

## Research Article



## Antiobesity Activity of Aqueous and Ethanol Extracts of *Aegle Marmelos* Leaves in High Fat Diet Induced Obese Rats.

Ankur Garg<sup>1,2</sup>, Randhir Singh\*<sup>2</sup><sup>1</sup>Department of Pharmacology, Translam Institute of Pharmaceutical Education and Research, Meerut, U.P., India.<sup>2</sup>Department of Pharmacology, M.M. College of Pharmacy, M.M. University, Mullana, Ambala, Haryana, India.\*Corresponding author's E-mail: [randhirsingh.dahiya@gmail.com](mailto:randhirsingh.dahiya@gmail.com)

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**ABSTRACT**

Obesity is a chronic disorder of global prevalence and associated with morbidity and mortality. The presents work evaluates the effect of ethanol (AmEe) and aqueous (AmAe) extracts of *Aegle marmelos* leaves in high fat diet (HFD) induced obese rats. Male Wistar rats weighing 150-200 g were divided into different groups i.e. Normal control, HFD control, Orlistat (lipase inhibitor), AmEe and AmAe at doses of 200, 400, 250 and 500 mg/kg. Treatment was started after 6 weeks upto 12 weeks along with HFD (except normal control). Oxidative stress was measured by measuring MDA, SOD and GSH level. AmEe and AmAe at 200, 400, 250 and 500 mg/kg/orally significantly attenuated oxidative stress as well as morphological parameters i.e. % body weight gain, BMI, WHR, obesity index, adiposity index respectively as compare to HFD control group. Similarly, serum glucose, triglyceride and total cholesterol were found to be attenuated as compare to HFD control group. Thus AmEe and AmAe exhibited antiobesity activity may due to decrease fat pad mass, by reduced adipocyte differentiation or by decreasing adipocyte hypertrophy in high fat diet induced obese rats.

**Keywords:** Herbal treatment; lipase inhibitor; Obesity; Oxidative Stress; Orlistat.**INTRODUCTION**

The effects of excess weight on morbidity and mortality have been known for more than 2000 yr. Obesity is closely related to hypertension, type 2 diabetes, coronary heart disease, cancer, respiratory complications, and osteoarthritis.<sup>1</sup> The primary treatment for obesity is dieting and physical exercise. If this fails, anti-obesity drugs or surgery is performed to reduce appetite or inhibit fat absorption.<sup>2</sup> Considerable efforts have been devoted to the discovery of antiobesity drugs worldwide. Despite the remarkable progress in the management of obesity by synthetic drugs, there has been a renewed interest in medicinal plants because of the side effects of synthetic drugs. The discovery of new drugs from traditional medicine is not a new phenomenon. Many Indian medicinal plants are reported to be useful in obesity. However, search for new antiobesity drugs continue.

*Aegle marmelos* (Linn) correa, (Rutaceae) (Synonym- bael or bel) is indigenous to India and is abundantly found in the Himalayan tract, Bengal, Central and South India. Chemical investigation on the different parts of the plant has resulted in the isolation of a large number of novel and interesting metabolites viz., alkaloids, coumarins, terpenoids, fatty acids and aminoacids, which are skimmianine, aegeline, lupeol, cineol, citral, citronella, cuminaldehyde, eugenol, marmelosin, marmesinine,  $\gamma$ -sitosterol,  $\beta$ -sitosterol, flavone, glycoside, oisopentenyl halfordiol, marmeline and phenylethyl cinnamamides.<sup>3-4</sup>

The different parts of *Aegle marmelos* (Rutaceae) are used for various therapeutic purposes, such as for treatment of asthma, anaemia, fractures, healing of

wounds, swollen joints, high blood pressure, jaundice, diarrhoea, healthy mind, brain, typhoid, troubles during pregnancy<sup>5</sup> and also used for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India<sup>6</sup>, Bangladesh<sup>7</sup> and SriLanka<sup>8</sup>. Aegeline 2, an alkaloidal amide isolated from the leaves of *Aegle marmelos* was found to regulate the lipid levels in dyslipidaemic hamster model.<sup>9</sup> Alcoholic extracts of the roots and fruits showed hypoglycaemic and antidiabetic activity.<sup>10-12</sup> As the antiobesity activity of *Aegle marmelos* leaves had not been investigated till date, the present study has been designed to evaluate the antiobesity activity of *Aegle marmelos* leaves aqueous and ethanol extract extract in high fat diet (HFD) induced obesity in Wistar albino rats.

**MATERIALS AND METHODS****Plant Material**

*Aegle marmelos* leaves were collected from herbal garden of Translam Institute of Pharmaceutical Education and Research, Meerut (U.P.), India, and authenticated by Dr. Shiddamallayyan N from the National Ayurveda Dietetics Research Institute, Bangalore, where a voucher specimen is preserved for further reference (Ref No. Drug authentication/SMPU/NADRI/BNG/2012-13/566)

**Preparation of Ethanol and Aqueous extracts of *Aegle marmelos* leaves.**

Powdered *Aegle marmelos* leaves were placed in thimble of Soxhlet apparatus and extraction was carried out by using ethanol as solvent for 72 h. The extracts were filtered; ethanol was distilled off using rotary evaporator to remove excess solvent. The 25 g of air dried ethanol



filtrate was soaked in 100 ml distilled water for 24 h. The extract was filtered by using muslin cloth and used for anti obesity activity.

### High Fat Diet-Induced Obesity

The male Wistar rats (150-200 g) were procured from animal house facility of Translam Institute of Pharmaceutical Education and Research, Meerut (U.P.), India and then housed in standard polypropylene cages and maintained under controlled room temperature ( $22 \pm 2^\circ\text{C}$ ) and humidity ( $55 \pm 5\%$ ) with 12 h light and 12 h dark cycle. All the rats were provided with commercially available rodent chow diet (Amrut rat feed, Nav Maharashtra Chakan Oil Mills Ltd., Delhi, India) and tap water *ad libitum*. After 1 week of acclimatization with free access to rodent chow diet and water, animals were used in the study. The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India were followed and protocol was approved by the Institutional Animal Ethics Committee. Rats were fed with prepared HFD and water *ad libitum* for the period of 12 weeks. Composition of the experimental diet (g/kg diet) was according to the formula of Srinivasan<sup>18</sup> with some modifications as shown in Table 1.

**Table 1:** Composition of HFD

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

### Experimental Design

In this study, a total of 42 rats were used and divided into seven groups of 06 rats each Group I: Normal Control rats were maintained on standard chow diet and water *ad libitum* for twelve weeks. No treatment was given to these rats.

Group II: High Fat Diet Control rats were maintained on high fat diet for twelve weeks to induce obesity.

Group III: Orlistat (Standard) (30 mg/kg/day p.o., 6 weeks) was administered to rats along with high fat diet at the end of sixth week and continued up to the end of the twelve weeks.

Group IV-V: Ethanol extract of *Aegle marmelos* leaves (200 & 400 mg/kg/day p.o., 6 weeks) was administered to

rats along with high fat diet at the end of sixth week and continued up to the end of the twelve weeks.

Group VI-VII: Aqueous extract of *Aegle marmelos* leaves (250 & 500 mg/kg/day p.o., 6 weeks) was administered to rats along with high fat diet at the end of sixth week and continued up to the end of the twelve weeks.

All the drugs were administered by oral gavage once a day. Food intake was measured daily for the period of 12 weeks at the same time on per cage basis and the average food consumed were calculated. At the end of the experimental period (on 85<sup>th</sup> day), the animals were anesthetized with Diethyl ether, following overnight fasting. Blood was drawn by retro-orbital method into a tube and the serum was obtained by centrifugation. After collection of blood, rats were sacrificed; Retroperitoneal (RET), epididymal (EPI), mesenteric (MES) adipose tissue and liver were excised immediately, rinsed with phosphate buffer saline and weighed. The serum, liver and adipose tissue samples were stored at  $-70^\circ\text{C}$  until analysis.

### Morphological Parameters to measure Obesity

The body weights were determined once a week. Body mass index (BMI), WHR, Adiposity index, Obesity index was calculated from formula:

- $\text{BMI} = \text{body weight (g)} / \text{length}^2 (\text{cm}^2)$ .<sup>13</sup>
- Waist-hip ratio.<sup>14</sup>
- Adiposity index = (sum of the weights of perirenal white adipose tissue (WAT), retroperitoneal WAT, and epididymal WAT divided by body weight  $\times 100$ ).<sup>14</sup>
- Obesity index = (body weight of rat/nasoanal length (mm)  $\times 10^4$ ).<sup>14</sup>

### Sample Collection

At the end of the experimental period, all rats were sacrificed and blood samples were collected. Sera were separated and stored in aliquots at  $-20^\circ\text{C}$  till used for estimation of lipid profile including; total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol by enzymatic colorimetric methods using commercial kits.

Then the abdomen were opened, liver and adipose tissues (Retroperitoneal, epididymal and mesenteric) were removed, washed three times in ice cold saline and blotted individually on ash-free filter paper, used for preparation of tissue homogenates for estimation of tissue Malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH) levels and for histological sections.

### Biochemical Estimation

#### Estimation of Total Cholesterol

Total serum cholesterol was estimated by using Bayer Diagnostic kit (Bayer Diagnostic India Ltd).



**Estimation of High Density Lipoprotein Cholesterol**

High-density lipoprotein cholesterol was estimated by using Bayer diagnostic kit (Bayer Diagnostic India Ltd.).

**Estimation of Triglycerides**

Triglycerides level was estimated by using Erba Diagnostics Manheim, Germany kit.

**Estimation of Serum Glucose**

Total serum glucose was estimated by glucose-peroxidase method.

**Methods for Assessment of Oxidative Stress****Estimation of Malondialdehyde (MDA)**

This method based on the formation of MDA as an end product of lipid per oxidation which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically at 532 nm and MDA standard was used to construct a standard curve against which readings of the samples were plotted.<sup>15</sup>

**Estimation of Superoxide Dismutase (SOD)**

The SOD activity was spectrophotometrically measured using a modified version of the method developed by Marklund and Marklund.<sup>16</sup> Briefly, SOD activity was detected based of its ability to inhibit superoxide-mediated reduction. One unit of SOD activity was defined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50% and was expressed as unit/g Hb and that from the tissue as unit/mg protein.

**Estimation of Reduced Glutathione (GSH)**

The method is based on the reduction of 5, 5 dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm by using a commercial kit was used (Biodiagnostic, Egypt).<sup>17</sup>

**Histopathological Analysis**

For histological examination adipose tissue was collected and fixed in 10% neutral buffered formalin, embedded in paraffin. Standard sections of 5 mm thickness were cut, which were then stained with haematoxylin and eosin, and examined by light microscopy.

**Drugs and Chemicals**

Orlistat was obtained from Ranbaxy Research Labs, Gurgaon, India; all other reagents used in this study were of analytical grade.

**Statistical Analysis**

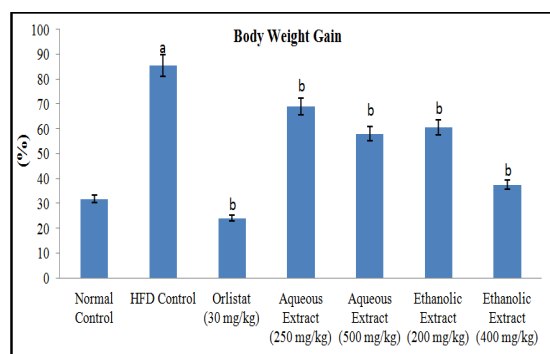
Statistical evaluation of analytical data was done by Student's t-test using the statistical software-GraphPad Prism 3.0. Data are expressed as the mean  $\pm$  standard error (SE). The biochemical data for random glucose, lipid profile and fat pad weights were statistically analyzed

using one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test  $p < 0.05$ . The effect of *Aegle marmelos* leaves aqueous and ethanol extract on feed intake, body weight, BMI, and Obesity index at different time points were statistically analyzed using repeated measure two way ANOVA followed by Bonferroni multiple comparison test  $p < 0.05$  was set to be statistically significant.

**RESULTS****Morphological Parameters****Effect of Orlistat and *Aegle marmelos* leaves extracts on Body Weight, Body Mass Index (BMI), Waist Hip Ratio and Feed Intake of Rats.**

Obesity was induced in normal rats by feeding a high-fat diet for 12 weeks. The mean body weights of the seven experimental groups were similar at the start of the experiment.

A significant increase in body weight, body mass index (BMI) and waist hip ratio along with decrease in feed intake was observed in rats of HFD control group after 12 weeks, as compared to normal control group. On the other hand, treatment with standard drug Orlistat (30 mg/kg, p.o.) once daily for six weeks, significantly ( $p < 0.05$ ) decreased the body weight, BMI, waist hip ratio and feed intake as compared to HFD control group. Whereas, once daily treatment for six weeks with AmEe and AmAe (200, 400, 250 & 500 mg/kg; p.o.), resulted in significant attenuation of body weight, BMI, waist hip ratio and feed intake as compared to HFD control group (Fig 1 & Table 2).



**Figure 1:** Effect of Orlistat and *Aegle marmelos* leaves extracts on % body weight gain of experimental rats. All values are represented as mean  $\pm$  S.E of n=6/group. Where, a =  $p < 0.05$  vs Normal Control; b =  $p < 0.05$  vs HFD Control.

**Effect of Orlistat and *Aegle marmelos* leaves extracts on Fat Pad Weights, Total Fat, Obesity Index and Adiposity Index of Rats.**

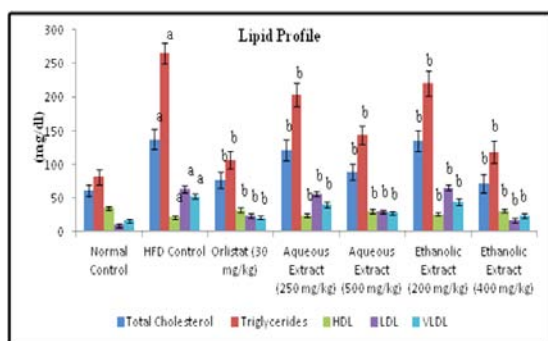
The fat pad weights (Epididymal, Mesenteric, Retroperitoneal and Total fat) significantly increased in HFD control rats, as compared to those of normal control rats. The once daily oral treatment of animals with standard drug (Orlistat), AmEe and AmAe (200, 400, 250

& 500 mg/kg, *p.o.*), for six weeks significantly ( $p < 0.01$ ) attenuated the fat pad weights, total fat, obesity index and adiposity index as compared to HFD control group (Table 2-3).

**Biochemical Parameters**

**Effect of Orlistat and Aegle marmelos leaves extracts treatment on High Fat Diet Induced Changes in Lipid Profile of Rats**

The evaluation of serum lipid profile of experimental animals was carried out for all groups. There was statistically significant ( $p < 0.01$ ) increase in total cholesterol (TC), triglycerides (TG) along with decreased high density lipoprotein (HDL) in HFD control group, as compared to normal control group. The once daily oral administration of Orlistat for six weeks along with HFD significantly decreased the levels of TC and TG with increase in HDL as compared to HFD control group. Also, the once daily treatment with AmEe and AmAe (200, 400, 250 & 500 mg/kg, *p.o.*), for six weeks significantly attenuated the levels of TC and TG with increase in HDL as compared to HFD control group and comparable to standard drug (Orlistat) treatment (Fig 2).

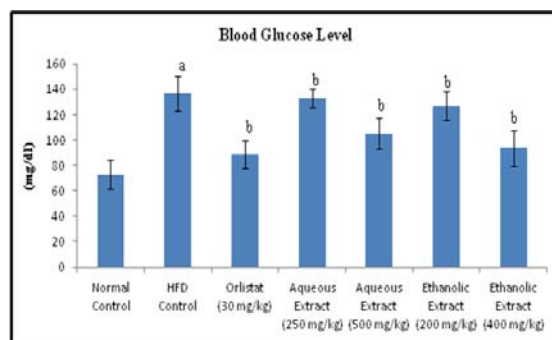


**Figure 2:** Effect of Orlistat and *Aegle marmelos* leaves extracts on lipid profile of experimental rats. All values are represented as mean  $\pm$  S.E. of  $n=6$ /group. Where, a =  $p < 0.05$  vs Normal Control; b =  $p < 0.05$  vs HFD Control.

**Effect of Orlistat and Aegle marmelos leaves extracts on High Fat Diet Induced Changes in Blood Glucose Level of Rats.**

Random blood glucose levels were measured at the end of study. Feeding with high fat diet for 12 weeks

significantly increased the blood glucose level in HFD control group as compared to normal control group. Further, the once daily per oral treatment with Orlistat (standard drug) 30 mg/kg for six weeks significantly decreased blood glucose level as compared to HFD control group. Also, the treatment of animals with AmEe and AmAe (200, 400, 250 & 500 mg/kg, *p.o.*), for six weeks show significant ( $p < 0.01$ ) difference in blood glucose level, as compared to HFD control rats (Fig 3).



**Figure 3:** Effect of Orlistat and *Aegle marmelos* leaves extracts on random blood glucose level of experimental rats. All values are represented as mean  $\pm$  S.E. of  $n=6$ /group. Where, a =  $p < 0.05$  vs Normal Control; b =  $p < 0.05$  vs HFD Control.

**Oxidative Stress Assessment**

**Effect of Orlistat, Aegle marmelos leaves aqueous and ethanol extracts on HFD Induced changes in MDA, SOD & GSH level of rats.**

A significant ( $p < 0.05$ ) decrease in reduced glutathione (GSH), superoxide dismutase (SOD) and along with increased malondialdehyde (MDA) in HFD control group, as compared to normal control group ( $p < 0.005$ ). The once daily oral administration of Orlistat for six weeks along with HFD significantly increased the levels of GSH and SOD with decrease in MDA when compared to HFD control group. Also, the once daily treatment with AmEe and AmAe (200, 400, 250 & 500 mg/kg, *p.o.*), for six weeks significantly attenuated the levels of GSH and SOD with decrease in MDA ( $p < 0.005$ ) as compared to HFD control group and comparable to standard drug (Orlistat) treatment (Table 4).

**Table 2:** Effect of various doses of *Aegle marmelos* leaves aqueous and ethanol extracts on HFD-induced changes on BMI, feed intake in kilocalories (Kcal) and in gram, WH Ratio, obesity index, adiposity index (%) on Day 84.

Parameter	Normal Chow Diet Control	High Fat Diet Control	Orlistat 30mg/kg	<i>Aegle marmelos</i>			
				Aqueous extract		Ethanol extract	
				250mg/kg	500mg/kg	200mg/kg	400mg/kg
BMI	1.13 $\pm$ 0.087	1.57 $\pm$ 0.10 <sup>a</sup>	0.98 $\pm$ 0.08 <sup>b</sup>	1.5 $\pm$ 0.12 <sup>b</sup>	1.15 $\pm$ 0.06 <sup>b</sup>	1.41 $\pm$ 0.10 <sup>b</sup>	1.1 $\pm$ 0.19 <sup>b</sup>
Feed Intake (gm)	21.66 $\pm$ 6.31	17.18 $\pm$ 2.88 <sup>a</sup>	4.17 $\pm$ 1.47 <sup>b</sup>	13.30 $\pm$ 1.26 <sup>b</sup>	8.86 $\pm$ 1.04 <sup>b</sup>	11.98 $\pm$ 0.81 <sup>b</sup>	7.52 $\pm$ 1.52 <sup>b</sup>
Feed intake (Kcal)	78 $\pm$ 22.74	61.86 $\pm$ 4.83 <sup>a</sup>	15.0 $\pm$ 5.29 <sup>b</sup>	47.88 $\pm$ 4.53 <sup>b</sup>	31.92 $\pm$ 3.76 <sup>b</sup>	43.14 $\pm$ 2.91 <sup>b</sup>	27.06 $\pm$ 5.48 <sup>b</sup>
WH Ratio	0.84 $\pm$ 0.023	1.09 $\pm$ 0.016 <sup>a</sup>	0.92 $\pm$ 0.43 <sup>b</sup>	1.03 $\pm$ 0.18 <sup>b</sup>	0.91 $\pm$ 0.08 <sup>b</sup>	1.01 $\pm$ 0.01 <sup>b</sup>	0.93 $\pm$ 0.021 <sup>b</sup>
Obesity Index	345.9 $\pm$ 8.07	368.5 $\pm$ 6.06 <sup>a</sup>	333.7 $\pm$ 3.17 <sup>b</sup>	365.5 $\pm$ 9.67 <sup>b</sup>	320.2 $\pm$ 8.14 <sup>b</sup>	345.3 $\pm$ 13.7 <sup>b</sup>	332.7 $\pm$ 9.14 <sup>b</sup>
Adiposity Index (%)	2.68 $\pm$ 0.08	5.00 $\pm$ 0.20 <sup>a</sup>	2.89 $\pm$ 0.23 <sup>b</sup>	5.00 $\pm$ 0.19 <sup>b</sup>	3.73 $\pm$ 0.12 <sup>b</sup>	4.99 $\pm$ 0.32 <sup>b</sup>	3.41 $\pm$ 0.18 <sup>b</sup>

All values are represented as mean  $\pm$  S.E.; a =  $p < 0.05$  vs Normal Chow Diet control, b =  $p < 0.05$  vs HFD control.



**Table 3:** Effect of various doses of *Aegle marmelos* leaves aqueous and ethanol extracts on HFD-induced changes on various fat pads on Day 84.

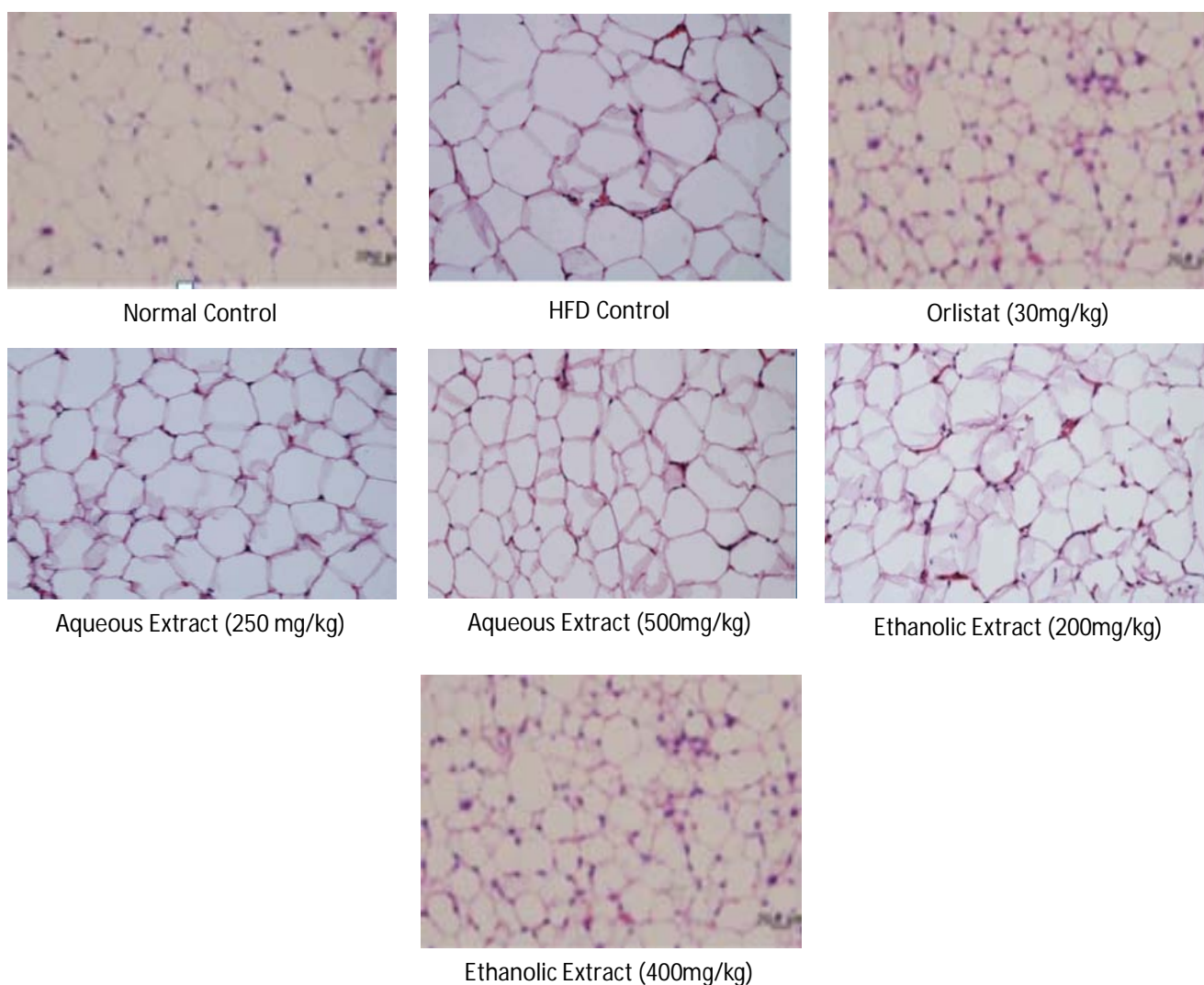
Parameter	Normal Chow Diet Control	High Fat Diet Control	Orlistat 30mg/kg	<i>Aegle marmelos</i>			
				Aqueous extract		Ethanol extract	
				250mg/kg	500mg/kg	200mg/kg	400mg/kg
Epididymal Fat (gm)	2.83 ± 0.49	7.4 ± 0.62 <sup>a</sup>	2.75 ± 0.69 <sup>b</sup>	6.34 ± 1.31 <sup>b</sup>	4.18 ± 0.81 <sup>b</sup>	6.28 ± 1.63 <sup>b</sup>	3.27 ± 1.12 <sup>b</sup>
Retroperitoneal Fat (gm)	1.26 ± 0.54	4.26 ± 0.65 <sup>a</sup>	1.22 ± 0.65 <sup>b</sup>	4.05 ± 0.61 <sup>b</sup>	1.8 ± 0.63 <sup>b</sup>	4.13 ± 0.32 <sup>b</sup>	2.20 ± 0.43 <sup>b</sup>
Mesenteric Fat (gm)	2.86 ± 0.25	7.06 ± 0.55 <sup>a</sup>	2.67 ± 0.52 <sup>b</sup>	6.48 ± 0.90 <sup>b</sup>	3.15 ± 0.78 <sup>b</sup>	5.98 ± 0.94 <sup>b</sup>	3.91 ± 0.69 <sup>b</sup>
Total Fat (gm)	6.97 ± 0.49	18.75 ± 1.34 <sup>a</sup>	6.63 ± 1.44 <sup>b</sup>	16.88 ± 1.96 <sup>b</sup>	9.13 ± 0.84 <sup>b</sup>	16.40 ± 2.00 <sup>b</sup>	9.38 ± 1.29 <sup>b</sup>

All values are represented as mean ± S.E; a =  $p < 0.05$  vs Normal Chow Diet control, b =  $p < 0.05$  vs HFD control.

**Table 4:** Effect of various doses of *Aegle marmelos* leaves aqueous and ethanol extracts on HFD-induced changes on antioxidant enzyme activities on Day 84.

Parameter	Normal Chow Diet Control	High Fat Diet Control	Orlistat 30 mg/kg	<i>Aegle marmelos</i>			
				Aqueous extract		Ethanol extract	
				250mg/kg	500mg/kg	200mg/kg	400mg/kg
MDA (nmol/g protein)	23.02 ± 0.88	32.68 ± 2.11 <sup>a</sup>	24.43 ± 0.65 <sup>b</sup>	30.23 ± 0.93 <sup>b</sup>	26.63 ± 1.27 <sup>b</sup>	28.94 ± 1.43 <sup>b</sup>	25.75 ± 1.14 <sup>b</sup>
GSH (µg/mg protein)	30.07 ± 3.86	12.26 ± 1.28 <sup>a</sup>	28.67 ± 2.14 <sup>b</sup>	22.25 ± 1.74 <sup>b</sup>	26.73 ± 1.31 <sup>b</sup>	23.72 ± 1.32 <sup>b</sup>	27.92 ± 1.73 <sup>b</sup>
SOD (unit/mg protein)	7.54 ± 0.17	5.48 ± 0.04 <sup>a</sup>	6.86 ± 0.12 <sup>b</sup>	4.22 ± 0.14 <sup>b</sup>	6.25 ± 0.28 <sup>b</sup>	4.91 ± 0.82 <sup>b</sup>	6.81 ± 0.72 <sup>b</sup>

All values are represented as mean ± S.E; a =  $p < 0.05$  vs Normal Chow Diet control, b =  $p < 0.05$  vs HFD control.

**Figure 4:** Effect of AmEe & AmAe (200, 400, 250 & 500 mg/kg, *p.o.*), on Adipose tissue of HFD fed Rats (Magnification-40x).

## DISCUSSION

The prevalence of obesity is rapidly rising. Rats fed with a lard-based HFD showed distinctive visceral adiposity, hyperglycemia, dyslipidemia and oxidative stress which are typically associated with human obesity.<sup>19</sup> It is well documented that the average energy intake of high fat diet fed mice was higher than that of normal diet fed mice.<sup>20-21</sup> Thus, in the present study, high fat diet (HFD) for 12 weeks was used to produce obesity in Wistar rats. It is well established that fat over-consumption lead to obesity in number of animals models including rats.<sup>22</sup> Obesity is also associated with an unfavorable lipid profile or dyslipidemia is well documented in various studies.<sup>23,24</sup> 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.<sup>25-26</sup> It is reported that high-fat diet promotes hyperglycemia and whole-body insulin resistance.<sup>27-28</sup> In the present study 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 in present study as the marker of hyperlipidemia and hyperglycemia. Obesity may result due to increased adipose mass.<sup>29</sup> Studies in animals, as well as in humans, demonstrate that body fat is more closely related to the amount of fat ingested than to total caloric intake.<sup>30-31</sup> The decrease in the weight may due to decrease fat pad mass by reduced formation of new adipocyte from precursor cells (adipocyte differentiation) or decreased adipocyte size due to fat storage (adipocyte hypertrophy).<sup>32</sup> It is earlier reported that the relative weight of the total visceral fat-depots (epididymal, mesenteric, and retroperitoneal) of the rats fed the HFD was significantly greater than the ND rats.<sup>19-21,33</sup> Therefore, three adipose tissues (epididymal, retroperitoneal, mesenteric fat depots) were weighed in the present study as an index of adiposity and size of adipose tissue was examined histologically using light microscope. Consumption of HFD also contributes to excessive formation of Reactive oxygen species (ROS) which leads to induced oxidative stress.<sup>34</sup> We determined oxidative stress by measuring the level of reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) and found that HFD feeding cause decrease in GSH, SOD and increase in MDA levels as compared to normal control group. Therefore in the present study body weight, BMI, obesity index, feed intake, weight of adipose tissue, adiposity index, serum glucose level, disturbed lipid profiles and assessment of oxidative stress were used as parameter to assess obesity.

In the present study, anti-obesity effect of aqueous (AmAe) and ethanol extracts (AmEe) of *Aegle marmelos* leaves (Rutaceae) was investigated using a HFD-induced obese rat model. In epidemiological studies, BMI is widely

used as a measure of fatness because it is highly correlated with body fat and is nearly independent of height.<sup>35</sup> In our study, significant reduction in BMI of AmAe and AmEe treated group was observed. Excessive growth of adipose tissue results in obesity which involves two growth mechanisms: hyperplasia (cell number increase) and hypertrophy (cell size increase).<sup>36</sup> Reduction in body weight gain of HFD-fed rats was accompanied by a depletion of body fat stores, since treatment with AmAe and AmEe 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 AmAe and AmEe have inhibitory effect on hypertrophy and hyperplasia of adipose tissue induced by high-fat diet, thus results in decrease of body weight gain.

In addition to its weight reducing effect, AmAe and AmEe treated HFD rats showed significantly lowered plasma TC, TG levels, increased HDL-C levels and improved glucose tolerance. It is reported that obesity, especially abdominal obesity, is associated with dyslipidemia, characterized by elevated TG and reduced HDL-C concentrations.<sup>26</sup> TGs are involved in the ectopic accumulation of lipid stores in the liver and are associated with a number of diseases such as metabolic syndrome. VLDL-C transports cholesterol and TG to the tissues, while high HDL-C is helpful in transporting excess cholesterol to the liver for excretion in the bile.<sup>37</sup> High TC and LDL-C levels and lower HDL-C level are risk factors for coronary heart disease.<sup>38</sup> Rao<sup>39</sup> reported that aqueous extract of leaves given in the dose equivalent to 1 gm powder/kg/day produced significant ( $p < 0.01$ ) anti-hyperglycemic effect within three days in alloxan induced diabetic rabbits while similar treatment in normal rabbits produced decrease upto 35.3% in blood glucose level after 4 hours of administration. AmAe and AmEe have shown good antihyperglycemic and antidyslipidemic property. Obesity and hyperlipidemia synergistically promote systemic oxidative stress-imbalance between tissue free radicals, reactive oxygen species (ROS) and antioxidants.<sup>40</sup> ROS could react with polyunsaturated fatty acids, which lead to lipid peroxidation.<sup>41</sup> Malondialdehyde is a by-product of lipid peroxidation and reflect the degree of oxidation in the body.<sup>15</sup> Possible mechanisms that generate oxidative stress in obesity include hyperglycemia, elevated lipid levels and inadequate antioxidant defences.<sup>42</sup>

In our study, the activities of GSH (a major endogenous antioxidant) and SOD decreased and MDA activity get increased in HFD-fed rats. Treatment of HFD-fed rats with AmAe and AmEe had reversed the activities of these enzymatic antioxidants. Therefore AmAe and AmEe treatment improves oxidative balance in HFD-fed obese rats.

The beneficial effect of high dose of *Aegle marmelos* 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. Moreover, our histological examinations revealed that the sizes of the adipocytes were significantly reduced in AmAe and AmEe treated rats (Fig4). However, AmAe and AmEe supplementation noticeably attenuated the extent of steatosis, suggesting that AmAe and AmEe may regulate lipid storage and mobilization in adipocytes.

## CONCLUSION

Literature proves substantial progress that links obesity with crude extracts and bioactive scaffolds from edible and medicinal plants.

Till date, there are numerous reports for the anti-obesity activity of the different parts and different extracts of *Aegle marmelos*. In continuing our focus on anti obesity potential of *Aegle marmelos* leaves, in this study, we have observed the antiobesity effect of aqueous and ethanol extract of *Aegle marmelos* leaves.

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