

Anti-obesity and hypo-lipidemic activity of *Eleusine indica* against high fat diet-induced obesity in rats

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Abstract

In present study the pharmacological activity of *Eleusine indica* extract for Anti-obesity and Hypo-lipidemic effects in animal model. The phytochemical components of extract like terpenoids, saponin, alkoids and flavonoids, which already implicated for anti-hyperlipidemic activity. There was a comparative reduction in body weight of methanolic extract of *Eleusine indica* treated groups at “200”and ‘400 mg’/kg, atorvastatin “10 mg’/kg .when compared to the high fat diet administered rats. The results also suggested that methanolic extract of *Eleusine indica* at “200mg’/kg and 400 mg/kg are capable preventing the body weight gain this result was in accordance with the result reported from previous study where a dose dependant decrease in the body weight was observed. This study also proved remarkable increased in locomotor activity when compared with high fat administered rats. Whereas in obese rats there was reduction in locomotors activity. Present study also reported that methanolic extract of *Eleusine indica* significant [P<0.001] decreased the level of LDL and Triglycerides also apart from reduction in weight, supplementation with MEEI was observed to attenuate significantly [P<0.01] decreased the levels of total cholesterol and significantly increased [P<0.01] the HDL level in rats feed with HFD.

Keyword: *Eleusine indica*; Methanolic extract; Anti-obesity; Hypo-lipidemic

1. Introduction

According to the World Health Organization (WHO), obesity is the accumulation of excessive body fat negatively affecting health. It was declared one of the top ten health risk conditions in the world, affecting about 300 million people worldwide (60 million adults in the United States alone;” 1”As simple as the definition of obesity appears, establishing the best technique to measure obesity has been a matter of dispute. The most accurate techniques to assess body fat, such as hydrostatic weighing, absorptiometry, bioelectrical impedance and computed tomography are also the most technically challenging, demanding specialized training and equipment and, therefore, the most expensive. For these reasons, skin fold thicknesses and weight-and-height indexes have been adopted as substitute measurement methods in clinical and public health works. Skin fold thickness is a measurement of the thickness of the double layer of skin and subcutaneous tissue obtained with specialized calipers.”2” this method can be used to estimate fatness and characterize its distribution. The main advantage of this measurement is the possibility to determine adiposity levels. However, this assessment has important limitations such as standardization and reproducibility of measurements. Because of these limitations, weight-and-height indexes are most commonly used worldwide as an assessment of obesity.”3-5”

Worldwide, more than one billion adults are overweight and about 300 million are obese. Obesity and overweight have increased dramatically in several countries. For example, in less than 10 years in China (1989 – 1997) the prevalence of overweight individuals doubled in women and tripled in men”6”

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Based on BMI values, according to data from the National Health and Nutrition Examination Survey (NHANES III, 1999-2000), almost 65% of the American adult population is overweight. These data represent an increase from the already high incidence rate of 56% found in the previous assessment (NHANES III, 1988-1994). The incidence of obesity in adults paralleled this tendency and increased from 23% to 31% during the same period of time⁸⁻⁹. Interestingly, according to the NHANES III (1988-1994), the prevalence of obesity in American adults was higher in women than in men. A higher prevalence of overweight women was found in African-American (37%) and Mexican-American (33%) populations than in the Caucasian population (22.7%). This pattern was not observed for men.¹⁰ This difference in obesity prevalence between genders has been tentatively explained by the regulation of body fat distribution by sex hormones. Usually, women have higher proportion of fat relative to total body weight than men. This characteristic is thought to be evolutionarily advantageous because of the metabolic burden of birth and care of children associated with women.¹¹

1.1. Plant Materials

The whole plant part (fruit, Leaves, stems, Roots) of *Eleusine indica* was used for investigation of Antiobesity and Hypolipidemic activity.

2. Material and method

2.1. Collection and authentication of plant materials.

The fresh whole plant of *Eleusine indica* were collected in the month of October 2017 from the natural habitat at Pusad in Yavatmal district region, Maharashtra, India. The plant was authenticated by Dr. Marathe sir of Botany Department: Nanded S.R.T.M University, India. A voucher specimen [No: DDK-001] was deposited at Herbarium, Department of Botany, SRTM University Nanded.

2.2. Preparation of crude extract

The collected whole plant were dried under shade, coarsely powdered in electric blender and subjected to successive extraction by soxhlets extract or using methanol as solvent until the termination of extraction. The extract were concentration by evaporation at room temperature Then the yield was calculated with reference to air dried basis finally the extract were transferred to air tight container and stored for further pharmacological activity.¹²

2.3. Preliminary phytochemical screening

2.3.1. Test for Carbohydrates

To 0.5ml of filtrate, "0.5 ml" of Benedict's reagent is added. The mixture is then heated on a boiling water bath for 2 min. A characteristic colored precipitate indicates the presence of sugar.

2.3.2. Test for Alkaloids

To a few drops of plant sample extract, 2 drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids. Test for Amino acids To 2 ml of aqueous filtrate, 2 drops of Ninhydrin solution is added. Appearance of purple color indicates the presence of amino acids

2.3.3. Test for Theo bromine

The extract is dissolved in 2 test tubes with '1ml" of hot water and "1 ml" of cold water each. If the extract dissolves in hot water and does not dissolve in cold water, it indicates the presence of Theo bromine.

2.3.4. Test for Saponin

The extract is diluted with distilled water and made up to '20 ml". The suspension is shaken for 15 min. Formation of foam indicates the presence of Saponin.

2.3.5. Test for Glycosides

To 2 ml of filtered hydrolyses, "3ml" of chloroform is added, shaken and the chloroform layer is separated, and 10% ammonia solution is added to it. Pink color indicates presence of glycosides.

2.3.6. Test for Phytosterols

The extract is dissolved in “2 ml” of acetic anhydride. To this, 1 or 2 drops of concentrated sulfuric acid is added slowly along the sides of the test tube. An array of color change shows the presence of Phytosterols.

2.3.7. Test for Flavonoids

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

2.3.8. Test for Tannins

To a few drops of extract, “2ml” of dilute ferric chloride solution (5%) is added. Formation of violet color indicates the presence of tannins.

2.3.9. Test for Terpenoids

To a “2 ml” of extract, few drops of saturated solution of trichloro acetic acid are used. Formation of colored precipitate confirms the presence of Terpenoids.

2.4. Pharmacological study

Wister rats (150-200) of either sex procured for studying the ability of methanolic extract of *Eleusine indica* to offer Antiobesity and Hypolipidemic activity in rat model. In - vivo study using experiential model is the best way of validating and testifying the results in order determine efficacy, potency and therapeutic nature of a drug. There are no of way by which screening method have been to validate the efficacy of various herbal drug. Animal study is best method to obtained authentic results concerning activity of any drug. No study can be accomplished without help of animal model and animal study. There for selection of proper animal model and screening method is very important for the success of overall study. Although ultimate beneficiaries of any in vivo study are human being, but it is not possible to use human for this purpose. Hence appropriate model where design which closely resemble to the human body environment and can be there for to be used to validate these results. To investigate possible potential of methanolic extract of *Eleusine indica* as therapy for anti-obesity and hypo lipid emic activity , the present study examine the effect of *Eleusine indica* on experimentally high fat diet induce obesity in rat .”13” the Protocol was approved by the Institution of Animal Ethics committee (Reg.no. 1865/PO/Re/S/16/CPCSEA19/02/2021). Rajarshi Shahu College of pharmacy, Buldana

2.5. Estimation of Blood Related Parameter

2.5.1. Triglycerides

Centrifuge the blood sample at 3000 RPM for 10 minutes to separate the serum. Transfer the serum to a clean test tube. Take 3 clean test tubes and add the reagents as follows by using micropipettes. Mix all the tubes well and incubate at “37 °C” for 10 minutes and the read the absorbance of Blank, Standard and Test using UV-spectrophotometer at 546 nm against distilled water as blank.”14-15”

2.5.2. HDL-cholesterol

Add “200 µl” of serum sample and “300 µl” of HDL-precipitating reagent in a clean test tube. Mix gently for 5 min. and stand at room temperature for 10 min. Centrifuge it at 3000 RPM for 10 minutes and separate the supernatant into another test tube. Use the supernatant for the estimation of HDL concentration in the serum sample. Take 3 clean test tubes and add the reagents as follows by using micropipettes. Mix all the tubes well and incubate at “370 C” for 10 minutes and the read the absorbance of Blank, Standard and Test using UV-spectrophotometer at 505 nm against distilled water as blank.

3. Result and Discussion

The phytochemical investigation of plant contain alkaloids, terpenes, flavonoids, tannins, anthraquinones, saponin and cardiac glycosides thus revealing an enormous medicinal value of this plant. The phytochemical components of extract like terpenoids, saponin, alkaloids and flavonoids, which already implicated for anti hyperlipidemic activity.

Table 1 Preliminary phytochemical analysis of *Eleusine indica*

Phytochemicals	Methanol extract	Aqueous extract	Ethanol extract
Flavonoids	+++	++	++
Tannins	+	-	-
Anthraquinones	+	+	++
Alkaloids	++	+++	++
Saponins	-	-	-
Cardiac glycosides	++	+	+
Anthracene glycosides	++	-	+

3.1. Effect of *Eleusine indica* extracts on Total Cholesterol in different treatment groups

The mean value of cholesterol in high fat diet treated group was found to be significantly high ($p < 0.01$) when compared with normal group. Whereas significantly ($p < 0.01$) decreased in cholesterol level in extract (200 mg/kg and 400 mg/kg) treated and atorvastatin (10 mg/kg) treated group was observed when compared with high fat diet groups.

Table 2 Total Cholesterol in different treatment groups

SN	Control	HFD	Low dose (200g/kg)	High Dose (400g/kg)	Standard
1	140.22	235.52	170.51	168.48	152.30
2	138.22	232.25	168.56	165.23	150.25
3	140.24	235.36	170.24	162.25	145.19
4	145.34	238.21	160.00	158.21	160.36
5	130.20	240.24	172.41	170.24	156.45
S.E. M	138.844±5.5	236.31±3.044a**	168.344±4.8 ^{aa}	160.236±4.7 ^{aa}	152.91±5.81 ^{aa}

Values represents as Mean ±SEM (n=5). **P=0.01, High fat group group compared against control group; aa P= 0.01, Extract treated(200 & 400 mg), atorvastatin groups compared against HFD control group. Significance determined by ANOVA followed by Dunnett's test.

3.2. Effect of *Eleusine indica* extract on Total Triglycerides in different treatment group

Table 3 Total Triglycerides in different treatment group

Sr. No	Control	HFD	Low dose [200mg/kg]	High Dose [400mg/kg]	Standard [10mg/kg]
1	132.20	240.26	180.21	160.41	150.52
2	125.33	230.47	183.40	150.28	145.31
3	135.42	232.21	175.34	163.42	130.36
4	140.25	200.24	180.27	165.37	132.52
5	127.74	210.84	179.34	160.57	135.23
S.E.M	132.188±2.66	222.804±7.430**	179.712±1.293 ^{aa}	160.0±2.603 ^{aa}	138.788±3.893 ^{aa}

Values represents as Mean ±SEM (n=5).; **P=0.01, High fat diet group group compared against control group; ^{aa} P= 0.01, Extract treated(200 & 400 mg), atorvastatin groups compared against HFD control group. Significance determined by ANOVA followed by Dennett's test. ANOVA followed by Dennett's test.

The mean value of Triglycerides in high fat diet treated group was found to be significantly high ($p < 0.01$) when compared with normal group. Whereas significantly ($p < 0.01$) decreased in Triglycerides level in extract (200 mg/kg

and 400 mg/kg) treated and atorvastatin (10 mg/kg) treated group was observed when compared with high fat diet groups.

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3.3. Effect of *Eleusine indica* extract on Total HDL in different treatment group

Table 4 Total HDL in different treatment group

Sr. No.	Control	HFD	L.D (200mg/kg)	H.D (400mg/kg)	Standard (10mg/kg)
1	52.21	23.32	27.31	35.42	37.34
2	62.34	29.46	30.47	35.18	46.25
3	68.46	29.27	32.46	40.00	45.68
4	58.12	27.34	30.45	38.42	47.25
5	60.42	28.45	33.52	40.23	45.26
S.E.M	60.31±5.93	27.56±2.5 **	30.84±2.3 ^{aa}	37.85±2.4 ^{aa}	44.35±3.99 ^{aa}

Values represents as Mean ±SEM (n=5); **P=0.01, High fat group group compared against control group.^{aa} P= 0.01, Extract treated (200 & 400 mg), atorvastatin groups compared against HFD control group Significance determined by ANOVA followed by Dennett's tes

The mean value of HDL in high fat diet treated group was found to be significantly low ($p < 0.01$) when compared with normal group. Whereas significantly ($p < 0.01$) high in HDL level in extract (200 mg/kg and 400 mg/kg) treated and atorvastatin (10 mg/kg) treated group was observed when compared with high fat diet groups.

Table 5 Total LDL in different treatment group

Sr.no	Control	HFD	L.D (200mg/kg)	H.D (400mg/kg)	Standard (10mg/kg)
1	42.00	103.00	61.29	60.15	46.25
2	34.00	98.04	54.73	61.75	37.7
3	42.00	100.54	58.08	47.97	35.47
4	45.00	119.86	47.61	44.63	52.75
5	48.00	115.91	57.09	72.98	49.77
S.E.M	40.61±3.281	107.47±4.36**	55.62±2.36 ^{aa}	57.56±5.10 ^{aa}	44.29±3.4 ^{aa}

Values represents as Mean ±SEM (n=5); **P=0.01, High fat diet group compared against control group; ^{aa}P= 0.01, Extract treated(200 & 400 mg), atorvastatin groups compared against HFD control group. Significance determined by ANOVA followed by Dennett's test.

The mean value of LDL in high fat diet treated group was found to be significantly high ($p < 0.01$) when compared with normal group. Whereas significantly ($p < 0.01$) low in LDL level in extract (200 mg/kg and 400 mg/kg) treated and atorvastatin (10 mg/kg) treated group was observed when compared with high fat diet groups

3.4. Blood Analysis Summery Data

There was a comparative reduction in body weight of methanolic extract of *Eleusine indica* treated groups at 200 and 400 mg/kg, atorvastatin 10mg/kg .when compared to the high fat diet administered rats. The results also suggested that methanolic extract of *Eleusine indica* at 200mg/kg and 400 mg/kg are capable preventing the body weight gain this result was in accordance with the result reported from previous study where a dose dependent decrease in the body weight was observed

Table 6 Blood Analysis Summary Data

Group	Parameters			
	Total cholesterol	Triglycerides	HDL	LDL
Control	138.844±5.503	132.188±2.66	40.61±3.281	40.61±3.281
HFD	236.31±3.044a**	222.804±7.430**	107.47±4.36**	107.47±4.36**
Low dose[200mg/kg]	168.344±4.8 ^{aa}	179.712±1.293 ^{aa}	55.62±2.36 ^{aa}	55.62±2.36 ^{aa}
High dose[400mg/kg]	160.236±4.7 ^{aa}	160.0±2.603 ^{aa}	57.56±5.10 ^{aa}	57.56±5.10 ^{aa}
Standard	152.91±5.811 ^{aa}	138.788±3.893 ^{aa}	44.29±3.4 ^{aa}	44.29±3.4 ^{aa}

3.5. Locomotors Activity

This study also proved remarkable increased in locomotor activity when compared with high fat administered rats. Whereas in obese rats there was reduction in locomotor activity. Further dyslipidemia is another importance hallmark in the pathogenesis of obesity characterized by hypertriglydemia with decreased level of LDL and VLDL

Table 7 Locomotors Activity

Sr. No	Control Group	High fat diet	Low dose [200mg/kg]	High dose [400mg/kg]	Standard [10mg/kg]
Day 1	35.56±2.33	60.34±7.51	56.33±4.33	43.65±4.22	39.55±3.55
Day 15	36.38±4.22	62.69±6.42	58.64±3.66	40.75±3.45	38.54±3.45
Day 30	36.42±3.44	64.85±8.35	52.24±5.24	38.66±4.66	37.66±4.21
Day 45	37.20±4.35	66.96±7.65	51.78±4.57	38.42±3.45	35.45±3.24

Present study also reported that methanolic extract of *Eleusine indica* significant [P<0.001] decreased the level of LDL and Triglycerides also apart from reduction in weight, supplementation with MEEI was observed to attenuate significantly [P<0.01] decreased the levels of total cholesterol and significantly increased [P<0.01] the HDL level in rats feed with HFD. The increase in the level of HDL was found to be in a dose dependent manner; that is, supplementation with MEEI at a dose of 400mg/kg shows a better effect in comparison to 200mg/kg.

4. Conclusion

The phytochemical investigation of plant contain alkaloids, terpens, flavonoids, tannins, anthraquions, saponin and cardiac glycosides thus revealing an enormous medicinal value of this plant. The phytochemical components of extract like terpenoids, saponin, alkaloids and flavonoids, which already implicated for antihyperlipidemic activity. On the basis of result obtained in present investigation it can be concluded that *Eleusine indica* has potent antiobesity and hypolipidemic activity which has nearly close to standard drug atorvastatin. Further study is required to evaluate mode of action of methaolic extract of *Eleusine indica* in the manegment of obesity and hyperlipidemia.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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