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EVALUATION OF *IN VIVO* ANTIDIABETIC ACTIVITY OF *AEGLE MARMELOS* FRUITS USING GLYCOSYLATED HAEMOGLOBIN (HBA1C) METHOD

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ABSTRACT

Aegle marmelos, commonly known as bael, Bengal quince, golden apple, stone apple, wood apple, bili, is a species of tree native to India. In the system of Ayurveda this drug was used in a number of diseases such as gastro intestinal diseases, piles, oedema, jaundice, vomiting, obesity, pediatric disorders, gynecological disorders, urinary complaints and as a rejuvenative. In our research we evaluated the antidiabetic activity of the fruits of Aegle *marmelos* by glycosylated haemoglobin method and found the pant fruit having potent activity.

Keywords: *Aegle marmelos,* Ayurveda drug, glycosylated haemoglobin method, Antidiabetic activity.

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slender, aromatic tree, 6.0 -7.5 m in height, and 90 to 120 cm in girth, with a somewhat fluted bole of 3.0-4.5 meter growing wild throughout the deciduous forests of India, ascending to an altitude of 1200 meter in the western Himalayas and also occurring in Andaman island. This is generally considered as sacred tree by the Hindus, as its leaves are offered to Lord Shiva during worship. Aqueous extract of *Aegle marmelos* leaves, was evaluated for hypoglycemic and antioxidant effect by Upadhya, by using alloxon induced diabetes in male albino rats and proposed AML may be useful in the long-term management of diabetes.

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future, is important to allow rational planning and allocation resources. The prevalence of diabetes for all age –groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.the prevalence of diabetes is higher in men than women, but there are more women with diabetes than men. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people >65 years of age.(1)

In modern medicine, that an imbalance in physiological homeostasis generates several diseases also recognizes that life is based on a complex and finely tuned network of reduction-oxidation (redox) reactions that are under homeostasis control. Cells or organisms are constantly subjected to factors that can alter this redox balance, often resulting in overt generation of free-radicals (oxidative stress). Oxidative stess is one of the major causative factor for development of number of diseases like diabetes mellitus, athrosclerosis, cancer, neurodegerative diseases and disorder of ageing, due to imbalance of oxidant and antioxidant status in the human body. Therefore, modern science have been initiated several global programmes to harness and harvest natural antioxidant rich resources and boost antioxidant defense in the human body by various means. (1&2).

HbAic is formed by a post transitional, non-enzymatic, substrate-concentration depedent irreversible process of combination of aldehyde (CHO) group of glucose and other hexoses with the amino-terminal(NH2) valine of the –chain of haemoglobin. The estimation of HbA1c has provided a dependable method of assessing glycemic control in diabetes. Amongst the various markers of glycemic control, glycated haemoglobin has now been established as the most reliable, though many other proteins are also glycated in the diabetic and non-diabetic states.(3)

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Table.1 HbA_1C levels in diabetes

Diabetic of control	HbA1c	Mean blood glucose levels
Non diabetic	4.0 - 6.0%	61 – 124 mg/dl
Goal	6.0 - 7.0%	124 – 156 mg/dl
Good control	7.0 - 8.0%	158 – 188 mg/dl
Diabetic	> 8.0%	> 188 mg/dl

Figure.1. The fresh fruits of *Aegle marmelos*

Materials and methods

Collection and authentication of plant

The fresh fruits of *Aegle marmelos* were collected from the Guntur district, Andhrapradesh. They were identified and authenticated in Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India and a specimen was preserved.

Preparation of the extract

Fresh fruits of *Aegle marmelos* were collected and air dried in shade under the room temperature. The dried fruit material was powdered mechanically and sieved through No.20 mesh sieve. The fine powder was kept separately in an airtight container until the time of use. Around 100 g of finely powdered bark material was

evenly packed in a soxhlet apparatus and the extraction was done with chloroform and Pet ether for 48 hours. The solvent was then evaporated under reduced pressure. The percentage (%) yield of the extract was calculated. The extracts were named CAM and PEAM respectively (5,6).

Experimental animals

Wistar albino rats weighing about 150-250 g maintained under standard laboratory conditions were used at a temperature of 23±ºC, with a light/dark period beginning at 6:00 hours. The animals were caged with a maximum of two animals in a polypropele cage and were fed with standard food pellets (Hindustan Lever Ltd) and water *ad libitum*. The study was conducted after obtaining ethical committee of institution.

***In vivo* antidiabetic activity by glycosylated Haemoglobin method**

Induction of experimental diabetes was done by single peritoneal injection of streptozotocin (65 mg/kg) to wistar albino rats. 72 hours lafter the confermation of diabetes, the rats were divided into four groups of six rats each. Group 1 served as control treated with normal saline, group 2 as diabetic control (streptozotocin 65 mg/kg), group 3 treated with 750 mg/kg of phytic acid as test, group 4 received 3.5 mg/kg of glimepiride as reference standard. Blood glucose, glycosylated haemoglobin (HbA1c) were evaluated by standard commercial kits and body weight were estimated on 0, 7, 14 and 28 days of treatment (7, 8, 9, 10).

Results and Discussion

Body Weight

Test drug treated group shows significant increase in body weight, comparable with that of standard and control. (Table.2)

Table 2: Effect of Test compound on body weight

Treatment	Body weight (gms)			
	0 day	7^{th} day	14^{th} day	28^{th} day
Control (Normal saline 10 ml/kg)	244.5±1.70	250.30±1.68	258.66±1.78	262±1.43
Diabetic control (streptozotocin 65 mg/kg)	234.16±2.07	202.5±3.09	182.3±3.84	175.16±2.88
Test drug (400 mg/kg)	255.0±1.82	243.6±1.94	259.5±2.78	264.5±2.23
Glimepiride (3.5 mg/kg)	245.0±1.82	246.0±3.27	250.83±2.33	265.5±2.27

Data are expressed as mean ± SEM; n=6 in each group

P<0.01 vs Diabetic control

Glycosylated haemoglobin (HbA1C) levels

The levels of glycosylated haemoglobin for the test drug treated group shows considerable improvement as tha of standard drug glimepride.(Table.3)

Table 3: Effect of Test compound on Glycosylated haemoglobin (HbA1C)

Treatment	Glycosylated haemoglobin	
	0 day	28^{th} day
Control (Normal saline 10 ml/kg)	4.205±0.876	4.861±0.356
Diabetic control (streptozotocin 60 mg/kg)	4.98±0.567	10.2983±0.950
Test drug (400 mg/kg)	4.98±0.180	5.273±0.280
Glimepiride (3.5 mg/kg)	4.63±0.254	4.601±0.276

Data are expressed as mean ± SEM; n=6 in each group

P<0.01 vs Diabetic control

Blood glucose level of experimental rats

The test group animals shows significant reduction in the blood glucose level, which are comparable with standard drug glimepride. (Table.4)

Table 4: Effect of Test drug on blood glucose level of experimental rats

Treatment	Blood glucose level (mg/dl) on		
	0 day	**7th day**	**28th day**
Control (Normal saline 10 ml/kg)	77.88±1.66	85.00±1.26	98.66±1.28
Diabetic control (streptozotocin 60 mg/kg)	79.33±1.38	313.66±12.49	441.83±10.83
Test drug (400 mg/kg)	77±1.34	184.66±4.31	94.76±2.57
Glimepiride (3.5 mg/kg)	74.5±1.02	169±5.42	96.33±1.64

Data are expressed as mean ± SEM; n=6 in each group

P<0.01 vs Diabetic control

Conclusion

The test compound possesses considerable anti-diabetic activity. Also the glycosylated Hemoglobin method proves to be a sensitive alternative method for determining the levels of disease status in diabetes. Further studies are needed to isolate the active ingredients of the test extract and structure of the compound to be elicited for a better anti-diabetic drug for human mankind.

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