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**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.1185151>Available online at: <http://www.iajps.com>**Research Article****HYPOLIPIDEMIC ACTIVITY OF AEGLE MARMELOS LEAVES
EXTRACT ON ALBINO WISTAR RATS****Dr. Venu Sampath Kumar. G¹, Fathima Nilesh Karanam², CH. S. Phani Kumar³ and
B. T. N. Vamsi Krishna⁴**¹ A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam,
Andhra Pradesh, India.² Viswanadha Institute of Pharmaceutical Sciences, Mindivanipalem, Visakhapatnam,
Andhra Pradesh, India.³ Vikas Institute of Pharmaceutical Sciences, Nidigatla, Rajamahendravaram,
Andhra Pradesh, India.⁴ Viswanadha Institute of Pharmaceutical Sciences, Mindivanipalem, Visakhapatnam,
Andhra Pradesh, India.**Abstract:**

Aegle marmelos commonly known as bael, wood apple and stone apple, belongs to the family Rutaceae. It is a deciduous shrub. Young leaves are pale green or pinkish, finely hairy while mature leaves are dark green and completely smooth and are used extensively in the indigenous system of medicine as an anti-diabetic agent and also as hypolipidemic agent. This study is aimed to evaluate the hypolipidemic activity of aqueous leaf extract of *Aegle marmelos*. The hypolipidemic activity of the plant were studied by administering aqueous leaf extract on Albino Wistar rats, using serum lipid profile i.e., high density lipo-protein (HDL), very low-density lipo-protein (VLDL), total cholesterol (TC) and triglyceride (TG) profile. The animals were divided into six groups, each group consists of six rats and the study is designed by following standard protocol for the evaluation of hypolipidemic activity. In this study, the treatment with standard hypolipidemic drug Pioglitazone is compared with the treatment of aqueous leaf extract of *Aegle marmelos* at dose levels of 100, 200 and 400mg/kg body weight. Hypolipidemic activity was observed best with all the dose levels based on lipid profiles (VLDL, HDL, TC and TG).

Key words: *Aegle marmelos*, Hypolipidemic activity, VLDL, HDL, TC and TG.**Corresponding author:****K. Fathima Nilesh**

Viswanadha Institute of Pharmaceutical Sciences,
Mindivanipalem, Visakhapatnam,
Andhra Pradesh, India.

Email: nileshkaranam@gmail.com

Mobile: +91-8977481593

QR code



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INTRODUCTION:

Bael (*Aegle marmelos*) has been known to be one of the most important medicinal plants of India since Charak (1500 B.C). More than 100 phytochemical compounds have been isolated from various parts of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins. These compounds are well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders [1]. *Aegle marmelos* is a traditional medicinal plant grows in India, Srilanka, Myanmar and Malaysia, commonly known as bale, wood apple and stone apple, belongs to the family Rutaceae. With respect to the available literature, the leaves consist of constituents (flavonoids) to treat hyperlipidemic conditions [2] Every part of *Aegle marmelos* plant such as its fruits, stem, bark, and leaves possesses medicinal property and is used for treating various eye and skin infections [3]. Leaf is considered to be one of the highest accumulatory parts of the plant containing bioactive compounds which are synthesized as secondary metabolites [4].

Cholesterol is the building block for cell membrane and a precursor of steroid hormones [5]. It forms several distinct particles with lipoproteins, mainly high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) [6]. It is well established that LDL and VLDL cholesterol levels are atherogenic whereas HDL-cholesterol has protective effects on the development of atherosclerosis [7]. Increased LDL and VLDL levels are the major independent risk factor for cardiovascular events whereas low level of HDL and elevated triglycerides (TG) are also recognized as residual risk for cardiovascular diseases [8]. Agents with the ability to decrease LDL/VLDL or total cholesterol levels, increase HDL cholesterol or lower TG have beneficial effects on preventing cardiovascular diseases. Hyperlipidemia is a risk factor contributing to atherosclerosis and occurrence of coronary heart disease and cerebrovascular accidents [9]. Hence, hypolipidemic molecules gain importance in curing those diseases. Hence, extracts of different doses were tried to know the hypolipidemic activity on diabetes mellitus (DM) induced Albino wistar rats by administering through intra-muscular injection. The effect of dose on hypolipidemic activity was studied based on serum lipid profile i.e. high-density lipoprotein level (HDL), very low-density lipoprotein level (VLDL), total cholesterol and triglyceride levels.

MATERIALS AND METHODS:

1. Preparation of leaves extract:

The leaves of *Aegle marmelos* was washed thoroughly with fresh water and dried under shade. The dried leaves were grounded finely in a mortar using pestle. The resultant powder was passed through sieve number 60. Then, 50 grams of powder was weighed on electronic balance and soaked in 250 ml distilled water for 8 hrs and mixed occasionally. The resultant mixture was passed through muslin cloth and filtered. The filtrate was centrifugated at 10000 rpm under room temperature and solids are separated by passing through a filter paper. The resultant filtrate was concentrated to reach 100 ml using a rotary vacuum pump and the concentrate was standardized to know the strength.

2. In vivo studies:

A) Preparation of animals:

Albino wistar rats were selected based on their body weight i.e., not less than 180-200 grams, housed, feeded and maintained with respect to CPCSEA guidelines for 15 days to suit the laboratory conditions.

B) Induction of diabetes mellitus (DM):

The rats were induced with DM by administering 50 mg/kg body weight Streptozotocin [10] in normal saline solution through intra-peritoneal injection. The diabetes level was observed for 72 hrs and the rats with greater than 200mg/dl glucose levels were considered for study.

C) Experimental design:

All the rats were fasted overnight and divided into six groups and each group consists of three rats, and DM induced rats were feeded with glucose solution to avoid hyper-glycemic condition at night times and study design was as follows:

Group I: Normal rat - as untreated control, administered with normal saline solution by intra-muscular route.

Group II: DM induced rats as untreated diabetic control, administered with normal saline solution by intra-muscular route.

Group III: Treatment with standard Pioglitazone.

Group IV: DM induced rats, administered with 100 mg/kg body weight per rat by intra-muscular route.

Group V: DM induced rats, administered with 200 mg/kg body weight per rat by intramuscular route.

Group VI: DM induced rats, administered with 400 mg/kg body weight per rat by intramuscular route.

D) Collection of blood samples, separation and analysis of serum:

1 ml of blood was collected from the rat by following eye vein puncture method and serum was separated by standing the blood until it was clotted and centrifuged for 10 minutes at 4000 rpm. The serum was collected using auto

pipette and lipid profile (HDL, VLDL, TC and TG) was analyzed using Lyons *et al.* (1992) [11] method.

E) Statistical analysis:

Values are presented as mean \pm S.E.M. Statistical difference between treatments and the controls were tested by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test using the "Stat" statistics computer program [12]. A difference in the mean values of $P < 0.05$ was considered to be statistically significant.

RESULTS:

Table 1: Effect of Pioglitazone, selected plant extract on blood glucose levels in normal and Streptozotocin induced diabetic rats during 4 weeks of study.

Group (n= 6)	Treatment	0th Day	7th Day	14th Day	28th Day
I	Normal control (1% gum acacia)	98.67 \pm 1.5	101 \pm 2.4 ^{ns}	93.2 \pm 1.6 ^{ns}	97.4 \pm 1.94 ^{ns}
II	Diabetic control	412.1 \pm 2.4	419.2 \pm 2.2 [#]	433.6 \pm 0.7	439 \pm 0.79
III	Pioglitazone (10 mg/kg)	476.2 \pm 4.0 [#]	358.5 \pm 4.54 [#]	157 \pm 3.25	101 \pm 3.27 [#]
IV	<i>Aegle marmelos</i> extract (100mg/kg)	436.41 \pm 5.31 [#]	364.2 \pm 2.5 [#]	189 \pm 2.75 [#]	145.7 \pm 1.56 [#]
V	<i>Aegle marmelos</i> extract (200mg/kg)	418.41 \pm 5.31 [#]	328.2 \pm 2.5 [#]	141 \pm 2.75 [#]	121.7 \pm 1.56 [#]
VI	<i>Aegle marmelos</i> extract (400mg/kg)	430.83 \pm 5.62 [#]	320.4 \pm 6.32 [#]	134.2 \pm 1.45 [#]	112.2 \pm 1.36 [#]

$P < 0.05$ [#], $P < 0.01$ [#], $P < 0.001$ [#] significantly decreased Blood glucose levels when compared with disease control and $P < 0.001$ [#] significantly increased when compared with normal control followed by one way ANNOVA followed by Dunnett's multiple comparison test.

Table 2: Blood serum profile (BSP) of various groups of Albino Wistar Rats.

Group (n=6)	Treatment	HDL Values (mg/dl)	VLDL values (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
I	Normal control (1% gum acacia)	23.08±1.61	17.36±0.68	99.27±2.41	124.46±3.15
II	Diabetic control	17.02±2.16	25.76±3.24	188.72±2.25	199.75±2.21
III	Pioglitazone (10mg/kg)	19.42±2.23	24.25±0.29	177.12±1.10	187.01±1.26
IV	<i>Aegel marmelos</i> extract (100mg/kg)	24.19±0.64	23.01±1.56	138.51±1.23	173.86±1.48
V	<i>Aegel marmelos</i> extract (200mg/kg)	31.79±1.62	22.58±1.83	120.93±1.86	156.92±1.77
VI	<i>Aegel marmelos</i> extract (400mg/kg)	40.10±0.94	18.24±2.71	100.43±0.78	130.27±1.84

P<0.05*, P< 0.01 **, P< 0.001 *** significantly increased the % change when compared with disease control and P<0.001# significantly increased when compared with normal control and P>0.05^{ns} non-significant, analyzed by two way ANNOVA followed by Bonferroni post-tests.

Result description of Blood Glucose Levels:

Table No.1: Summarize the effect of different treatment groups on reduction of serum FBS of STZ induced albino wistar rats. The reduction of serum FBS after treatment from 0th week to 4th week was found to be 98.67 ± 1.5 to 97.4 ± 1.94 in Normal control; 412.1 ± 2.4 to 439. ± 0.79 in Disease control; 476.2 ± 4.0 to 101 ± 3.27 in (STD/pioglitazone); 436.41 ± 5.31 to 145.7 ± 1.56 in low dose (100mg/kg); 418.41 ± 5.31 to 121.7 ± 1.56 in mid dose (200mg/kg); 430.83 ± 5.62 to 112.2 ± 1.36 in high dose (400mg/kg); depicted in **Graph No. 1**

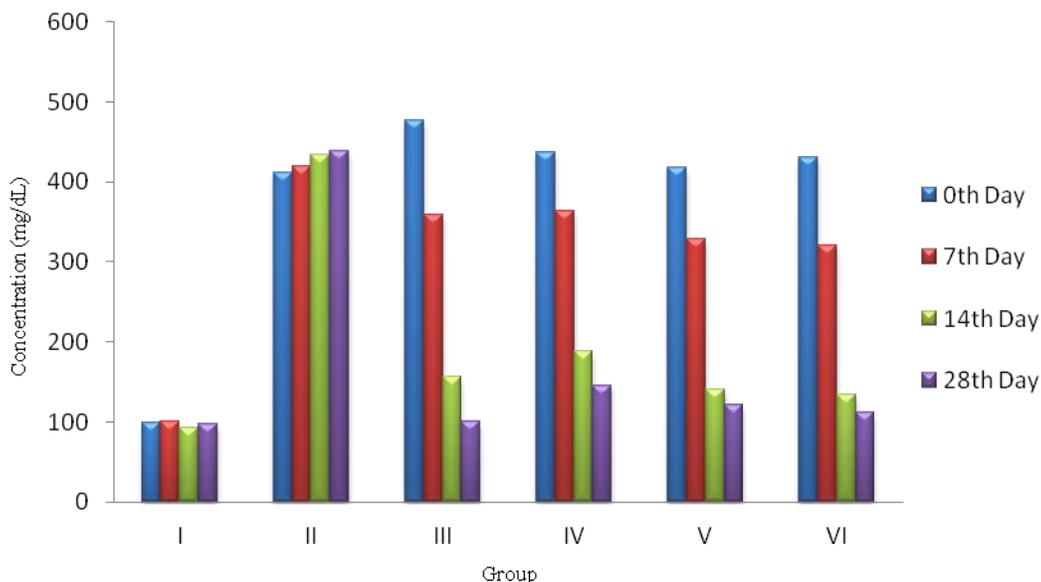
Result description of Lipid Profile:

Table No.2: Summary of the effect of treatment groups on lipid profile at the end of the study was described as, The serum HDL (mg/dl) levels of 0th to 4th week were significantly decreased in diabetic rats (17.02±2.16) when compared to normal control rats (23.08 ± 1.61), whereas the different treatment and standard groups significantly increased the HDL (mg/dl) levels in a dose dependent manner when compared to diabetic rats is depicted on **Graph No.2**. The order of % change of HDL is more in pioglitazone (40.10±0.94) and various doses of plant

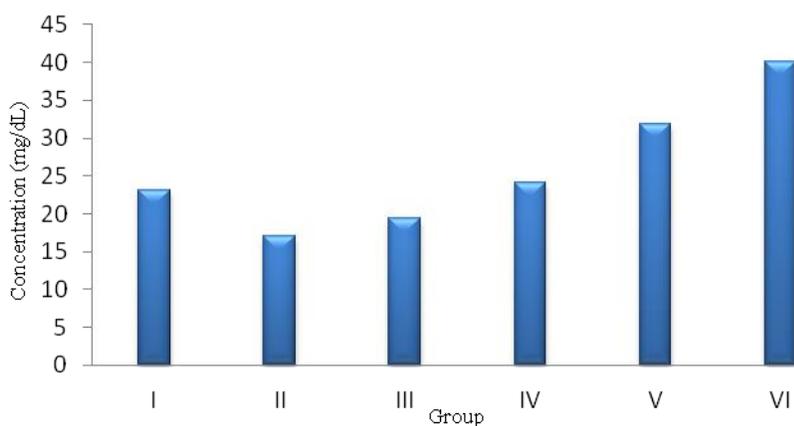
extract have shown (19.42±2.23, 24.19±0.64 & 31.79±1.62). The serum VLDL (mg/dl) levels of 0th to 4th week were significantly increased in diabetic rats (25.76±3.24) when compared to normal control rats (17.36 ± 0.68), whereas the different treatment and standard groups significantly decreased the VLDL (mg/dl) levels in a dose dependent manner when compared to diabetic rats is depicted on **Graph No.3**. The order of % reduction of VLDL is more in pioglitazone (18.24±2.71) and various doses of plant extract have shown (24.25±0.29, 23.01±1.56 & 22.58±1.83). The serum Total cholesterol (TC) (mg/dl) levels of 0th to 4th week were significantly increased in diabetic rats (188.72±2.25) when compared to normal control rats (99.27 ± 2.41), whereas the different treatment and standard groups significantly decreased the TC (mg/dl) levels in a dose dependent manner when compared to diabetic rats is depicted on **Graph No.4**. The order of % reduction of TC is more in pioglitazone (100.43±0.78) and various doses of plant extract have shown (177.12±1.10, 138.51±1.23 & 120.93±1.86). The serum Triglycerides (mg/dl) levels of 0th to 4th week were significantly increased in diabetic rats (199.75±2.21) when compared to normal control rats

(124.46 ± 3.15), whereas the different treatment and standard groups significantly decreased the Triglycerides (mg/dl) levels in a dose dependent manner when compared to diabetic rats is depicted on

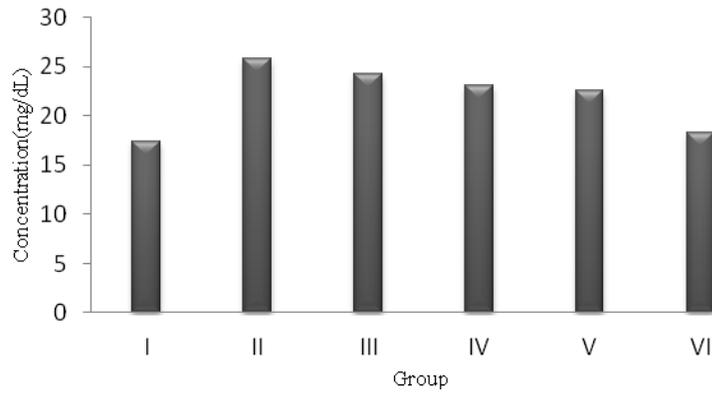
Graph No.5. The order of % reduction of Triglycerides is more in pioglitazone (130.27 ± 1.84) and various doses of plant extract have shown (187.01 ± 1.26 , 173.86 ± 1.48 & 156.92 ± 1.77).



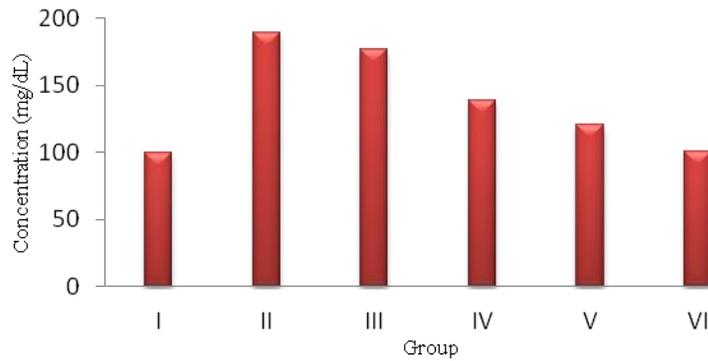
Graph 1: Effect of different treatment groups on reduction of serum FBS of STZ induced Albino Wistar Rats



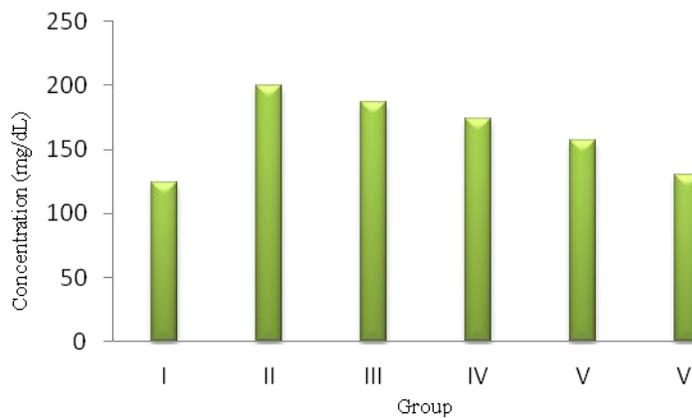
Graph 2: HDL Profile of Rat Serum for different groups



Graph 3: VLDL Profile of Rat Serum for different groups



Graph 4: Total cholesterol Profile of Rat Serum for different groups



Graph 5: Total Triglycerides Profile of Rat Serum for different groups.

DISCUSSION:

Anti-diabetic activity:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both [13]. Long term diabetes leads to a series of metabolic aberration causing vascular pathology. Diabetes patients usually show varied symptoms of polyurea, polydypsea and polyphagia. In severe forms weight loss can be seen, while in some cases, symptoms may be absent and consequently hyperglycemia may remain undetected. Many synthetic drugs, though capable of managing the disease, produce number of adverse effects. Hence, a new approach of plants or herbs with blood glucose lowering capacity was preferred.

In present study, a single i.p. injection of STZ (45 mg/kg of body wt.) to rats resulted in severe hyperglycemia, damage of Islets of Langerhans and β cells, elevation in cholesterol level and decreased body weight in albino Wistar rats. STZ has been widely used for the induction of diabetes mellitus in various experimental animals, it produce diabetes mellitus by cytotoxic action on pancreatic β - cells results in insulin deficiency. Extract of these medicinal plants were given orally with the help of a gastric tube to STZ induced diabetic rats. Further, samples of blood were collected 0th, 7th, 14th and 28th in multi dose treatment study (Sub acute study). Control animals received equal volume of 1% gum acacia.

The results of blood glucose level estimation obtained from STZ induced diabetic rats, in single dose treatment study, indicated that the ethanolic extract of *Aegle marmelos* ($P < 0.01$) and standard drug- Pioglitazone ($P < 0.01$) showed highly significant antidiabetic activity. Whereas in multidose treatment study these extracts and Pioglitazone had shown highly significant ($P < 0.01$) antidiabetic activity when compared with diabetic control rats.

Induction of diabetes with STZ is associated with a characteristic loss of body weight, which is due to increased muscle wasting, loss of tissue proteins. In this study, highly significant prevention of reduction in body weight observed from 14th day onwards ($P < 0.001$), during the period of treatment of the rats, with *Aegle marmelos* (400 mg/kg/day) and Pioglitazone (10 mg/kg/day) treated group. This indicates the possible action including acting through pancreatic mechanism or by inhibition of glucose absorption through gastrointestinal tract.

Effect of selected plant extract *Aegle marmelos* on serum lipid profile:

The Cholesterol and triglycerides are the major plasma lipids, essentially used for the synthesis of cell membrane and hormone. The levels of serum lipids are usually elevated in diabetes mellitus. This abnormal high level of serum lipids is mainly due to inhibition of antilipolytic actions of insulin as it is being resistance in diabetes. It is reported that the hypercholesterolemia and hypertriglyceridemia occurs in STZ induced diabetic rats [14]. As the selected plant extracts have antihyperglycemic activity they ameliorate the insulin resistance. The increased insulin level might be responsible decreasing lipolysis and inhibit further release of free fatty acid from the lipids.

The selected plant extract showed a significant decrease in the levels of TC, TG, VLDL, LDL and significant increase in HDL- levels in a dose dependent manner.

CONCLUSION:

From the above results, we have concluded that the leaf extracts of *Aegle marmelos* showed better hypolipidemic activity at different dose levels by comparing with that of the standard (Pioglitazone) and at all dose levels the HDL values showed increase in the dose dependent manner and vice versa for VLDL, TC and TG. Hence, the leaves of *Aegle marmelos* have Hypolipidemic activity which can be used for performing further studies and also in the development of new Pharmaceutical activities.

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