

**HYPOGLYCAEMIC, ANTI-INFLAMMATORY AND  
ATPASE EFFECT OF THE DRIED FLOWER  
EXTRACTS OF *Aegle marmelos* (BAEL FRUIT)**

By

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PhD

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## DECLARATION BY CANDIDATE

The work described in this thesis, was carried out by me under the supervision of Dr. T. Sugandhika Suresh (Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura), Prof. Kamani Samarasinghe (Department of Pathology, Faculty of Medical Sciences, University of Sri Jayewardenepura) and Dr. Shiroma Handunnetti (Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo) and a report on this has not been submitted in whole or in part to any university for another Degree/Diploma.

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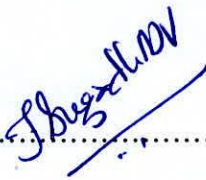
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**Hypoglycaemic, anti-inflammatory and ATPase effect of the  
dried flower extracts of *Aegle marmelos* (bael fruit)**

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**TO MY BELOVED FAMILY**

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## ABBREVIATIONS

ABTS	2, 2'-Azino-bis (3- ethylbenzothiazoline-6-sulphonic acid
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
AST	Aspartate amino transferase
ATPase	Adenosine tri phosphatase
CIS	Commercially available instant sachets
COX	Cyclo-oxygenase
DM	Diabetes Mellitus
DPPH	2,2 -Diphenyl-1-picrylhydrazyl
EAF	Ethyl acetate fraction
EC50	Half maximal effective concentration
EEAM	Ethanollic extract of dried flowers of <i>A. marmelos</i>
FTIR	Fourier Transform Infra Red spectroscopy
GCMS	Gas Chromatography – Mass Spectrum
GK	Glucose kinase
GLUT	Glucose transporter
GSK	Glucose synthase kinase
$\gamma$ -GT	Gamma glutamyl transferase
Hb	Haemoglobin
HbA1C	Glycated haemoglobin
HDL	High density lipoprotein
HF	Hexane fraction
IC50	Half maximal inhibitory concentration



LD50	50 % concentration of the lethal dose 50
LDL	Low density lipoprotein
OGTT	Oral glucose tolerance test
NMMA	N-monomethyl-L-arginine acetate salt
NO	Nitric oxide
NSAID	Non steroidal anti-inflammatory drugs
PBS	Phosphate buffered saline
PFE	Powdered dried- flower extract
Rf	Retention factor
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TEAC	Trolox equivalent anti-oxidant capacity
TLC	Thin layer chromatography
TPE	Traditionally prepared extract
UV	Ultra violet
WEAM	Water extract of dried flowers of <i>A. marmelos</i>



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# **Hypoglycaemic, anti-inflammatory and ATPase effect of the dried flower extracts of *Aegle marmelos* (bael fruit)**

By Kumaragamage Dona Krishanthi Peshala Kumari

## **ABSTRACT**

*Aegle marmelos* Correa (L.) is a highly reputed medicinal plant used in the traditional medicine systems of Asian countries and possesses many medicinal properties. Although many parts of this plant have been extensively investigated, there is no experimental evidence on the flower extracts of the plant. The drink made by boiling the dried flowers of *A. marmelos* is popular in Sri Lanka. The objectives of the present study were to evaluate the hypoglycaemic, anti-inflammatory and ATPase effect of the flower extracts of this plant.

The oral hypoglycaemic effect of the water extract of dried flowers of *A. marmelos* (WEAM) was evaluated in healthy and alloxan induced diabetic Wistar rats. Time course and dose curve were studied in healthy rats. The optimum dose was 500 mg/kg ( $p < 0.001$ ), which gave the highest reduction of post glucose load serum glucose concentration (30 %) at 2 h after the administration of glucose. Administration of multiple doses of WEAM in both healthy and diabetic rats showed a gradual reduction of the fasting and post glucose load serum glucose concentrations. In diabetic rats following a 42 day consecutive administration of the test extract, there was a 40 % ( $p < 0.01$ ) reduction in fasting serum glucose concentration and 31 % ( $p < 0.01$ ) reduction in the post glucose load serum glucose concentration. The level of HbA1c ( $p < 0.01$ ) was also reduced significantly in comparison to the control. The effectiveness



of the WEAM was comparable with the reference drugs metformin and glibenclamide in both healthy and diabetic rats. To evaluate the hypoglycaemic mechanisms of WEAM (500 mg/kg) in diabetic rats; the intestinal glucose content, liver glycogen content, serum glycogen synthase kinase, serum glucokinase and serum insulin levels were estimated. The glucose concentration of the intestinal content of the Test group was significantly reduced ( $P < 0.05$ ) by 33 % compared to the Control group. Serum concentration of insulin increased significantly ( $P < 0.05$ ) by 55.1 % in the Test group. Among the enzymes tested, serum concentration of glucokinase increased significantly ( $P < 0.001$ ) by 50.7 % and there was a significant reduction ( $P < 0.05$ , 20 %) in serum glycogen synthase kinase level in the Test group. The glycogen content of the livers of Test rats increased by 64 % ( $P < 0.05$ ) after long term feeding of the test extract.

To evaluate possible toxicity, the rats received a single dose (500 mg/kg) of the Test extract for 42 consecutive days. There was no statistically significant ( $P > 0.05$ ) difference between concentrations of serum ALT, AST, ALP,  $\gamma$ -GT, creatinine and Hb levels of Test group compared to the Control group. No histopathological changes were observed in liver, heart and kidney tissues of Control and Test groups and this suggested that the WEAM does not exert any possible adverse effects on organs. The alterations of blood parameters and histopathology in diabetic rats were reversed by the long term administration of the test extract.

To evaluate the hypoglycaemic effect in healthy volunteers ( $n = 30$ ) and Type 2 diabetic patients ( $n = 30$ ), the WEAM (85 mg/kg) was given continuously for 14 days and fasting and post glucose load serum glucose concentrations were measured. The individuals were monitored afterwards and serum ALT, AST, ALP,  $\gamma$ -GT, creatinine and Hb levels were measured. In healthy subjects the fasting serum glucose concentration was reduced

by 6 % ( $P < 0.001$ ) while, the post glucose load serum glucose concentration was reduced by 20 % ( $P < 0.001$ ) after 14 days. In diabetic patients, following consumption of the test extract for 14 days, the fasting serum glucose concentration was reduced by 20.0 % ( $P < 0.001$ ) and the post glucose load serum glucose concentration was reduced by 35.5 % ( $P < 0.001$ ). No adverse effects were reported during and after the experiment and the serum levels of tested liver enzymes, creatinine and Hb were not significantly ( $P > 0.05$ ) altered in human subjects after the treatment of test extract.

Anti-inflammatory effect of the test extract was determined by evaluating the percent inhibition of rat paw oedema, induced by carrageenan in healthy and diabetic rats. The maximum percentage inhibition of paw oedema (69.9 %) was shown by the dose of 200 mg/kg at 2 h and the activity was comparable to that of indomethacin. The anti-inflammatory effect was exerted through multiple mechanisms including the inhibition of nitric oxide production, anti-histamine effect, membrane stabilization activity and the high antioxidant capacity of the WEAM.

The effect of the test extract on  $\text{Na}^+/\text{K}^+$  ATPase activity in small intestine, liver and erythrocytes were tested in normal and diabetic rats. The  $\text{Na}^+/\text{K}^+$  ATPase activity in the erythrocytes and liver were significantly lower ( $P < 0.001$ ) in diabetic rats compared to normal rats. It was significantly higher ( $P < 0.001$ ) in both tissues of diabetic rats following administration of a single dose of the test extract. The  $\text{Na}^+/\text{K}^+$  ATPase activity in the small intestine was significantly higher ( $P < 0.01$ ) in diabetic rats compared to normal rats and it was further increased significantly ( $P < 0.01$ ) by 24.4 % after administration of the test extract. The pathological alterations of  $\text{Na}^+/\text{K}^+$  ATPase activity in tissues during diabetes appeared to have been reversed by the test extract. In humans, erythrocyte  $\text{Na}^+/\text{K}^+$  ATPase is a peripheral marker that reflects the activity of



this enzyme in neurons. Following consumption of the test extract, Na<sup>+</sup>/K<sup>+</sup> ATPase activity in erythrocyte membranes in human volunteers was significantly (P < 0.001) increased by 63 % and it provides potential evidence for the stimulatory effect exerted by test extract.

The ethanol extract of flowers was subjected to activity guided fractionation using gel filtration chromatography, column chromatography and thin layer chromatography. The separated fractions were tested for hypoglycaemic and anti-inflammatory effect in rats. Fourier Transform Infra Red (FTIR) spectra were taken for the most active fractions and the active hypoglycaemic fraction comprised a mixture of flavonoids and coumarins and active anti-inflammatory fraction composed of triterpenoids.

Different preparations of the WEAM and black tea were given to 30 volunteers and a questionnaire was used for sensory evaluation. The traditionally prepared *beli mal* drink had appreciable organoleptic properties which are comparable to that of black tea. However the properties of the extract prepared by the powdered dried flowers and commercially available instant sachets were poor in comparison to that of tea or the traditionally prepared drink.

As hypoglycaemic and anti-inflammatory effects are scientifically proven by this pioneer study, this beverage can be promoted as a health drink among the population.