

## Antidibetic, antihyperlipidemic and antioxidative effects of *Aegle marmelos* and silymarin on alloxan induced diabetes in mice

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### Abstract

This project was designed for the evaluation of the antidibetic, antihyperlipidemic and antioxidative effect of *Aegle marmelose* (L.) Correa ex Roxb., and silymarin on alloxan induced diabetes in mice. A total of 15 mice were used which were divided into five groups as A, B, C, D and E. Group A was treated with normal chick's diet. Alloxan were given at 150 mg kg<sup>-1</sup> body weight through abdominal region, to all groups with the exception of control. Group B was diabetic mice, group C was treated with silymarin (200 mg kg<sup>-1</sup>) of body weight, group D was treated with *A. marmelose* (180 mg kg<sup>-1</sup>) of body weight and Group E treated with silymarin (200 mg kg<sup>-1</sup>) and *A. marmelose* (180 mg kg<sup>-1</sup>) of body weight. Blood samples were taken from the coccygeal vein of the mice for the estimation of glucose, total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, malondialdehyde, oxidative stress marker, glutathione, superoxide dismutase and catalase activity. Results showed that *A. marmelos* and silymarin reduced the oxidative stress and increased the level of glutathione, superoxide dismutase, catalase and thiobarbituric acid reactive substances. It was concluded from the present study that *A. marmelos* and silymarin can reduce the diabetes in mice.

**Keywords:** *Aegle marmelos*, antidiabetic, antihyperlipidemic, mice.

### Introduction

A set of metabolic disorders having a frequent attribute of hyperglycemia is known as diabetes mellitus. The defect in secretion and action of insulin cause hyperglycemia in diabetes. Diabetes mellitus is the central intimidation to human health due to alteration in human behavior and standard of living and resulted in increase in the occurrence of diabetes worldwide (Zimmet *et al.*, 2001; Wild *et al.*, 2004). Diabetics showed anomalous antioxidant status that was due to autooxidation of glucose and excess glycosylated proteins (Ceriello *et al.*, 1991; Mak *et al.*, 1996). Oxidative stress is responsible for tissue damage and shows troubles including retinopathy, nephropathy and coronary heart disease (Lyons, 1991). Anyhow, risk of heart problems could be reduced by improving blood pressure, blood glucose and lipid levels. Oxidative stress is encountered in the biological system during the metabolic process. Moreover, this stress has been observed in relation with diabetes and its related harms in humans (Nirmala *et al.*, 2011). Oxidative

stress is responsible for inactivation of enzymes, changes in collagen that are related to its structural functions and glycation of proteins (Boynes, 1991).

Alloxan, is used for the induction of diabetes, showed its activity in damaging the beta cells of pancreas and responsible for the release of oxygen radicals (Halliwell and Gutteridge, 1985). Chemically, alloxan is a derivative of pyrimidine. Alloxan becomes amass in insulin producing cells by uptake through glucose transporter. Alloxan generates reactive oxygen species and its toxic action is produced in beta cells by free radicals in redox reaction. Studies showed that diabetes is not induced in humans by alloxan. Even in high doses, it shows no diabetes in humans most likely due to contrary glucose uptake mechanisms in rodents and human (Eizirik *et al.*, 1994; Tyrberg *et al.*, 2001).

Bael (*Aegle marmelos*) belongs to family Rutaceae, is also known as Bengal quince, golden apple, stone apple, wood apple, bili. The species of tree native to India and is also available throughout Southeast Asia (Anonymous, 2013). The bael fruit

has a smooth, woody shell with a green, gray, or yellow peel and are larger like grape fruit or pomelo. Fruit has abundant medicinal properties like antipyretic, antidiarrheal, astringent, antidysenteric, antiscourbutic, haemostatic and antidote to venom of snake. It is also used for the curing in diabetes mellitus (Alam *et al.*, 1990). Oral administration of aqueous solution of *A. marmelos* root-bark showed hypoglycemic effect in normal fasted rats. In addition, the same extract completely prevented peak rise of blood sugar at 1 h in oral glucose tolerance test (OGTT) (Karunanyake *et al.*, 1984). Extract of *A. marmelos* has been used to decrease the level of glucose in the serum and found antioxidant activity *ex vivo* (Sabu and Kuttan, 2004). Another important plant is *Silybum marianum* that belongs to family Asteraceae, commonly known as milk thistle and has been used for the cure of liver diseases. Plant derives its name due to its milky veins in the leaves. Silymarin is derived from *S. marianum*, has antioxidative properties and shows its effects by decreasing the lipid peroxidation in hepatocytes and also for averting liver glutathione depletion (Skottova *et al.*, 2003; Sbolova *et al.*, 2006).

In the present study, effect of *A. marmelos* and silymarin on blood sugar levels and markers of oxidative stress (i.e. lipid peroxidation, catalase, glutathione and superoxide dismutase) was assessed in alloxan treated mice.

## Materials and Methods

Fifteen albino mice with initial body weight 15-30 g were divided into five groups (A to E), each group consisting of 3 mice. Group A was served as control while the remaining groups were given alloxan (1 mg kg<sup>-1</sup> body weight). Group C and D were given silymarin (200 mg kg<sup>-1</sup>) and *A. marmelos* (180 mg kg<sup>-1</sup>), respectively to check their independent role in diabetic mice, while in Group E synergistic role was investigated of both silymarin (200 mg kg<sup>-1</sup>) and *A. marmelos* (180 mg kg<sup>-1</sup>).

**Table 1: Different treatments given to mice**

Treatments	
Group A	Control
Group B	Alloxan (1 mg kg <sup>-1</sup> body weight)
Group C	Alloxan + Silymarin 200 mg kg <sup>-1</sup> body weight)
Group D	Alloxan + Standardized extract of <i>A. marmelos</i> 180 mg kg <sup>-1</sup> body weight)
Group E	Alloxan + Silymarin (200 mg kg <sup>-1</sup> body weight) + <i>A. marmelos</i> (180 mg kg <sup>-1</sup> body weight)

The sample were processed and analyzed by spectrophotometrically for superoxide dismutase activity, following the method of Kakkar *et al.* (1984). Glutathione was estimated according to the method of Moron *et al.* (1979). Lipid peroxidation level in the serum samples were expressed by malondialdehyde (MDA) according to method of Ohkawa *et al.* (1979). Catalase activity was determined by measuring the decrease in hydrogen peroxide concentration at 230 nm by the method of Aebi (1974). Blood glucose, total cholesterol, high density lipoprotein (HDL), low density lipoprotein and triglyceride were estimated by using human diagnostic enzymatic kits.

## Results and Discussion

Statistical analysis showed highly significant differences among the effect of silymarin and fruit extract of *A. marmelose* on glucose level in mice received alloxan as compared to control. Alloxan injected mice showed highly increased level of total cholesterol, triglycerides and blood glucose and reduction in high density lipoproteins level as compared with control group. This abnormal lipid profile was restored by inducing mice with *A. marmelose* and silymarin. Maximum recovery rate of 89%, 87%, 83%, 62% and 32% in total cholesterol, high density lipoproteins, low density lipoproteins, triglycerides and blood glucose was recorded in mice treated with combination dose of *A. marmelose* + silymarin as compared normal control group. The levels of glutathione, superoxide dismutase, malondialdehyde and catalase were maximally restored by the combination therapy of silymarin and *A. marmelose* with optimum recovery rate of 68%, 59%, 62% and 49%, respectively.

Mice receiving alloxan had increased level of total cholesterol, low density lipoproteins, triglyceride, blood glucose and malondialdehyde as compared to normal controls. Interestingly, all mice group (C, D and E) receiving silymarin and *Aegle marmelose* therapy showed time course recovery towards normalcy i.e. decreased level of total cholesterol, low density lipoproteins, triglyceride, Blood glucose, malondialdehyde and increased level of high density of lipoproteins as well as restored levels of total cholesterol and antioxidative enzymes. Maximum restoration of all the above said parameters in group E suggested that combined therapy of silymarin and *A. marmelose* is more effective than used in isolation and both extracts have a synergistic effect tending to normalize the hepatic enzymes. *A. marmelose* decreases blood glucose level by improving

glucose tolerance and also has lipid-lowering and antioxidant properties (Kannur *et al.*, 2006). Extract of *A. marmelos* has been used to decrease the level of glucose in the serum and found antioxidant activity *ex vivo* (Sabu and Kuttan, 2004). Liver fibrosis is caused by oxidative stress

and this stress is main feature of hepatitis due to multiple conditions (Wasmuth *et al.*, 2003). Moreover, oxidative stress is involved in chronic ailments of diabetes and also related to increasing levels of lipid peroxidation (Elangovan *et al.*, 2000).

**Table 1:** Effect of oral administration of *Aegle marmelos* and silymarin on total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides and blood glucose on alloxan induced diabetes in mice.

Treatments	Total cholesterol (mg dL <sup>-1</sup> )	High density lipoproteins (mg dL <sup>-1</sup> )	Low density lipoproteins (mg dL <sup>-1</sup> )	Total Glucose (mg dL <sup>-1</sup> )	Blood glucose (mg dL <sup>-1</sup> )
Control (healthy)	105.103±2.02 c	55.69±4.07 ab	38.70±2.86 b	66.69±2.88 d	106.44 ± 0.71 d
Alloxan	121.91 ± 4.04 a	50.39 ± 0.81 bc	46.91 ± 2.51 a	139.14 ± 1.83 a	351.43 ± 2.28 a
Alloxan with silymarin	117.58 ± 1.52 ab	49.56± 3.80 c	45.92± 1.52 a	125.25 ± 3.00 b	216.95 ± 7.03 b
Alloxan with <i>Aegle marmelos</i>	114.57± 4.71 b	51.25± 2.00 bc	40.25± 1.00 b	117.91± 1.52 b	145.40 ± n 12.32 c
Alloxan with <i>Aegle marmelos</i> + silymarin	107.95 ± 1.57 c	58.12 ± 1.80 a	38.91 ± 0.56 b	86.91 ± 10.05 c	111.23 ± 1.99 d

**Table 2:** Effect of oral administration of *Aegle marmelos* and silymarin on reduced glutathione, superoxide dismutase, malondialdehyde and catalase on alloxan induced diabetes in mice.

Treatments	Reduced Glutathione (mg dL <sup>-1</sup> )	Superoxide Dismutase (µg mL <sup>-1</sup> )	Melondialdehyde (µ mol mL <sup>-1</sup> )	Catalase (µ mol mol <sup>-1</sup> of protein)
Control (healthy)	1.85±0.11 a	46.43±0.74 b	4.84±0.51 c	33.22±2.00 b
Alloxan	1.34 ± 0.10 c	30.44± 1.28 d	9.39 ± 1.02 a	19.24 ± 1.01 e
Alloxan with silymarin	1.49 ± 0.06 bc	41.29± 1.05 c	6.83± 0.52 b	28.6± 1.51 c
Alloxan with <i>Aegle marmelos</i>	1.60 ± 0.07 b	41.59± 3.21 c	7.25± 1.00 b	23.58± 2.09 d
Alloxan with <i>Aegle marmelos</i> + silymarin	1.98± 0.05 a	51.58 ± 4.49 a	5.83 ± 0.51 bc	39.58 ± 1.54 a

Values are expressed as means ± SD; n=05 for each treatment group

Means values within a row not sharing a common letter were significantly different at P≤0.05 as determined by Duncan's Multiple Range test.

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