

## Hypoglycaemic effect of the traditional drink, the water extract of dried flowers of *Aegle marmelos* (L.) Correa (*bael* fruit) in Wistar rats

<sup>1</sup>Kumari K D K P, <sup>2</sup>Samarasinghe K & <sup>1</sup>Suresh T S\*

<sup>1</sup>Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangadowila, Nugegoda, Sri Lanka,

<sup>2</sup>Department of Pathology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangadowila, Nugegoda, Sri Lanka  
E-mails: kripeshala@gmail.com; kamani62@yahoo.com; malalavidhane@yahoo.com

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This study was designed to prove scientifically the medicinal benefits of a traditional drink. In this study the oral hypoglycaemic effect of the water extract of dried flowers of *A. marmelos* (L.)Correa (WEAM) was evaluated using Wistar rats. Healthy and alloxan-induced diabetic rats were kept on overnight fasting and they received the WEAM, drugs or distilled water, 30 min prior to glucose loading (3 gm/kg). Blood was collected after 2 hrs and serum glucose concentrations were determined by the glucose oxidase method. This procedure was repeated to determine the optimum dose and time, to evaluate the effect of the multiple doses and to compare the activity with reference drugs, Metformin and Glibenclamide. The extract gave statistically significant ( $p < 0.05$ ) hypoglycaemic effect in both healthy and diabetic rats. The optimum activity was showed by 500 mg/kg at 2 hrs after the administration of glucose. The effectiveness of the WEAM was comparable with the reference drugs Metformin and Glibenclamide. The administration of the extract for a period of 42 days showed an increased hypoglycaemic ( $p < 0.01$ ) effect and glycated haemoglobin level was reduced significantly ( $p < 0.01$ ) in diabetic rats. The water extracts of dried flowers of *Aegle marmelos* posses a significant oral hypoglycaemic effect.

**Keywords:** Traditional drink, *Aegle marmelos*, Hypoglycaemic, Diabetes, Rats

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*Aegle marmelos* (L.) Correa (Family: Rutaceae) is a highly reputed medicinal plant in the traditional systems of medicine which is used to treat a wide variety of disorders. This plant, commonly known as the *bael* fruit tree is found in many Asian countries including Sri Lanka<sup>1</sup>. Many parts of the tree such as root, leaf, trunk and fruit are used in traditional systems of medicine and extensive investigations done on different parts, revealed medicinal properties such as anti-inflammatory, antifungal, antibacterial, hepato-protective, hypoglycaemic effects<sup>2</sup>.

A drink prepared by boiling dried flowers has been a popular brew in Sri Lanka for centuries which has refreshing, soothing and calming effects. This was especially popular among the hard working farmer communities where it helped them to refresh themselves during work as well as at the end of a laborious day. During the recent past, much attention has been given to functional foods and nutraceuticals which contain beneficial factors for health other than

nutrients. This beverage has gained much attention among the urban population due to the claims by indigenous medical practitioners as possessing many medicinal properties. The traditional medicinal systems states that it is used as an anti-dysenteric, anti-diabetic, diaphoretic and as a local anesthetic drug<sup>3,4</sup>. It is also used in epilepsy and as an expectorant<sup>5</sup>.

Diabetes mellitus (DM) is one of the major health problems in the world and it is associated with excessive morbidity and mortality<sup>6</sup>. Even though the commonly used synthetic drugs are very effective in treatment of DM, they are associated with certain adverse effects<sup>7</sup>. A novel trend has emerged during the last few decades to investigate plant materials in search of new oral hypoglycaemic compounds as they are considered to be safe with minimal side effects<sup>8</sup>.

Therefore, the present study was planned to evaluate the hypoglycaemic effect of the water extract of the dried flowers of *A. marmelos* in healthy and diabetic Wistar rats and the results of this study will help to prove the medicinal benefits of this traditional

\*Corresponding author

brew. This is an attempt to promote traditional foods and beverages among the population as functional foods with beneficial medicinal properties.

## Materials and methods

### Ethical clearance

Ethical approval was obtained for the animal studies from the Ethics Review Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. The approval Ref. No is 432/09 (06/05/2009).

### Plant material

The fallen flowers are collected and sundried by the farmers and sold for commercial purposes. These were purchased from farmers from different provinces of the country and pooled together. Required amounts were taken from this pool for the preparation of extracts. The plant material was collected in July 2010 and authenticated by Prof P Tissera (Professor of Botany) of the Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura. A voucher specimen (USJP FMS 6/2010) has been deposited at the herbarium of the Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura.

### Animals

Male, Wistar rats (250-300 gm) were purchased from the Medical Research Institute, Colombo 8 and housed under standardized conditions ( $23^{\circ} \pm 2^{\circ}\text{C}$ , 60%-70% relative humidity and 12 hrs photo period) at the Animal House. The rats were fed with a standard diet and water *ad libitum*. In all experiments, six rats were used in each group.

### Preparation of extract

The water extract was prepared daily by boiling 25 gm of dried flowers of *A. marmelos* in 500 ml water until reduced to 50 ml.

### Administration of extracts and drugs

All preparations were orally administered to rats via Sondi needles. Metformin and glibenclamide were purchased from the State Pharmaceutical Co-corporation, Sri Lanka. Suspensions of powdered tablets were used for oral administration.

### Studies with healthy rats

#### Single dose experiment

The oral hypoglycaemic effect of a single dose of the water extract of dried flowers of *A. marmelos*

(200 mg/kg) was carried out in two groups of healthy, Wistar rats. The Test and Control groups were fasted for 8-10 hrs and received the test extract and distilled water respectively. This was followed by the administration of glucose (3 gm/kg) after half an hour. Blood was drawn from the lateral tail vein after 2 hrs under light anaesthesia with diethyl ether. The serum glucose concentrations were measured using glucose oxidase reagent kits (Biolabo, France).

#### Dose curve

Eight groups of healthy, male Wistar rats were fasted overnight and administered graded doses (50, 100, 200, 400, 500, 750 and 1000 mg/kg) of the extract and distilled water as the control. Glucose was given  $\frac{1}{2}$  hr later and blood was drawn at 2 hrs to determine serum glucose concentrations.

#### Time course

The time course was done with optimal dose (500 mg/kg) with 2 groups of rats (Test and Control). The same protocol as above was carried out. Serum glucose was determined at 1 hr, 2 hrs and 3 hrs.

#### Comparison with known oral hypoglycaemic agents

To compare the activity of the test extract with commonly used oral hypoglycaemics, rats were divided into 4 groups where Group 1, 2, 3 and 4 received a single dose of the extract (500 mg/kg), metformin (15 mg/kg), glibenclamide (0.1 mg/kg) and distilled water, respectively. The same protocol as in the single dose experiment was followed.

#### Multiple dose experiment

To evaluate the hypoglycaemic effect of the long term administration of the test extract, the Test and Control groups received a single dose (500 mg/kg) of the extract and distilled water respectively for 7 consecutive days. Following an overnight fast, fasting and post glucose load serum glucose levels were measured on Day 8.

### Studies with diabetic rats

#### Induction of Diabetes

Experimental DM was induced in rats by the intravenous administration of 40 mg/kg of Alloxan Monohydrate. Fasting blood glucose levels were determined after 72 hrs and rats with a serum glucose concentration of  $> 7.00$  mmol/L were selected.

#### Single dose experiment

The oral hypoglycaemic effect of a single dose of the test extract was carried out on 2 groups of

diabetes-induced, male Wistar rats. Fasting serum glucose was determined in Test and Control rats prior to administration of the extract (500 mg/kg) and water respectively. Glucose was given (3 gm/kg) ½ h later. Blood was drawn after 2 hrs and the serum glucose concentrations were measured.

#### Comparison with oral hypoglycaemic agents

The efficacy of the test extract was compared with the activity of reference drugs. The diabetic rats were divided into 4 groups; Group 1, 2, 3 and 4 which received a single dose of the extract (500 mg/kg), metformin (15 mg/kg), glibenclamide (0.1 mg/kg) and distilled water respectively and the test was carried out as described for healthy rats.

#### Multiple dose experiment

The diabetic rats were divided into 3 groups and group 1 received distilled water as the control. The group 2 received the extract (500 mg/kg) while the third group was given the reference drug metformin (15 mg/kg). The rats received the extracts and drugs continuously for 42 days as a single dose between 8.00 – 8.30 am everyday. On day 8, 21 and 42, fasting and post glucose load serum glucose were determined and the glycated haemoglobin (GlyHb) levels were also measured on day 42.

#### Statistical analysis

Analysis of variance (ANOVA) was tested in SPSS and the results were further subjected to the Student's *t* test. A *p*-value < 0.05 was considered as significant. The results are presented as the mean ± SEM.

### Results

#### Studies with healthy rats

##### Single dose experiment

The single dose of the extract (200 mg/kg) showed a statistically significant (*p* = 0.003) oral hypoglycaemic activity in healthy rats. The mean serum glucose level of the test group was 7.70 ± 0.2 mmol/L, while it was 9.00 ± 0.1 mmol/L in the control group. There was a 15 % reduction in the mean serum glucose of the Test group compared to control.

##### Dose curve

All the doses of the water extract gave statistically significant (*p* < 0.05) reductions in serum glucose concentrations (Table 1) of the Test groups. The optimum dose was 500 mg/kg (*p* = 0.0003), which gave the highest reduction of 30%.

#### Time course

The optimum activity was given at 2 hrs after the administration of glucose (Table 2).

#### Comparison with known oral hypoglycaemic agents

When compared with oral hypoglycaemics, the percentage reduction of serum glucose level of the Test group was 29 % (6.40 ± 0.5 mmol/L) compared to the Control (9.02 ± 0.1 mmol/L) group, while it was 27 % (6.52 ± 0.6 mmol/L) and 26 % (6.70 ± 0.1 mmol/L) for metformin (*p* = 0.02) and glibenclamide (*p* = 0.02) groups, respectively.

#### Multiple dose experiment

On day 8, fasting serum glucose levels of the Test and Control groups were 4.7 ± 0.2 mmol/L and 6.15 ± 0.5 mmol/L respectively while the post glucose load serum glucose levels were 5.56 ± 0.4 mmol/L and 8.70 ± 0.2 mmol/L respectively. The reduction in Test group was statistically significant while the percentage reduction of serum glucose was 23.18 % (*p* = 0.04) in fasting rats and 38.5 % (*p* = 0.003) after glucose administration in the Test group when compared with the Control.

Table 1—The effect of graded doses of water extract of dried flowers of *Aegle marmelos* in healthy Wistar rats

Treatment	Serum glucose Concentrations (mmol/L) (Mean ± SEM)
Control	8.89 ± 0.1
WEAM (50 mg/kg)	7.72 ± 0.2 (13.1%)*
WEAM (100 mg/kg)	7.71 ± 0.2 (15.4%)*
WEAM (200 mg/kg)	7.66 ± 0.2 (16.0%)*
WEAM (400 mg/kg)	7.65 ± 0.3 (16.2%)*
WEAM (500 mg/kg)	6.63 ± 0.1 (29.5%)**
WEAM (750 mg/kg)	7.34 ± 0.2 (23.5%)**
WEAM (1000 mg/kg)	7.33 ± 0.2 (21.3%)*

(*p*<0.01)\* (*p*<0.001) \*\* Figures in parentheses indicate the percentage reduction.

Table 2—The effect of water extract of dried flowers of *Aegle marmelos* (500 mg/kg) on serum glucose concentrations at different time intervals in healthy Wistar rats

Time	Serum glucose concentrations Test	(mmol/L) (Mean ± SEM) Control
1 hr	4.99 ± 0.4 (21.4%) **	6.34 ± 0.5
2 hrs	5.34 ± 0.3 (28.8%)*	7.49 ± 0.4
3 hrs	6.79 ± 0.2 (15.0%) **	8.00 ± 0.2

(*p*<0.05)\* (*p*<0.01) \*\* Figures in parentheses indicate the percentage reduction.

## Studies with diabetic rats

### Single dose experiment

Administration of a single dose of the extract (500 mg/kg) in diabetic rats showed a statistically significant ( $p = 0.02$ ) oral hypoglycaemic activity. The mean fasting serum glucose levels of Test and Control were  $10.56 \pm 2.0$  mmol/L and  $11.48 \pm 1.9$  mmol/L while it was  $13.67 \pm 1.5$  mmol/L and  $17.20 \pm 2.1$  mmol/L after glucose consumption. The percentage reduction of serum glucose level in the Test group was 21 % when compared to the Control.

### Comparison with oral hypoglycaemic drugs

When the hypoglycaemic activity was compared with reference drugs, the percentage reduction of serum glucose level of the Test group was 21 % ( $14.43 \pm 1.4$  mmol/L) with respect to the Control ( $18.27 \pm 0.3$  mmol/L) group, whereas it was 26 % ( $13.45 \pm 1.8$  mmol/L) and 25 % ( $13.61 \pm 1.7$  mmol/L) for metformin and glibenclamide groups respectively.

### Multiple dose experiment

Fig. 1 shows the effect of the long term feeding of the extract on plasma glucose levels of diabetic rats on day 8, 21 and 42. Apart from the reduction in serum glucose, the long term administration of the WEAM reduced the level of GlyHb significantly ( $p = 0.005$ ) in comparison to the Control. The mean GlyHb levels of the groups receiving the test extract and metformin was 6.1 % and 7.1 % respectively, while it was 10.5 % in control group.

## Discussion

This study was experiments. This dose exerted the maximum hypoglycaemic effect at 2 hrs after oral administration of glucose and therefore in all experiments, blood was drawn for estimation of serum glucose concentration at 2 hrs following administration of glucose. Once the oral hypoglycaemic activity was established, the most effective dose was compared with commonly used oral hypoglycaemic agents, metformin and glibenclamide. Here, it was observed that the effect of the extract was comparable to these drugs as judged by the percentage reduction in the serum glucose in test extract, metformin and glibenclamide groups (29 %, 27 % and 26 % respectively) comparative to the Control. With the single dose experiments the acute hypoglycaemic effect became evident. To determine whether a chronic hypoglycaemic effect was exerted, initially the dose of 500 mg/kg was

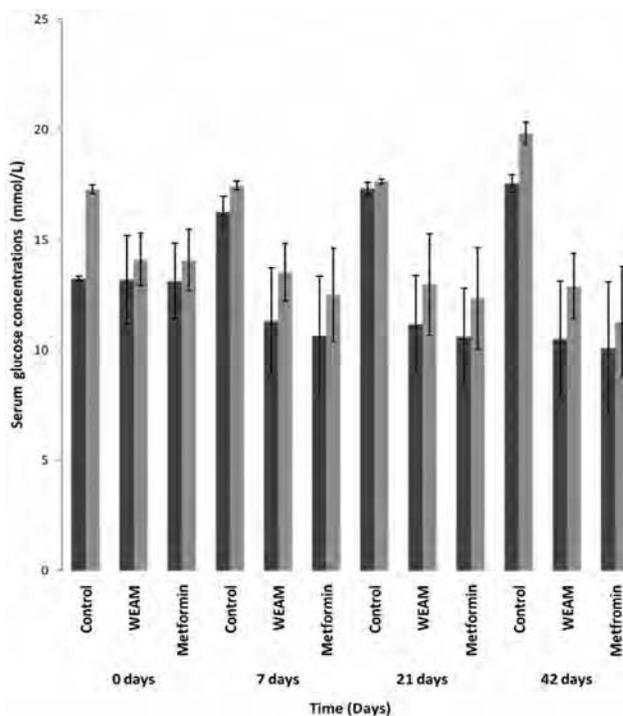


Fig.1- The effect of long term feeding of the water extract of dried flowers of *A. marmelos* (WEAM) on the serum glucose concentrations of diabetic Wistar rats

given for one week to the Test group and subjected to challenge with glucose on day 8. The chronic effect was evident by the statistically significant reduction in the fasting serum glucose levels of the healthy rats ( $p = 0.003$ ), where the blood sample was obtained on day 8, approximately 24-25 hrs after the last dose was given on day 7. Similar results were obtained by Lanjhiyana *et al.*<sup>11</sup> in a study done with root bark extract of *A. marmelos*, when it was given for 7 days to normal rats.

Having determined the hypoglycaemic effect of the test extract in healthy rats, it was studied in alloxan-induced diabetic rats. Alloxan monohydrate induces diabetes by destroying pancreatic beta cells<sup>12</sup>. A single as well as multiple doses of the extract exerted statistically significant oral hypoglycaemic effects in diabetic rats. When administered for 6 weeks the glycated haemoglobin concentration, which is a good indicator of long term glycaemic control, was significantly lower in the Test group. Therefore the potential therapeutic benefit of this extract solicits further investigation. In addition to this, the hypoglycaemic effect is comparable to that of metformin and glibenclamide, in the diabetic rats. Lanjhiyana *et al.*<sup>11</sup> and Kamalakkannan *et al.*<sup>13</sup> observed similar reduction of glycated haemoglobin

in diabetic rats after feeding extracts of root bark and fruit of *A. marmelos* respectively.

Sujeewani and Suresh<sup>14</sup> have reported the presence of flavanols, a group of flavonoids and Saponin in the water extract of the dried flowers of *A. marmelos*. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats<sup>15</sup>. The hypoglycaemic activity of test extract may be due to the presence of more than one anti-hyperglycaemic principle and their synergistic properties. Phytochemical studies have revealed<sup>5</sup> that different parts of the plant contain several types of coumarins, sterols, tannins, flavonoids, alkaloids, etc.

Oxidative stress has a significant effect in the aetiology of diabetes as well as diabetes related complications in human beings. As reported by Sujeewani and Suresh<sup>14</sup>, the flower extract exhibited DPPH scavenging activity and it is capable of reducing the oxidative stress in Wistar rats. Kuttan & Sabu<sup>16</sup> and Upadhyaya *et al.*<sup>17</sup> observed that the leaf extract of *A. marmelos* was capable of reducing oxidative stress by scavenging lipid peroxidation and enhancing certain anti oxidant levels which cause lowering of elevated blood glucose. This suggests that the herbal formulations with simultaneous antioxidant effects are better candidates to be used in the management of DM. Therefore, the water extract of dried flowers of *Aegle marmelos*, which is a popular beverage among Sri Lankan population, can be promoted as a functional food with valuable medicinal benefits.

### Conclusion

The water extract of dried flowers of *Aegle marmelos* exerts a statistically significant oral hypoglycaemic effect in healthy and alloxan-induced diabetic rats. The optimal dose is 500 mg/kg and the optimal time of activity is 2 hrs after administration of the extract. The efficiency of the water extract was comparable with metformin and glibenclamide.

This is the first ever study reported on the water extract of the dried flowers of *Aegle marmelos* and its health effects. Although this drink is a very popular beverage among the rural community of Sri Lanka, it is not so in the urban areas. With the western world turning more towards functional foods and nutraceuticals, this traditional drink if given the due recognition could be promoted as a

health drink. Possible measures will be taken to educate the public regarding the findings of our study and to promote this drink. Attempts will be made to identify active constituents from this extract in future studies.

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