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The bioactive role of aqueous extract of *aegle marmelos* leaves on obese rats

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***Abstract*---**The aim of this study was to evaluate the effect of *Aegle marmelos* aqueous extract (AMAE) leaves on obese rats. Twenty-five adult male albino rats (Sprague-Dawley strain), weighing about (200±10g) were divided randomly into two main groups as follow: the first group (-Ve control= 5 rats) was fed on basal diet. The second group (20 rats) was fed on high fat diet (HFD) for 4 weeks then divided equally to 4 groups from group 2 to group 5. Group 2 (+Ve control) fed on basal diet. Group 3, 4 and 5 fed on HDF administrated orally with 1, 2 and 3 ml of AMAE, respectively. At the end of the experimental period (4 weeks), rats were scarified and serum was collected to determine levels of lipid profile, glucose, leptin kidneys and liver functions. The results showed that the body weight of rats and serum levels of lipids profile, leptin, glucose, kidneys functions and liver enzymes were elevated by HFD administration (positive control group) compared with negative control group, while HDL-C decreased. It was also found that oral administration of AMAE diet with reversed these changes that caused by HFD. The study recommends that intake of *Aegle marmelos* leaves may be beneficial for patients who suffer from obesity.

Keywords*---Aegle marmelos*, High fat diet, Obesity, Rats.

Introduction

Obesity is defined as the excess of adipose tissue resulting from the positive energy balance and is associated with the imbalance between the anabolic and catabolic processes involved in lipid metabolism (Bautista *et al.*, 2019 and Kim *et al.*, 2021). Among the parameters adopted for the classification of body weight, the Body Mass Index (BMI) is the most used worldwide, in which BMI values >30 kg/m2 are classified as obesity (Mehrzad, 2020). Its etiology is the result of the interaction of several factors with emphasis on genetic and environmental factors, mainly excessive food intake and lifestyle (Fernandez-Navarro *et al.*, 2019). Estimates indicate that by 2025, 18% of the male population and 21% of the female population will be obese (NCD Risk Factor Collaboration, 2016). Obesity is associated with the development of numerous pathologies such as diabetes mellitus (Carbone *et al.*, 2019), arterial hypertension (Nurdiantami *et al.*, 2018), cardiovascular diseases (Xia *et al.*, 2019), liver diseases (Polyzos *et al.*, 2019), some types of cancers (Wang *et al.*, 2019), allergies (Michalovich *et al.*, 2019), osteoporosis (Qiao *et al.*, 2020) and obstructive sleep apnea (Ger *et al.*, 2020).

Considerable efforts have been devoted to the discovery of antiobesity drugs worldwide. The discovery of new drugs from traditional medicine is not a new phenomenon. Many Indian medicinal plants are reported to be useful in obesity (Garg and Singh, 2015). *Aegle marmelos*, known as bael in India is a plant of Rutaceae family, is a moderate sized, slender, aromatic tree used as ethnomedicines against various human ailments (Reddy *et al.*, 2012 and Sankeshi *et al.*, 2013). The young leaves and shoots are eaten as a vegetable as well as they are a salad green in Thailand (Karmase *et al.*, 2013). Various phytoconstituents like flavonoids, alkaloids, sterols, tannins and flavonoids glycosides have been isolated from the various parts of Aegle marmelos which is responsible for its antioxidant property (Chandel *et al.*, 2020). It has documented antidiabetic, antimicrobial, anti-inflammatory, hepatoprtective and anticancer activity (Chockalingam *et al.*, 2012 and Sankeshi *et al.*, 2013). Ethanolic extract of Bael leaves also inhibited the elevation of serum cholesterol and triglycerides level in hyperlipidaemic rat (Vijaya *et al.*, 2009).

There-fore there is possibility that the *A. marmelos* extract and compounds may act by lipolysis. The present study was designed to evaluate the antiobesity activity of *Aegle marmelos* leaves aqueous extract in high fat diet (HFD) induced obesity in albino rats.

Materials and Methods

A. Materials

Leaves of *Aegle marmelos* were collected from in El-Zohrya botanical garden, Giza, Egypt. Casein, all vitamins, minerals, cellulose and choline were obtained from El-Gomhoria Company, Cairo, Egypt. Starch, corn oil and sucrose were obtained from the local market. Twenty-five adult male albino rats (Sprague Dawley strain), weighing about 200±10 g b.wt. were obtained from the Laboratory Animal Colony, Agricultural Research Center, Giza, Egypt.

B. Methods

Preparation of *Aegle marmelos* Aqueous Extract (AMAE):
20 gm of dried *Aegle marmelos* leaves ground were submerged in 100 ml of distilled water and allowed to soak overnight, then filtered to obtain a liquid extract. A known concentration of AM aqueous extract was given orally by stomach tube.

Induction of Obesity:
Rats will be fed on high fat diet (HFD) containing (saturated fat 19%, soybean oil 1% to provide essential fatty acids, sucrose 10%, casein 20%, cellulose 5%, vitamin mixture 1%, salt mixture 3.5%, choline chloride 0.25% and the remainder is corn starch) for four weeks to induce obesity in rats (Min *et al.*, 2004).

Diet Composition and Experimental Animal Design:
The basal diet was formulated according to AIN-93M diet (Reeves *et al.*, 1993). Twenty-five male rats were housed in well conditions in biological studies Research Labs, Agricultural Research Center, Giza, Egypt. They were left for seven days as adaptation period and they were allowed to feed standard laboratory food and water. After the period of adaptation, animals were divided into two main groups, as follows: the first group (5 rats) was fed on basal diet and served as a negative control group (-ve), the second group (20 rats) was fed on HFD (Min *et al.*, 2004). After four weeks, this group was divided equally to six subgroups as follows:-

Subgroup (1): Obese rats, served as positive control group were fed on HFD only.
Subgroup (2): Obese rats were fed on HFD and received 1 mL/day of AMAE.
Subgroup (3): Obese rats were fed on HFD and received 2 mL/day of AMAE.
Subgroup (4): Obese rats were fed on HFD and received 3 mL/day of AMAE.

At the end of the experimental period (4 weeks), rats were fasted overnight before scarifying and blood samples were collected from each rat and were centrifuged at 3000 rpm for 15 min to obtain the serum for biochemical analysis.

Biological Evaluation:
Feed intake (FI), body weight gain percent (BWG %) and feed efficiency ratios (FER) were determined according to Chapman *et al.*, (1959) using the following equation:
BWG% = Final body weight - Initial body weight / Initial body weight × 100
FER = Weight gain / Feed intake.

Biochemical Analysis of Serum:
Serum glucose and leptin were determined according to the methods described by Trinder (1969) and Zhang *et al.*, (1995), respectively. Aspartate aminotransaminase (AST) and alanine aminotransaminases (ALT) were determined according to Young, (2001), and alkaline phosphates (ALP) was determined according to Roy, (1970). Serum urea and creatinine were assayed according to Young, (2001), uric acid was determined according to Milena, (2003). Serum total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-c) was determined according to Richmond, (1973); Wahlefeld, (1974) and

Albers *et al.*, (1983), respectively. Regarding to serum low density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were calculated according to Fridewald *et al.*, (1972).

Statistical analysis

Results were expressed as the mean standard error ± SE. Data were statistically analyzed for variance "ANOVA" test at $P \leq (0.05)$ using SPSS statistical software, version 20 was used for these calculations (Armitage and Berry, 1987).

Results and Discussion

Results illustrated in Table (1) showed the effect of diet supplemented with leaves of *Aegle marmelos* aqueous extract (AMAE) on body weight gain (BWG%), feed intake (FI), feed efficiency ratio (FER) and peritoneal fat (PF) of obese rats. Data revealed that FI increased in positive control group (+ve) when compared with the negative control group (-ve). HFD caused a significant ($P< 0.05$) increased in BWG%, FER and PF when compared to the negative control group. AMAE administration decreased FI compared with positive control. Group 4 (2 ml) and 5 (3 ml) showed that AMAE administration significantly reduced ($P< 0.05$) in BWG%, FER and PF when compared to positive control, while group 3 (1 ml) showed no significant compared with +ve group. Results indicated that AMAE attenuated body weight gain as compared HFD control group were in agreement with Garg and Singh, (2015) who found that AM extracts attenuated body weight gain and exhibited anti-obesity activity may due to decrease fat pad mass, by reduced adipocyte differentiation or by decreasing adipocyte hypertrophy in high fat diet induced obese rats. Also, Maqbool *et al.,* (2019) and Siddiqui *et al.,* (2019) reported that *Aegle marmelos* is effective in restoration the body weight to normal levels.

Data in Table (2) showed that HFD group caused a significant elevated ($P< 0.05$) in serum TC, TG, LDL-c and VLDL-c when compared with the negative control group. On the other hand, all treated groups with AMAE significantly decrease ($P<0.05$) in the levels of TC, TG, VLDL compared to the control positive group. Also, it was observed that group 3 and 4 had no significant in LDL level while group 5 showed a significant decreased when compared to +ve group. Regarding serum HDL-c level, results showed a significant ($P< 0.05$) decrease in serum HDL level in HFD group when compared to the negative control group. Rats that administrated with 2 ml and 1 ml of AMAE were significant increased ($P< 0.05$) in HDL level and there was no significant in group 5 when compared to the positive control groups.

Obesity is also associated with an unfavorable lipid profile or dyslipidemia is well documented in various studies (Kelley *et al.*, 2002 and Malnick and Knobler, 2006). Lipid abnormalities related to obesity include an elevated serum concentration of fatty acids, TC, LDL-c, VLDL-c and TG, as well as a reduction in serum HDL-c (Velasquez and Bhathena, 2007), as found in the present study. AMAE improved levels of lipid profile that affected by HFD as found in the in the present results were confirmed by Vijaya *et al.,* (2009) and Bhuvaneswari and Sasikumar, (2013). The authors reported that phytochemical screening revealed

the presence of alkaloids, flavonoids, tannins, saponins and sitosterol in the AM leaves and results obtained suggested marked antihyperlipidemic activity of the extracts of AM leaves. On the other hand, Narender *et al.*, (2007) reported that Aegeline, the major constituent in the AM leaves has shown good antihyperglycemic activity. Aegeline has also antidyslipidemic property and it has reduced plasma triglyceride, total cholesterol and free fatty acids accompanied with increase in high density lipoprotein in dyslipidemic hamster model at the dose of 50 mg/kg body weight.

The concentrations of serum leptin and glucose were recorded in Table (3), results indicated that leptin and glucose levels in the HFD control group significantly increased ($P< 0.05$) compared to normal group (-ve). While all treated groups with AMAE significantly ($P< 0.05$) decreased compared to the positive control group. It was observed that glucose levels in group 5 had nonsignificant when compared with negative control. These results were in the same line with Hadžović-Džuvo *et al.*, (2014) who reported that HFD caused elevated in serum levels of leptin and glucose. Karmase *et al.*, (2013) demonstrated that umbelliferone and esculetin were the most active compounds found in AM leaves which reduced glucose level in their respective HFD groups. Furthermore, Ramesh and Pugalendi (2005); Ramesh *et al.*, 2007 and Ahmed *et al.*, (2011) The most active compounds screened in the lipolysis assay, umbelliferone and esculetin also have been reported for the anti-diabetic activity and their effect on the lipid profile has also been documented in the streptozotocin-induced diabetic rats' model.

Results in Table (4) indicated that serum urea, uric acid and creatinine levels significantly increased ($P< 0.05$) in the positive control group compared to negative control. While all administrated groups with AMAE significantly ($P< 0.05$) decreased when compared with the positive control group. Also, it was observed that group 5 (3 ml of AMAE) showed no significant in urea, uric acid and creatinine levels when compared to -ve control. Obesity is as an important independent risk factor for kidney disease. This risk is probably explained by renal intracellular lipid accumulation (Corpeli *et al.*, 2009). Kore *et al.,* (2011) confirmed that AEAM reduced serum creatinine, urea and blood urea nitrogen levels, indicating that AEAM leaves possesses the nephroprotective activity.

As seen in Table (5), serum concentrations of AST, ALT and ALP were significantly elevated ($P< 0.05$) in the positive control group compared with negative control group. It was observed a significant ($P< 0.05$) reduce in serum AST, ALT and ALP levels for all groups treated with AMAE when compared to the positive control group. Lasker *et al.*, (2019) confirmed that AST, ALT and ALP activities were increased by HFD. AMAE restored liver enzymes to normal levels compared to negative group. These findings were confirmed by Modi *et al.,* (2012) and Ibrahim *et al.,* (2018) who found that administration of AM restored hepatic enzymes to normal levels, reduced free radical generation and enhanced the antioxidants armory. They confirmed AM as a potential hepatoprotective agents.

Conclusion

The present study demonstrated that *Aegle marmelos* aqueous extract leaves are a promising ani-obesity agent and this activity of AMAE may be due to its phenolic content.

Table (1): Effect of *Aegle marmelos* on Body Weight Gain (BWG), Feed Intake (FI), Feed Efficiency Ratio (FER) and Peritoneal Fat (PF)of Obese Rats

parameter / Groups	BWG %	FI (g/day/ra)	FER	PF
G1: Control (-ve)	10.33±0.66^{b}	17	0.021±0.001^{c}	1.115±0.039^{d}
G2: Control (+ve)	17.39±0.61^{a}	20	0.034±0.001^{a}	2.314 ±0.102^{a}
G3: 1 ml AMAE	15.32±0.32^{a}	16.7	0.031±0.001^{ab}	2.059±0.069^{ab}
G4: 2 ml AMAE	12.13±0.34^{b}	15.9	0.027±0.001^{b}	1.805±0.123^{bc}
G5: 3 ml AMAE	6.12±0.57^{c}	15.5	0.014±0.001^{d}	1.382±0.115^{cd}

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
*AMAE: *Aegle marmelos* aqueous extract.

Table (2): Effect of *Aegle marmelos* on Serum Triglyceride (TG), Total Cholesterol (TC), Very Low Density Lipo- protein Cholesterol (VLDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and High-Density Lipoprotein Cholesterol (HDL-C), of Obese Rats

parameter / Groups	TG	TC	HDL-c	LDL-c	VLDL-c
	mg/dl				
G1: Control (-ve)	93.00±0.57^{d}	86.33±1.20^{e}	55.00±0.15^{a}	19.26±0.24^{c}	12.06±0.76^{e}
G2: Control (+ve)	133.33±0.67^{a}	206.00±1.15^{a}	41.00±0.57^{d}	24.00±0.34^{a}	141.00±0.28^{a}
G3: 1 ml AMAE	116.66±0.18^{b}	173.34±1.66^{b}	43.00±0.15^{cd}	23.33±0.43^{a}	107.00±0.41^{b}
G4: 2 ml AMAE	115.65±0.18^{bc}	158.00±1.52^{c}	46.00±0.75^{bc}	23.13±0.29^{ab}	88.86±0.57^{c}
G5: 3 ml AMAE	105.33±0.65^{c}	122.33±1.88^{d}	49.00±0.52^{b}	21.06±0.76^{bc}	52.21±0.48^{d}

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
*AMAE: *Aegle marmelos* aqueous extract.

Table (3): Effect of *Aegle marmelos* on Serum Leptin and Glucose of Obese Rats

parameter / Groups	Leptin	Glucose
	µg/L	mg/dL
G1: Control (-ve)	10.90±0.26^{d}	83.17±1.45^{d}
G2: Control (+ve)	17.83±0.56^{a}	131.66±1.72^{a}
G3: 1 ml AMAE	14.86±0.23^{b}	117.00±1.15^{b}
G4: 2 ml AMAE	13. 05±0.17^{b}	96.23±1.73^{c}
G5: 3 ml AMAE	11.70±0.40^{c}	89.86±1.71^{d}

*Mean values are expressed as means ± SE.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
*AMAE: *Aegle marmelos* aqueous extract.

Table (4): Effect of *Aegle marmelos* on Urea, Uric Acid and Creatinine of Obese Rats

parameter / Groups	Urea	Uric Acid	Creatinine
	mg/dl		
G1: Control (-ve)	19.16±0.44^{c}	3.36±0.88^{c}	0.97±0.012^{c}
G2: Control (+ve)	52.33±0.88^{a}	6.56±0.14^{a}	1.94±0.014^{a}
G3: 1 ml AMAE	23.32±0.98^{b}	4.46±0.15^{b}	1.18±0.011^{b}
G4: 2 ml AMAE	21.85±0.86^{bc}	4.36±0.12^{b}	1.12±0.024^{b}
G5: 3 ml AMAE	20.13±0.64^{bc}	3.70±0.11^{c}	1.03±0.010^{c}

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
*AMAE: *Aegle marmelos* aqueous extract.

Table (5): Effect of *Aegle marmelos* on Serum Asparta Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline phosphatase (ALP) of Obese Rats

parameter / Groups	AST	ALT	ALP
	(µ/L)		
G1: Control (-ve)	27.66±0.88^{d}	37.34±1.21^{c}	51.00±1.73^{b}
G2: Control (+ve)	46.39±0.96^{a}	50.65±1.01^{a}	77.33±1.21^{a}
G3: 1 ml AMAE	44.00±0.57^{ab}	44.67±1.20^{b}	53.69±1.76^{b}
G4: 2 ml AMAE	41.00±0.86^{b}	42.69±1.21^{b}	52.39±1.11^{b}
G5: 3 ml AMAE	35.49±0.52^{c}	40.18±0.98^{bc}	51.67±1.20^{b}

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.
*AMAE: *Aegle marmelos* aqueous extract.

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