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Original Article

Effect of Aqueous Extract of *Aegle marmelos* Fruit and Leaf on Serum Insulin on Non Diabetic and Type 1 Model Diabetic Rats

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Abstract

Introduction: *Diabetes mellitus (DM) is one of the commonest endocrine and metabolic disorders affecting mankind all over the world. Plants are considered to be source of therapeutic agents. Aegle marmelos are used extensively in the indigenous system of medicine as an antidiabetic agent.*

Methods: *To see effect of aqueous extract of Aegle marmelos fruit and leaf on serum Insulin on non diabetic and type 1 model diabetic rats, this present study was carried out in Biomedical Research Group (BMRG), Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorder (BIRDEM), Dhaka. A total of 47 Long-Evans rats were taken for the experiment among which 23 were nondiabetic and 24 was diabetic rat. Both the Diabetic and non diabetic animals were fed with both extracts at a dose of 250 mg/kg/body weight/day.*

Results: *In the diabetic rats serum insulin values did not change significantly in the leaf extract group; however there was about 30% increase of insulin level in the fruit extract group which was not significant also.*

Conclusion: *It was concluded that that Aegle marmelos fruit and leaf had no significant effect on insulinaemic value on Diabetic or nondiabetic rat models.*

Key words: *Aegle marmelos, Insulinaemic value.*

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Introduction

Diabetes mellitus (DM) is one of the commonest endocrine and metabolic disorders of mankind all over the world. It is widely recognized as one of the leading causes of death and disability worldwide.[1]

Conventionally, Type 1 Diabetes Mellitus (T1DM) is treated with exogenous insulin and Type 2 Diabetes Mellitus (T2DM) with synthetic oral hypoglycemic agents like sulphonylureas and biguanides. A substantial proportion of T2DM also require insulin.

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However, the existing therapeutic agents have considerable limitations in the management of this complex disorder and search for alternate agents are continuing all over the world.[2,3] Plants are considered to be source of treatment of diseases, as there are multiple therapeutic properties like antiulcer, antidiabetic, antihyperlipidaemic, anticancer, antimicrobial, radioprotective, antipyretic, analgesic and antispermatogenic effects.[4-7] The plant kingdom has become a target for multinational drug companies and research institutes for the discovery of new biologically active compounds and potential drugs.[8] The World Health Organization has recommended, specially in developing countries, the initiation of programmes designed to use medicinal plants more effectively in the traditional health care system.[7] The resolution of the 31st World Health Organization Assembly requested a complete inventory, and a thorough evaluation of the efficacy, safety and standardization of medicinal plants for the treatment of diabetes.[10] *Aegle marmelos* was originated in India and is presently growing in most of the countries of Southeast Asia. *Aegle marmelos* are used extensively in the indigenous system of medicine as an antidiabetic agent, so *A. marmelos* leaf has already shown great potential as a source of antidiabetic agent.[2,9,10] In fact, turning this potentiality into a scientifically validated product needs further work on the mechanism of action of the plant extract. Although some works have been done on the insulin releasing or B cell regenerating action of the leaf extract, a number of potential targets have not yet been investigated.[9,10]

Materials and methods

Aegle marmelos leaves and fruits were collected from Chapainawabgonj, a district of Rajshahi division. The aqueous extract of *A. Marmelos* fruit and leaf was made by standard method. A total of 47 Long-Evans rats were taken for the experiment among which 23 were nondiabetic and 24 were diabetic. *Aegle marmelos* fruit pulp was dried in sunlight for 5/6 days, coarsely powdered by grinder machine and stored in room temperature until the making of extracts. All the collected leaves were washed carefully again and again to get neat and clean leaves. Wet leaves were primarily spread over brown paper to soak the extra water. After soaking the water the leaves were spread over aluminum foil and crisp dried for 3 days at 48^0C in an oven. The dried leaves were crushed in an electric grinder to make a fine powder stored in room temperature until the making of extracts.

Every 100 gram *A. marmelos* fruit and leaf powder with 500 ml distilled water in a beaker was mixed. This mixer was mixed nicely with a spoon. The beaker was covered by aluminum foil and placed over magnetic stirrer with a magnate inside the beaker. The mixer was rotated at a rotation cycle of 600 rotations /min. The mixer was stirred for 8 hours. The mixer was sieved in beaker. The hard pellet was again mixed with 100 ml distilled water and again stirred for one hour and sieved. Combined liquid was taken in a round bottom flask and evaporated and concentrated in rotary evaporator under a temperature ranging from 40°-50°C and under a rotation at 60 rotations/min.

It was concentrated till the total liquid was changed into a viscous pest. This pest was freeze dried under vacuum of gel solid yield. The dry sample was stored in a reagent bottle at 4^{o}C in a freezer. The experiments were carried out on adult Long-Evans rats (125-220g) of both sexes, bred at BIRDEM animal house and maintained at a constant room temperature of 22 ± 5^{0}C with humidity of 40-70 % and the natural 12 hours day-night cycle. The rats were fed on a standard laboratory pellet diet and water supplied *ad libitum*, except during the day of experimental procedure. The influence of circadian rhythms was avoided by starting all experiments at 8.30 a.m. These diabetic model rats were induced by single injection of Streptozosin (STZ). After 7days of injection, injected rats were tested whether they had T1DM or not. The drug dose was 250 ml/Kg/10 ml. On '0' day after 12 hours fasting, body weight was taken and blood was collected from the tail tip by giving mild anaesthesia, centrifuged and serum used for measuring Insulin. Then for 14 days extract was feed with normal diet. On the 15^{th} day, blood was taken from tail tip by giving mild anaesthesia on them. Again blood was centrifuged and serum used for measuring Insulin. Values were calculated by SPSS (V. 19) and result was made.

Results

Table 1 shows effects of aqueous extracts of *A. marmelos* fruit and leaf on insulin of nondiabetic rats during 0-15 days time period. Insulin (ng/dl, Mean ± SD) of the *A. marmelos* fruit group (n=9) was 1.41±0.47 (on `0´ day) and 1.08±0.44 (on `15´ day), *A. marmelos* leaf group (n=4) was 0.59±0.34 (on `0´ day) and 0.57±0.13 (on `15´ day). In *A. marmelos* fruit group percentage deviation '0' day versus '15' day (ng/dl, Mean ± SD) was 17.28±39.53 and in *A. marmelos* leaf group was 0.8±.22. In percentage deviation, no significant difference was found between two groups. In paired' test in *A.*

marmelos fruit and *A. marmelos* leaf group no significant difference was found.

Table 1: Effect of aqueous extracts of *A Marmelos* fruit and leaf on insulinaemic status of nondiabetic rats

Group & parameter	**0 day (M±SD) *Insulin(ng/dl)***	**15 day *Insulin(ng/dl)***	**t/p value**	**Deviation from 0 day (%)**
AMFE (n=9)	1.41±.47 (100)	1.08±.44 (82.72±39.53)	1.14/.199	17.28±39.53
AMLE (n=4)	.59±.34 (100)	.57±.13 (99.2±.22)	1.71/.185	0.8±.22

Note: Comparison between 0 day (baseline) and 15 day values of the same group was compared by Paired- t test. Values were expressed as Mean±SD. P<0.05 was considered as statistically significant, n= number of subjects; AMFE group=*Aegle Marmelos* fruit extract treated group, AMLE group=*Aegle Marmelos* leaf extract treated group.

Table 2: Effect of aqueous extracts of *A. marmelos* fruit and leaf on insulinaemic status of diabetic rats

Group & parameter	**0 day (M±SD) *Insulin(ng/dl)***	**15 day *Insulin(ng/dl)***	**t/p value**	**Deviation from 0 day (%)**
AMFE (n=8)	.25±.09 (100)	.32±.16 (130.55±46.05)	-1.79/.116	-30.55±46.05
AMLE (n=7)	.27±.18 (100)	.24±.17 (87±101.50)	.48/.647	13.73±101.50

Note: Comparison between 0 day (baseline) and 15 day values of the same group was compared by Paired- t test. Values were expressed as Mean± SD. P<0.05 was considered as statistically significant, n= number of subjects; AMFE group=*Aegle marmelos* fruit extract treated group, AMLE group=*Aegle marmelos* leaf extract treated group.

Discussion

It was evident that in nondiabetic rats (who have healthy beta cells) serum insulin values increased over a period of 15 days which may be a normal fluctuation over a growing period. In the two treatment groups, the value did change from baseline and there is no consistent difference between the groups. In the diabetic rats, serum insulin values did not change significantly in the Leaf Extract group; however there was about 30% increase of insulin level in the Fruit Extract group. It seems that the hypoglycaemic effect of the aqueous extract of *A. marmelos* fruit is mediated by increased secretion of insulin in the diabetic model rats. It was also previously[3-6] suggested that the antidiabetic effect of *A. marmelos* fruit may probably be due to the potentiation of insulin secretion from ß cells. It is unclear whether the rise in insulin level is due to stimulation of secretion in existing beta cells or prevention of further damage in the cells. The regeneration of cells may also be conceived and all these need further investigation with *in vivo* as well as *in vitro* techniques.

In contrast to the fruit extract the leaf extract of *A. marmelos* did not show any hypoglycaemic activity in nondiabetic or diabetic model rats in the present study. Also it did not show any significant effect on serum insulin level of the rats. The finding in nondiabetic rats is in contrast with the finding[11-13] where it was found the antihyperglycemic and insulin releasing effects of *A. marmelos* leaves on normal fasted rabbits. The finding in diabetic rats is also in contrast with the observation[11] which had reported hypoglycaemic and insulin releasing properties of *A. marmelos* leaf extract in similar model rats.

Conclusion

It may be concluded that *Aegle marmelos* fruit and leaf has no significant effect on insulinaemic value on Diabetic or nondiabetic rat.

Contribution of the authors

First author was the chief researcher, second and third authors helped in data collection. Fourth author was the guide of the research work.

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