ORIGINAL ARTICLE

Effect of Ethanolic Extract of *Emblica officinalis* (Amla) on Glucose Homeostasis in Rats Fed with High Fat Diet

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Abstract:

Background: Emblica officinalis contains many biological active components which are found to have some medicinal properties against diseases. *Aim and Objectives:* To assess hypolipidemic and glucose regulatory actions of Ethanolic Extract of *Emblica officinalis* (EEO) on High Fat Diet (HFD) fed experimental rats. *Material and Methods:* Twenty four rats were divided into four groups, having six rats in each group as following; Group I- Control (20% fat); Group II (EEO 100 mg/kg/b w); Group III (30% fat) and Group IV (30% fat + EEO 100 mg/kg/b w). The treatment was continued for 21 days. Gravimetric parameters and lipid profiles of all the groups were measured. Oral Glucose Tolerance Test (OGTT), fasting and postprandial glucose and fasting insulin levels were estimated. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated. Statistical analysis was done. *Results:* Significant alteration in serum lipid profile, fasting and post prandial blood glucose levels and fasting insulin level were observed in rats of Group III fed with high fat diet. Supplementation of EEO improved both of glycemic and lipid profiles in rats of Group IV fed with high fat diet. *Conclusion:* Results from the study indicate the beneficial role of EEO on dyslipidemia and glucose homeostasis in rats treated with high fat diet.

Keywords: *Emblica officinalis*, Dyslipidemia, Oral Glucose Tolerance Test, Homeostasis Model Assessment of Insulin Resistance

Introduction:

Diet and nutrition are very vital factors in maintaining health of human life [1]. In recent years, life styles are over influenced by an excess consumption of high fat diets [2]. Over-consumption of high fat diet may increase a positive energy balance and develops overweight and obesity states [3]. Subsequently long term excess consumption of high fat diet causes hyperlipidemia, diabetes type 2 and fatty liver diseases [4]. Hyperlipidemia occurs due to high levels of lipids especially total cholesterol, triglycerides and insufficient HDL which ultimately causing atherosclerosis and disease associated with it. Hyperlipidemia is associated with diabetes mellitus [5].

Diabetes and dyslipidemia are two main factors which revolve around the pathophysiological effects of abnormal lipid levels and insulin resistance [6]. Excess FFA storage from abnormal lipid metabolism leads to insulin resistance in peripheral cells, eventually causing hyper-insulinemia, hyperglycemia along with hyperlipidemia. However diabetes mellitus develops through pre diabetic state and land up in diabetic state if dietary lipid is not well regulated [4]. Hence retaining blood glucose homeostasis efficiently, has become indispensible concern in prevention of diabetes mellitus.

Emblica officinalis belongs to Euphorbiacae family, commonly known as Amla or Indian gooseberry. It has been reported that *Emblica officinalis* contains many biological active components like polyphenols, tannins, gallic acids, emblicanin A & B and flavonoids. Tannins and flavonoids present in *Emblica officinalis* were documented as possible glucose lowering, lipid lowering antioxidant [7]. Hyperlipidemia in association with Type 2 diabetes mellitus is found to be a burden of 10% to 73% of total population of India [8].

The present study was aimed to assess possible effects of Ethanolic Extract of *Emblica officinalis* (EEO) on glucose regulation in rats fed with High Fat Diet (HFD).

Materials and Methods:

Fresh, mature and good quality fruits of *Emblica officinalis* (Amla) were purchased from the local market, in the months of October-November 2015.

Preparation of extract:

Fruits of *Emblica officinalis* (Amla) were identified and authenticated by Department of Botany, KCP Science College, Vijayapur and Voucher Specimen No. BMPP/03 is deposited in our research laboratory for further reference. Fruits of *Emblica officinalis* were allowed to dry

and dried fruits were coarsely powdered. Four hundred and eighty gram of the dried powdered fruit material was extracted with 99% ethanol using Soxhlet apparatus at a temperature below 60°C for 24 hours. The solvent was evaporated under vacuum which was collected as semisolid mass with percentage yield (26%) with respect to the dried powder. This extract was stored as stock solution in refrigerator and diluted with distilled water when required [9].

Animals and Diet:

Healthy albino Wistar rats (n=24) of weight 180-250 g were selected for the study. All animals were allowed to acclimatize for 7 days to the laboratory atmosphere at 22-24^{0}C and were maintained 12 hr light/dark cycle. Institutional Animal Ethics Clearance (IEC ref. No. 664/15, dated 7/12/2015) was procured. As per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSCEA) guidelines animal care was taken during experiment [10]. Dietary composition of hyperlipidemic diet is mentioned in the following table 1 [11].

Experimental Protocol:

All 24 rats were divided into following four groups with 6 rats in each group. Group-I treated as control (fat 20%), Group II rats were supplemented with EEO 100 mg/kg b w with

Table 1: Composition of Diet

Diet Source	Ingredients	Control Diet (CD)	High Fat Diet (HFD)
Carbohydrate	Amylum	60%	50%
Protein	Casein	18%	18%
Fat	Vegetable oil	20 %	30 %
Salt and vitamins	NaCl and Multivitamins	2%	2%

normal diet for 21 days. Group-III rats fed with high fat diet (fat 30%) for 21 days, Group IV-fed with high fat diet (fat 30%) and EEO 100mg/kg b w for 21 days. Dose of high fat diet was 30 g of fat in 100 g of total diet. Dietary protocol was maintained on pair feeding [11].

Gravimetric Analysis:

The body weight of all rats were recorded in the initial step of experiment on first day and on the last day (22nd day) by using digital weighing machine (Practum1102-10IN). Also percent changes of weight gain of all rats were calculated.

Sample Collection

On 20th day, Oral Glucose Tolerance Test (OGTT) was performed on rats of all groups [12]. All animals were kept for an overnight fasting on 21st day. On 22nd day blood was collected in 10% Ethylene Diamine Tetra Acetic Acid (EDTA) tubes by retro orbital puncture using local anesthesia. Blood samples were centrifuged at 900 g for 10 min and the serum separated.

Biochemical Parameters:
Fasting and Postprandial Blood Glucose:

Fasting Blood Sugar (FBS) and Postprandial Blood Sugar (PPBS) of rats were assessed. Plasma insulin was estimated by ELISA technique by using rat insulin kit. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) Index was also estimated.

Lipid Profile:

Serum Total Cholesterol (TC), Serum Trigly-cerides (TG), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and High Density Lipoprotein (HDL) were analyzed by using commercial diagnostic kit (Erba Diagnostic kit).

Glucose Homeostasis:

OGTT was performed on rats in all groups after an overnight fast on 20th day. All rats were fed with 0.35 g of glucose/100 g of b w [12]. Blood samples were collected from the tail vein at the indicated time points (0 hr, 0.5 hr, 1 hr, 1.5 hr and 2 hr) and evaluated these samples with a commercial hand-held Glucometer (ACCU-CHEK Active; Roche).

Statistical Analysis:

Statistical analysis was done using SPSS software version 16. Values were expressed in terms of Mean ± SD. To determine the significance of inter group differences, one way ANOVA followed by *'Post Hoc t tests'* were done. $P \leqslant 0.05$ was considered statistically significant.

Results:

Body weight and percent body weight gain of rats were significantly higher in group III (HFD fed rats with 30% fat) as compared to group I (control with 20 % fat) and group II (EEO fed rats) on 22nd day. However, group IV (EEO + HFD fed rats) showed significant decrease in both final body weight and % body weight gain of rats compared to group III (HFD) rats (Table 2).

TC and TG levels were significantly increased in group III (HFD with 30% fat) compared with group I (control with 20% fat) and group II (EEO fed rats). In case of group IV rats showed significant reduction of TC and TG as compared to group III (HFD with 30% fat). No significant difference was observed in the values of LDL and VLDL among all groups of rats however, HDL levels of group IV rats showed significant increase as compared to group III (Table 3).

FBS and PPBS levels were significantly increased in group III as compared to group I (control) and II (fed with EEO), whereas group IV (HFD with

30% fat + EEO) rats have shown significant decrease of FBS and PPBS as compared to group III (HFD with 30% fat). Fasting insulin levels were found to be significantly lesser in group III as compared to group I and II. Decreased insulin levels are concomitant of HOMA-IR index in group III compared to group I and II whereas an increase in insulin levels with concomitant increase in HOMA IR index were also observed in group IV rats as compared to group III (Table 4). Figure 1 depicts OGTT of all the experimental rats. Results showed an impaired glucose tolerance in group III (HFD with 30% fat) while group IV (HFD with 30% fat + EEO) showed an improved glucose tolerance (Fig.1).

Table 2: Effect of EEO on Body Weight of Rats Fed with High Fat Diet

Parameter	Group I	Group II	Group III	Group IV	ANOVA	
					F Value	P value
Initial body weight (g on 1st day)	214.3±7.6	207.0±5.5	245.3±6.2	221.3±7.2	3.88	0.056
Final body weight (g on 22nd day)	235.3±6.6	224.3±6.6	283.6±6.5 [a, b]	241.3±7.09 [c]	10.9	0.003*
Percentage of body weight gain	8.8±0.6	7.6±0.9	13.3±2.8 [a,b]	8.2±0.7	5.2	0.02*

Values are expressed as mean ± SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Group I is control rats (fat 20%), Group: II EEO fed rats, Group III: HFD fed rats (fat 30%), Group IV: HFD(fat 30%) + EEO fed rats. Superscript a, b, c, d express significant differences between groups. a depicts comparison with group I, b depicts comparison with group II, c depicts comparison with group III and d depicts comparison with group IV.(≤ 0.05).*

Table 3: Effect of EEO on Lipid Profile of Rats Fed with High Fat Diet

Parameter (mg/dl)	Group I	Group II	Group III	Group IV	ANOVA	
					F value	P value
TC	116.6 ± 4.1	126.6±5.8	139±8.4 [a,b]	134.6±3.7 [c]	17.2	0.001*
TG	96.0 ± 10.0	112.0 ± 2.0	122±5.2 [a,b]	114.6±12.0 [c]	5.12	0.029*
LDL	73.2±1.2	74.2±2.1	75.1±3.0	73.1±4.3	0.308	0.819
VLDL	24.0±2.1	22.9±0.6	19.2±2.02	22.9 ±2.4	3.6	0.065
HDL	31.3±1.5	35.3±2.5	31.0±1.0	36.0±2.0 [c]	6.008	0.019*

Values are expressed as mean ± SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Group I is control rats (fat 20%), Group: II EEO fed rats, Group III: HFD fed rats (fat 30%), Group IV: HFD(fat 30%) + EEO fed rats. Superscript a, b, c, d express significant differences between groups. a depicts comparison with group I, b depicts comparison with group II, c depicts comparison with group III and d depicts comparison with group IV.(≤ 0.05). TC- Total Cholesterol, TG- Triglycerides, LDL- Low Density Lipoproteins, VLDL- Very Low Density Lipoproteins, HDL- High Density Lipoproteins*

Parameters	Group I	Group II	Group III	Group IV	ANOVA	
					F value	P value
FBS (mg/dl)	96.3± 0.57	100.3± 3.7	128.6±3.2^{a,b}	115.3±1.1^{c}	50.98	0.000*
PPBS (mg/dl)	86.3±0.67	96.3±4.7	102.0±7.2^{a,b}	98.0±2.0^{c}	20.74	0.000*
Fasting Insulin (µU/ml)	6.8± 0.6	6.4 ± 0.3	5.65±0.3^{a,b}	5.8 ± 0.5^{c}	36.2	0.000*
HOMA-IR index	29.0±0.9	28.4±2.05	32.1 ± 1.6^{a,b}	30.4 ± 1.06$^{a, b, c}$	70.42	0.000*

Table 4: Effect of EEO on Glucose Homeostasis in Rats Fed with High Fat Diet

Values are expressed as mean ± SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Group I is control rats (fat 20%), Group: II EEO fed rats, Group III: HFD fed rats (fat 30%), Group IV: HFD(fat 30%) + EEO fed rats. Superscript a, b, c, d express significant differences between groups. a depicts comparison with group I, b depicts comparison with group II, c depicts comparison with group III and d depicts comparison with group IV.(≤0.05). FBS- Fasting Blood Sugar, PPBS - Post Prandial Blood Sugar, HOMA-IR-Homeostasis Model Assessment of Insulin Resistance*
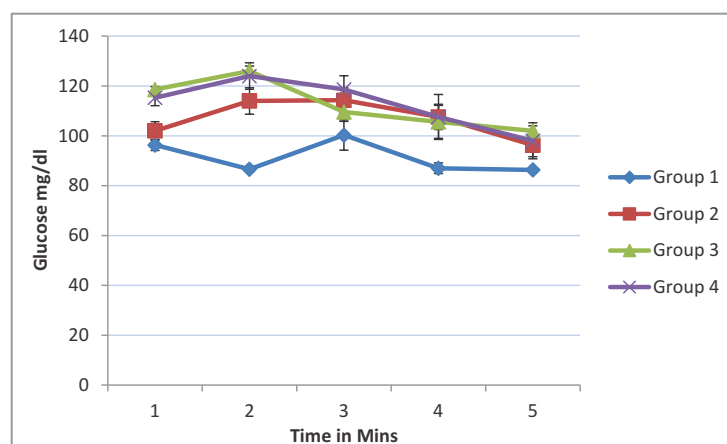

Fig. 1: OGTT of Rats Fed with High Fat Diet

Discussion:

In the present study, EEO was tested for its lipid lowering and glucose lowering activity in albino Wistar rats fed with hyperlipidemic diet. These protective effects of *Emblica officinalis* were evaluated by assessing lipid profile, fasting glucose, fasting insulin and HOMA IR.

Our results indicate that hyperlipidemic diet showed an increase in total body weight and percent of body weight gain of rats fed with HFD.

A possible explanation for the influence of high fat diet on weight gain is that diet induces a positive fat balance due to the loss of adjustment between fat oxidation and consumption. In the long term, this positive accumulation of fat can lead to weight gain and hence longer the duration of the diet, greater is the gain of body weight [13]. HFD with EEO supplementation prevents weight gain in rats, possibly due to altered regulation of lipid synthesis

[11]. *Emblica officinalis* supplemented to HFD fed rats showed significant changes in lipid profile by redistribution of lipoproteins possibly through its bioactive compounds like flavonoids which are capable to prevent LDL oxidation [7]. It has also been reported that ethanolic extract of *Emblica officinalis* may reduces cholesterol synthesis by inhibiting HMG Co-A reductase activity [14]. Another hypothesis is that the possible polyphenolic compounds of *Emblica officinalis* might have interfered and counteracted lipid peroxidation [14].

HFD is associated with insulin resistance and reduced insulin secretion by beta cells in the pancreas which may lead to altered glucose homeostasis in our study [4]. The possible link between hyperlipidemia and beta cell dysfunction of pancreas may be due to elevated plasma Free Fatty Acids (FFA). Lipotoxicity caused by elevation of FFA induces diabetogenic effect [15]. *Emblica officinalis* supplementation is effective in reducing blood glucose levels by regenerating and rejuvenating beta cells of pancreas and increasing insulin production and secretion 12[13]. *Emblica officinalis* is rich in polyphenolic contents (541.3 mg gallic acid equivalent/1 gm extract) might have glucose lowering effects [16]. Tannoids present in *Emblica officinalis* extract act as a strong inhibitors of aldose reductase [17].

Creg *et al*. (2016) reported in their study that after 6 weeks of hyperlipidemic, hypocholestremic diet, rats were presented with dyslipidemia, hyper-insulinemia and elevated levels of TG. These elevated TG increase glucose production, and acts on peripheral action of insulin [18]. It is reported that rats fed with high fat diet for 12 weeks developed insulin resistance along with increased levels of cholesterol [19]. Akkilar *et al.* (2016) observed that increased blood glucose, decreased plasma insulin with higher HOMA-IR in high fat fed rats clearly indicates an alteration of glucose homeostasis [20].

Our observation on EEO supplementation to HFD fed rats (Group IV) showing a reduction in fasting and postprandial blood glucose levels supports the study conducted by Akhtar *et al*. [21].

The decrease in TC and TG levels with supplementation of *Emblica officinalis* in our study hypothesizes that a possible inhibition of lipolysis in adipose tissue due to insulin sensitizing or insulin mimetic effect of polyphenolic compound of *Emblica officinalis*. This may suggest that *Emblica officinalis* have lipid lowering activity by acting and sensitizing insulin receptors [7].

The efficiency of *Emblica officinalis* in hyperlipidemea indicates a protective measure against dyslipidemia and regulation of blood sugar levels. Probably polyphenolic compounds like tannins, gallic acids, emblicanin (A & B) and flavonoids might have play the protective role by various means including antioxidant activities, lipid regulations and glucose homeostasis in high fat fed experimental animals.

Conclusion:

High fat diet causes dyslipidemia and altered glucose homeostasis in rats. Ethanolic extract of *Emblica officinalis* supplementation are found to be protective against high fat diet induced altered lipid and glucose metabolism.

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