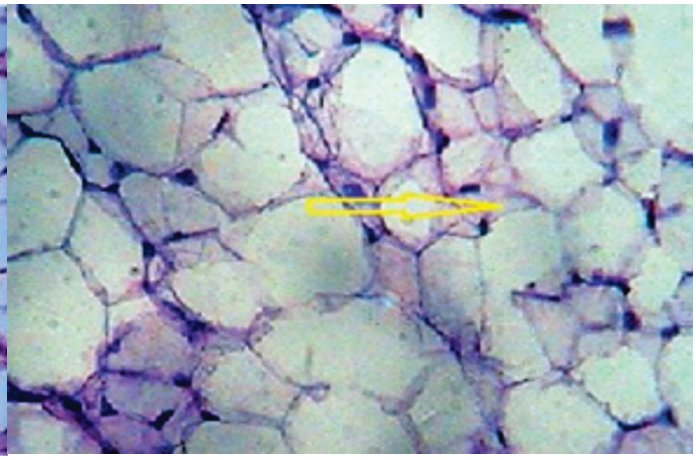

Figure 19 : Orlistat 120 mg/kg Group

Figure 11-19 : Effect of *Tamarindus Indica* P.E.E., C.E., Aqueous (250mg/kg-500mg/kg) extracts and Orlistate on Adipose tissue of High Fat Diet induced Rats (40x Magnification, haematoxylin and eosin stain)

DISCUSSION

Weight gain and obesity are result of positive energy balance due to a mismatch between energy intake (EI) and energy expenditure (EE). The EI in turn is subject to a wide range of influences, including appetite, gastro-intestinal signals such as distension of the stomach, chemical signals to the gastric mucosa and blood-borne metabolites such as glucose and fatty acid[24,25]. The prevalence of obesity is rapidly rising. Rats fed with a lard-based high fat diet (HFD) showed distinctive visceral adiposity, hyperglycemia, dyslipidemia and oxidative stress which are typically associated with human obesity [26]. Obesity may result due to increased adipose mass [27]. In the present study, high fat diet (HFD) for 60 days was used to produce obesity in Wistar rats. Obesity is also associated with an unfavourable lipid profile or dyslipidaemia is well documented in various studies [28]. Lipid abnormalities related to obesity include an elevated serum concentration of fatty acids, total cholesterol (TC), low-density-lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol and triglycerides (TG), as well as a reduction in serum high-density lipoprotein (HDL) cholesterol [29]. Study shows there was significant increase in serum glucose level in the HFD fed rats as compared to normal diet fed rats. Therefore, serum lipid levels (total cholesterol, HDL and triglycerides) and glucose levels were estimated as the marker of hyperlipidemia and hyperglycemia. Consumption of HFD also contributes to excessive formation of Reactive oxygen species (ROS) which leads to induced oxidative stress [14]. We determined oxidative stress by measuring the level of reduced glutathione (GSH), reduced lipid peroxidation oxidative stress (TBARS) and superoxide anion generation reduced nitroblue tetrazolium (NBT) and found that HFD feeding cause decrease in GSH, TBARS and increase in NBT levels as compared to normal control group. Anti-obesity effect of petroleum ether chloroform and aqueous extracts of *T. indica* fruit pulp was investigated using a HFD-induced obese rat model. Tamarind fruit pulp has a sweet acidic taste due to a combination of high contents of tartaric acid and reducing sugars [8]. Supplementation of obese rats with *T. indica* extract at 250 and 500 mg/kg conversely causes a remarkable reduction of body weights compared to the positive control group and significantly different with that of the normal control group. It has been suggested that the high digestible starch and acidic content of tamarind extract cause alterations in the colonic mucosa and affected the microfloral content of the colon thereby aggravating the adverse effects of fatty diet and obese condition [30]. Excessive growth of adipose tissue results in obesity which involves two growth mechanisms: hyperplasia (cell number increase) and hypertrophy (cell size increase)[31]. Reduction in body weight gain of HFD-fed rats was accompanied by a depletion of body fat stores, since

treatment with T. indica extract at 250 and 500 mg/kg also significantly reduced the weight of adipose tissues (epididymal, retroperitoneal and mesenteric fat) as compared with that of HFD-fed rats. These data confirmed that *T. indica* extract have inhibitory effect on hypertrophy and hyperplasia of adipose tissue induced by high-fat diet, thus *T. indica* extracts are capable to prevent body weight gain comparable helping in maintaining the current body weight. Similarities of these findings support the postulation that tamarind possesses weight reducing properties. Apart from weight reducing ability, it was observed that *T. indica* extract supplementation was found to significantly decrease the levels of total cholesterol, glucose and triglyceride and increase HDL-C level in plasma of the obese group treated with *T. indica* extract, which reversed the effect of high-fat diet consumption alone. Reduction of plasma triglycerides and cholesterol levels is believed to be associated with the epicatechins contents in *T. indica* [32,33]. *T. indica* pulp extract was observed to have strong antioxidant effect in hyperlipidemia and it prevents lipid peroxidation. In the present study, supplementation with *T. indica* pulp extract in obese induced animals reduced lipid peroxidation indicated with low MDA concentration respectively when treated with 250 and 500 mg /kg extracts in contrast to the high fat diet group without the treatment. Obesity and hyperlipidemia synergistically promote systemic oxidative stress-imbalance between tissue free radicals, reactive oxygen species (ROS) and antioxidants [34]. In contrast, the increased level of oxidative stress found in the positive group clearly demonstrated that high fat consumption attributes to increased oxidative stress. The groups treated with *T. indica* extracts showed a significant elevation in their GSH activities compared to the untreated rats. These results are associated with the reduction of GSH levels. The beneficial effect of high dose of *T. indica in* preventing the high fat diet induced body weight gain has been observed to be almost similar to the effect produced by Orlistat, well reported pancreatic lipase inhibitor. From the histopathological examinations, it was revealed that the adipocyte diameter is clearly increased high in High fat diet group, as compared to the control group. Treatment with petroleum ether (250 and 500mg/kg), chloroform (250 and 500mg/kg) and aqueous (250 and 500mg/kg) along with High fat diet has shown a smaller size of adipocytes, when compared with high fat diet group of rats. Correction in lipid profile, decrease in adiposite diameter and adipocyte index by *T. Indica* fruit pulp extract may be because of β-sitosterol content.

CONCLUSION

T. indica has therapeutic potential in the management of obesity. Treatment with petroleum ether, chloroform and aqueous extract of *T. indica* fruit pulp and Orlistat significantly attenuated the body weight, waist hip ratio, adiposity index, obesity index, serum cholesterol level, serum glucose level, serum triglyceride level, whereas increased the serum high density cholesterol level of body in comparison to high fat diet group rats in dose dependent pattern.

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