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Antidiabetic activity of leaf and callus extracts of *Aegle marmelos* in rabbit

Sevugan Arumugam[a], Subramanian Kavimani[b], Balamuthu Kadalmani[c], Abdul Bakrudeen Ali Ahmed[d], Mohammed Abdulkadar Akbarsha[c], Mandali Venkateswara Rao[d,*]

[a] National Taiwan University, Taipei 106, Taiwan
[b] Mother Theresa Institute of Health Science, Pondicherry, India
[c] Department of Animal Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620 024, India
[d] Department of Plant Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620 024, India

* Corresponding author, e-mail: mvrao_456@yahoo.co.in

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ABSTRACT: *Aegle marmelos* (the bael tree) is a popular medicinal plant in the Ayurveda and Siddha systems of medicine and folk medicines used to treat diabetes. The present study was undertaken to find the extent to which calluses obtained from leaf explant of *A. marmelos* has a potential for application in diabetes management compared to the ordinary plant material. Treatment using extracts from both leaf and callus produced significant decreases in blood sugar level in streptozotozin diabetic rabbits. Among the various extracts, the methanol extracts of the leaf and callus brought about the maximum anti-diabetic effect. The study revealed that the *in vitro* callus culture of *A. marmelos* has as much potential in diabetes management as the original leaf extract.

KEYWORDS: *Aegle marmelos*, callus culture, methanolic extraction, anti-diabetic activity

INTRODUCTION

Aegle marmelos (L.) Corr., (Rutaceae) is a popular medicinal plant in the Ayurvedic and Siddha systems of medicine and folk medicines used to treat a wide variety of ailments. The plant, popularly known as the bael tree, is native to the Indo-Malayan region[1] and is currently cultivated in India, Pakistan, Bangladesh, Sri Lanka, Burma, and Thailand[2]. The tree is a slender, aromatic perennial, 6.0–7.5 m tall and 90–120 cm in girth. It flowers from May to July and yields an annual average of 300–400 fruits (200–250 kg) per tree. Various parts of the tree, including the fruit, possess medicinal properties. The roots are useful for treating diarrhoea, dysentery, and dyspepsia[3]. The leaf is used for opthalmia, diabetes, and asthmatic complaints. Unripe fruit is useful for treating diarrhoea, dysentery and stomachalgia. The aqueous extracts of the stem and root bark are used to treat malaria, fever, jaundice, and skin diseases such as ulcers, urticaria, and eczema[4]. In pharmacological trials, both the fruit and root showed antiamoebic and hypoglycaemic activities[5,6]. The plant is rich in alkaloids, among which aegline, marmesin, marmin, and marmelosin are the major ones. Aqueous leaf extract and methanolic extract of the root bark of *A. marmelos* showed preventive effects on myocardial diseases[7,8]. The compounds luvangetin and pyranocoumarin, isolated from the seeds of *A. marmelos,* showed significant antiulcer activity[9]. Essential oil isolated from the leaf has antifungal activity[10]. The aqueous extract of leaf possesses a hypoglycaemic effect[11].

The incidence of diabetes is increasing. Worldwide, it affects 230 million people of which 30 million are in India. It has been estimated that by the year 2025, the global incidence of diabetes would increase to 350 million[12]. Management of diabetes is a huge burden. While therapeutic insulin production is not adequate to meet demands, the recombinant DNA approach to diabetes management originally considered as a panacea has faced several problems[13]. It is hypothesized that the ultimate therapy for type I and type II diabetes lies in the herbal approach[14]. However, herbs are not inexhaustible natural resources, and the demand for herbal medicines cannot be met by cultivation only[15]. Plant tissue culture is a boon and can help produce large quantities of the herbal material. However, it is speculated that plant materials produced through tissue culture are deficient in secondary chemicals of therapeutic importance[16]. This study was therefore undertaken to find out whether in the case of *A. marmelos*, extracts from calluses are more effective than those from ordinary leaf material in the management of diabetes.

MATERIALS AND METHODS

Aegle marmelos callus cultures were initiated from leaf explants. Explants were collected from a 40-year old plant, sterilized, and then cultured on a modified Murashige and Skoog medium[17], i.e., a B5 medium[18] supplemented with 2,4-dichlorophenoxy acetic acid (0.5 mg/l) and benzyl adenine (0.2 mg/l). The cultures were maintained under sterile control conditions at 25 ± 2 °C. A 16:8 h (light:dark) photoperiod and a light intensity of 25 µmol/m^{2}s were used[19].

About 5 g each of fresh as well as oven-dried (at 40 °C) leaf, immature leaflet, root and callus (1, 2, 3, 4 month-old) were placed separately in 100 ml distilled water and were allowed to stand for 24 h with occasional shaking. After filtration followed by evaporation on a water bath, the extract was finally concentrated in an oven at 40 °C. Fresh leaf and callus (90 days old) were dried in an oven at 40 °C and powdered. Using a Soxhlet apparatus 500 g of the powder was extracted serially in petroleum ether, benzene, chloroform, and methanol. The extracts were concentrated in a rotary evaporator.

Swiss albino rabbits (1 year old) of either sex, weighing 1.5–2.5 kg, were used as the test animal. The rabbits were fed on a standard pellet diet (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum* and maintained at 28–30 °C. After laboratory acclimation for 7 days, the rabbits were starved for 48 h and divided into groups of five. The animals were induced into a diabetic state by intraperitoneal injection of a freshly prepared solution of streptozotozin (STZ) (Sigma Chemical Co., St. Louis, MO, USA) in 0.05 mM citrate buffer (pH 4.5) at a dose of 45 mg/kg body weight per day for 3 days[20]. The control rabbits received citrate buffer alone. Rabbits with a blood sugar level of more than 300 mg/dl were selected for the study. The rabbits were divided into 3 treatments, and the common control group in each case received physiological saline.

In the first treatment, aqueous extracts of dry leaf powder, fresh leaf, immature leaflet, callus powder (1, 2, 3 and 4 month-old) and root powder were suspended in physiological saline and fed to STZ-diabetic rabbits in separate groups at a daily dose of 1.0 g/kg body weight using an oral gavage. Each rabbit received 3 doses of the preparation on consecutive days.

In the second treatment, petroleum ether, benzene, chloroform, and methanol extracts of dry leaf/callus were suspended in physiological saline and fed to STZ-diabetic rabbits daily at a dose of 1.0 g/kg body weight per day. Each rabbit received 3 doses on consecutive days.

Finally, in the third treatment, methanol extracts of leaf and callus suspended in physiological saline were fed at a daily dose of 1.0 g/kg body weight. Therapeutic human mixtard insulin (Torrent Pharmaceutical Ltd, Mehsana, India) (1 unit/kg body weight) was administered through the intraperitoneal. route to a separate subgroup of STZ-diabetic rabbits for 3 days in order to compare the antidiabetic potency of the extracts with that of insulin. Blood was collected on day 1, 5, 10, and 15 after the last dose.

The rabbits were fasted for 12 h and blood was collected directly from a pinna venule using a syringe carrying a #26 needle on day 1, 5, 10, and 15 after the last dose of treatment. Blood sugar was determined using a glucometer (Johnson & Johnson Co., USA).

RESULTS AND DISCUSSION

Treatment of crude aqueous extract of the leaf and the three month-old callus brought about a significant decrease in blood sugar (Table 1). All organic solvent extracts proved to be anti-diabetic. Among the various extracts, the methanol extract of leaf and callus brought about the maximum anti-diabetic effect (Table 2).

The effect of methanol extracts of leaf and callus in the diabetic rabbits was studied for different durations (Table 3). It can be seen that there is an immediate marked drop in blood sugar levels for the leaf and callus extract treatments. Among the various

Table 1 Effect of an aqueous extract of *Aegle marmelos* on blood sugar levels in STZ-diabetic rabbits.

Treatment	Blood sugar level (mg/dl) (Mean ± SD)
Control	310.2 ± 1.1
Immature leaflet	262.5 ± 1.5
Callus (1 month old)	255.5 ± 0.5
Root	251.1 ± 1.0
Fresh leaf	246.6 ± 2.5
Callus (2 month old)	236.1 ± 1.0
(3 month old)	209.1 ± 1.0
(4 month old)	226.0 ± 1.1
Dry leaf	217.4 ± 1.2

Table 2 Effect of different organic solvent extracts of leaf and callus of *Aegle marmelos* on blood sugar levels of STZ-diabetic rabbits.

Treatment	Blood sugar level (mg/dl) (Mean ± SD)	
	Leaf extract treated	Callus extract treated
Control	361.6 ± 2.8	360.9 ± 3.1
Petroleum ether Ex	236.5 ± 2.5	256.6 ± 1.5
Benzene Ex	222.5 ± 2.6	251.6 ± 1.6
Chloroform Ex	226.1 ± 2.0	255.0 ± 1.8
Methanol Ex	207.1 ± 2.0	210.1 ± 2.0

solvent extracts, methanol extract produced the maximum reduction of sugar level, and the highest reduction was observed on the tenth day. However, in the case of the insulin treatment, the blood sugar level was decreased to less than 100 mg/dl, irrespective of the duration. The results of this study indicate that the biosynthesis of the various secondary metabolites of therapeutic value in diabetes in the callus cultures of *A. marmelos* is similar to that found *in vivo* mature leaves. The callus cultures of *Cassia tora* produce ten times more anthraquinone derivatives than *in vivo* plant material[21].

STZ-induced β-cell death in the pancreas is due to the alkylation of DNA thereby producing hyperglycemia[22]. The immediate action of STZ is on β-cells, even at relatively moderate doses of 45 mg/kg of body weight in a single dose. In the present study STZ treatment of rabbits for 15 days increased blood sugar to a significant level[20].

The extracts have the potential of correcting diabetes by acting as an anti-hyperglycemic agent rather than by inducing hypoglycemia. This idea is supported by the demonstrated therapeutic importance of solvent extracts of *A. marmelos*[23]. The present findings confirm that the methanol extract can bring about greater reduction of sugar level in induced diabetic rabbits than other solvent extracts.

The present study shows the hypoglycemic effect of extracts of leaf and callus against STZ-induced diabetes. It appears that the leaf and callus extracts possess the ability to stimulate the insulin secreting cells of pancreas. This would, in turn, decrease the blood sugar as would do the leaf extract of *A. marmelos*[11]. All these findings suggest that both the leaf and callus extracts may be acting, through some mechanism, to improve the receptor-responsiveness to insulin causing increased sugar uptake by the tissue.

In the present study both the leaf and callus extracts brought about a significant hypoglycemic effect in rabbits. The hypoglycemic effect of D-400, a herbal formulation, on fasting blood sugar level in rat was attained on day 20, and thereafter the blood sugar level did not decrease even after 90 days[24]. However, those animals were not induced to be in a

Table 3 Effect of methanol extract of leaf and callus of *Aegle marmelos* and insulin on blood sugar level in STZ-diabetic rabbits.

Treatment	Blood sugar level (mg/dl) (Mean ± SD) (Days)			
	1	5	10	15
Control	317.56 ± 2.04	345.52 ± 3.62	349.04 ± 1.26	350.04 ± 2.25
Leaf-extract	208.51 ± 2.02	195.54 ± 2.05	123.76 ± 2.53	128.76 ± 3.71
Callus-extract	200.72 ± 5.25	187.72 ± 2.36	106.21 ± 1.05	109.05 ± 1.52
Insulin	95.22 ± 2.04	96.26 ± 1.42	97.07 ± 1.52	97.52 ± 2.24

diabetic state and therefore carbohydrate metabolism was already in homeostasis and hence no further fall could occur. *A. marmelos* would act like insulin in the restoration of blood sugar and body weight to normal levels in rat and was therefore recommended as a potential hypoglycemic agent[5,23]. A similar result was obtained in the present study with the callus extract. Furthermore, the callus extract-treated animals appeared healthier and less prone to fluctuation in the extent of the hypoglycemic condition than was observed in their insulin-treated counterparts.

These results suggest that both the leaf and callus materials contain anti-diabetic active principles, which would reduce the sugar level in STZ-diabetic rabbits. It is also inferred that the crude solvent extracts of leaf and callus powder may have some compounds in addition to the active anti-diabetic principles. Also, the solvents may affect the action of the principal compound responsible for the anti-diabetic effect. Further study using the purified active principle from the leaf and callus extracts may reveal the role of the respective preparations as hypoglycaemic agents in diabetes management.

In conclusion, the present findings indicate that the methanol extract of the callus powder of *Aegle marmelos* is as potent as the *in vivo* leaf extract in the management of diabetes.

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