

**Original Research Article**Evaluation of hypoglycaemic activity of *Aegle marmelos* alcoholic seed extract in experimental models of hyperglycemic rats

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**ABSTRACT**

The present study aimed to investigate the antidiabetic effect of alcoholic seed extract of *Aegle marmelos* in comparison with glibenclamide in in vivo using alloxan rat model. The diabetic rats were orally given glibenclamide and alcoholic seed extract of *Aegle marmelos* for 21 days. The effects were studied in vivo. Oral administration of alcoholic seed extract of *Aegle marmelos* decrease the level of serum glucose, total cholesterol (TCH), triglycerides (TG), low density lipoprotein (LDLP), very low density lipoprotein (VLDLP) significantly while increasing HDL-cholesterol. Alcoholic seed extract of *Aegle marmelos* was also evaluated for oral glucose tolerance (OGTT) characteristics. In conclusion, alcoholic seed extract of *Aegle marmelos* had potential antidiabetic activity. Further it has been observed that the seed extract have positive effect on liver & kidney parenchyma.

**Introduction**

The use of herbal medicine for the treatment of diabetes mellitus has gained importance throughout the world [1]. According to world Health Organization at least 30 million people are involved throughout the world, and the numbers of cases reported are increasing rapidly with the aging of populations, changes in lifestyle, and improvement in ascertainment (WHO, 1980) [2]. Diabetes mellitus is a chronic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels. Though different types of oral hypoglycaemic agents are available along with insulin for the treatment of diabetes mellitus, there is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycaemic agents [3-4]. A large number of herbal drugs stated in the ayurvedic system of medicine of india to possess anti-diabetic activity of which 1200 plants species were confirmed upon with the help of available research literature [5-6].

*Aegle marmelos* corr (Rutaceae) most commonly known as 'Bael' in Hindi is indigenous to India [7-8]. The bark, leaves and fruits of *Aegle marmelos* are used in ayurvedic medicine for the treatment of various ailments. Various crude extracts of

this plant have shown activities including antiulcer, antidiabetic, antihyperlipidemic, antioxidant, anticancer, antimicrobial, radioprotective, anti-inflammatory, antipyretic and analgesic [9-11].

**Material and Method****Plant materials**

*Aegle marmelos* (Rutaceae) fruits were collected from Noida (U.P), India and authenticated by Dr. S. K. Malhotra, Principal scientist of Horticulture the Department of Horticulture, India Council of Agriculture Research, Delhi. A voucher specimen (PSM/2011/70) of the seeds of *Aegle marmelos* was deposited at the Department of Horticulture, ICAR, Delhi, India.

**Preparation of extract**

Dried seeds of *Aegle marmelos* was macerated in methanol. The concentrated crude extract was converted into emulsion (oil in water) form in different concentration [12].

**Preliminary phytochemical screening**

The presence of phytochemicals, a preliminary phytochemical study with alcoholic seed extract of *Aegle marmelos* was performed[13-14].

### Drugs & Chemicals

Alloxane was purchased from sigma chemicals co. St. Louis Mo. USA, glibenclamide & Heparin from zydus cadila Ahemadabad India.

### Animals

Male Wistar rats (150-180gm), used in the present study were obtained from the central animal house of the institute. All the animal experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC). The animals were maintained under standard conditions of humidity (50±10%), temperature (22±2°C) and light (12 hour light and 12 hour dark). They were fed standard rodent pellet diet purchased from a commercial supplier and had free access to water. All animals were acclimatized for 1 week before start of experimentation and experiments on animals were performed in accordance with the CPCSEA guidelines.

### Acute Toxicity Studies

The acute oral toxicity study was carried out according to the guidelines set by organization for Economic co-operation and development (OECD) guideline 423. Male Wistar rats (150-180gm) were used for this study. Animals were divided into different groups containing three animals each. The different doses of alcoholic seed extract of *Aegle marmelos* (5, 50, 300, 2000 mg/kg) were given orally. Animals were observed individually at least once during the first 30 minute after dosing, periodically during the first 24 hour (with special attention given during the first 4 hour), and daily then after, for a total of 14 days. During these days the animals were examined for behavioural, neurological & autonomic profiles[15].

### Induction of diabetes in rats

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate at a dose of 120 mg/kg. The individual dosagic amount of the drug (alloxan) was weighed and then solubilised in saline just prior to injection. Two days after the injection, blood samples were collected by retro-orbital plexus puncture and the serum glucose level was estimated using glucose oxidase-peroxidase method and rats with serum glucose level of >200 mg/dl were segregated for the study. Treatment with seed extract was started 48 h after alloxan injection[16].

### Experimental design

Animals were randomly assigned to six groups (n=5) Group I: normal control rats; Group II: diabetic control; Group III: diabetic rats received glibenclamide (2.5 mg/kg); Test Group

IV: diabetic rats received alcoholic seed extract of *Aegle marmelos* (100 mg/kg); Test Group V: diabetic rats received alcoholic seed extract of *Aegle marmelos* (200 mg/kg); Test Group VI: diabetic rats received alcoholic seed extract of *Aegle marmelos* (400 mg/kg) daily for 21 days. Blood samples were drawn by retro-orbital puncture either anesthetized overnight fasted rats and collected in eppenndroff's tubes. Serum was separated by centrifugation at 3000 rpm for 10 min at room temperature and used for estimation of glucose level using glucose-oxidase method (Trinder, 1969) with optical density measured by UV spectrophotometry at 520nm using standard kits and fasting blood glucose levels were estimated on 0, 7, 14 and 21 day. All the lipid profile parameters were determined. Total cholesterol (TCH), triglycerides (TG), HDL. Low density lipoprotein (LDLP), very low density lipoprotein (VLDLP) were analyzed from serum and body weights were determined.

### Effect of alcoholic seed extract of *Aegle marmelos* on Oral Glucose Tolerance Test (OGTT) in rats

On 21 day the oral glucose tolerance test was performed in overnight fasted rats. Glibenclamide 2.5 mg/kg and dose of 100mg/kg, 200 mg/kg and 400 mg/kg per oral of alcoholic seed extract of *Aegle marmelos*. Glucose 2g/kg was administered orally 30 minutes after the administration of alcoholic seed extract of *Aegle marmelos*. Blood was withdrawn from the retro-orbital sinus plexus just prior to glucose administration and at 0, 30, 60, 90, 120 min after glucose loading. Serum was separated and blood glucose levels were measured immediately by glucose oxidase method

### Histopathological examination

The Liver & kidney tissues from each animals were removed after sacrificing and collected in 10% formalin solution. After 24 hrs stored tissues were embedded in molten paraffin with the help of metallic blocks, covered with flexible plastic moulds and kept under freezing plates to allow the paraffin to solidify. Cross section (5µ thick) of the fixed tissues were cut using microtome. The Section of liver were stained with periodic acid Schiff's stain (PAS) & kidney & liver with hematoxyline & eosine stain (H & E) for histological examination[17-19]. The section were observed with light microscope and photograph with digital camera(Canon, Japan).

### Statistical analysis

All data were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA), followed by Dunnett's test. The value of P < 0.005 was considered significant.

### Result

#### Preliminary phytochemical Screening

The percentage yield of alcoholic seed extract of *Aegel marmelos* was found to be 11.28%. The methanol extract contained triterpenoids, steroids, alkaloids, flavonoids, proteins, tannins, phenol & glycosides.

#### Acute Toxicity Study

The various observations showed the normal behaviour of the treated rats. No toxic effects were observed at a higher dose of 2g/kg body weight. Hence there were no lethal effects in any of the groups till the end of the study.

#### Changes in body weight

Throughout the experiment the body weight of all the experimental rats were monitored daily. (Fig1) shows the observations of body weight of experimental rats during the experiment. The body weight of alcoholic seed extract of *Aegel marmelos* methanolic 100mg/kg, 200mg/kg and 400mg/kg and standard drug treated group, increased significantly ( $P<0.01$ ) by 13%, 14% & 14.3%.

#### Anti diabetic activity of alcoholic seed extract of *Aegel marmelos* in alloxane induced diabetic rats

The results from the study clearly indicated that the alcoholic seed extract of *Aegel marmelos* exhibited significant hypoglycaemic activity in alloxane induced diabetic rats. At the end of 21 days of treatment, there was 10 %, 31.1%, and 47% decrease ( $P<0.01$ ) of blood glucose levels with the alcoholic seed extract of *Aegel marmelos* 100mg/kg, 200mg/kg and 400mg/kg (fig 2). The standard drug glibenclamide also indicated a significant decrease (61%) of serum glucose levels.

#### Effect of alcoholic seed extract of *Aegel marmelos* on Oral Glucose Tolerance Test (OGTT)

There was a significant increase in blood sugar level in diabetic animals. Group receiving glibenclamide showed a significant decrease in blood glucose level in every 30 minutes while other groups receiving alcoholic seed extract of *Aegel marmelos* (group V & VI) showed a continuous decrease in blood sugar level till 120 minutes levels significantly ( $P<0.001$ ) as compared to the diabetic control group. (fig 3) however group IV showed a minor appreciable decrease in blood glucose levels compared to group V and group VI.

#### Effect of alcoholic seed extract of *Aegel marmelos* on lipid profile

When compared to the diabetic control rats, significant ( $P<0.01$ ) reduction of 6.3%, 14%, 21% CHL (cholesterol), 9 %, 21.1%, 28.1% TG, 11%, 30%, 41%, LDL, 9%, 21%, 28% VLDL, were found after treatment with alcoholic seed extract of *Aegel marmelos* 100mg/kg, 200mg/kg and 400mg/kg. Also

there was a significant ( $P<0.05$ ) increase of 23%, 52%, 56% HDL cholesterol in treated diabetic rats. (fig 4)

#### Changes of histopathological of the kidney and liver

After 21 days treatment period, the histopathological examination of the liver which was stained with PAS. The periodic acid Schiff's technique was used to demonstrate the presence of high proportion of carbohydrate macromolecules (glycogen, glycoprotein, proteoglycans). The control rats showed normal architecture of hepatocytes that appeared mostly mononuclear with extensive cytoplasm densely stained with coarse granular PAS-positive deposits (fig a). The alloxanated diabetic rats showed marked diminution in polysaccharide content in their hepatocyte (fig b). The standard group showed a normal liver architecture with moderate staining (fig c). Mild increase in polysaccharide content in hepatocytes was observed in the group of diabetic rats treated with alcoholic seed extract of *Aegel marmelos* (100mg/kg, 200mg/kg and 400mg/kg) when compared with the diabetic control (fig d, e, f).

#### Examination of the liver and kidney with Haematoxylin & Eosin staining

Examination of the liver with haematoxylin & eosin, the control rats showed the hepatocytes are seen as cords radiating from the central veins. The portal tracts were composed of portal vein radicle, hepatic artery radicle and bile duct radicle. The central veins appeared normal. The alloxanated diabetic rats showed the hepatocytes global microvesicular steatosis, the portal tracts appeared normal and the central veins appeared congested. The standard group showed a most of the liver lobules remained normal and in certain lobes, the hepatocytes in midzonal regions appeared regenerating. The cells were prominent, moderately crowded and contained large nuclei with condensed chromatin. The diabetic rat treated with aegle marmelos 100mg/kg no significant changes in the liver when compared with diabetic control but diabetic rat treated with alcoholic seed extract of *Aegel marmelos* 200mg/kg & 400mg/kg, the hepatocytes portal tracts and central veins appear normal, no steatosis was observed.

Examination of the kidney of the control rats revealed normal glomeruli with thin glomerular basement membranes, normal cellularity and patent capsular space surrounding proximal and distal tubules. Alloxanated diabetic rats showed massive cellular infiltration and hemorrhage in interstitial tissue section of the kidney and another area of the same group showed completely degenerated glomeruli with thickening of Bowman's capsule lobulation with wide urinary space. The standard group showed a most of the partial prevention of the hyalinization but mild to recover the glomerulosclerosis to the normal condition. Kidney from alloxanated diabetic rat subjected to 100 mg/kg of aegle marmelos showing only mild cellular infiltrate in the interstitial tissue, some glomeruli showed lobulation and others showed degeneration with wide urinary space. Section of the kidney of an alloxanated diabetic

rat treated with 200mg/kg of alcoholic seed extract of *Aegel marmelos* showing glomerular degeneration with thickening of Bowman's capsule and cell debris in some tubular lumina. Section of the kidney of an alloxanated diabetic rat treated

with 400 mg/kg of alcoholic seed extract of *Aegel marmelos* showing lobulation of some glomeruli and vacuolar degeneration in some tubular epithelial cells.

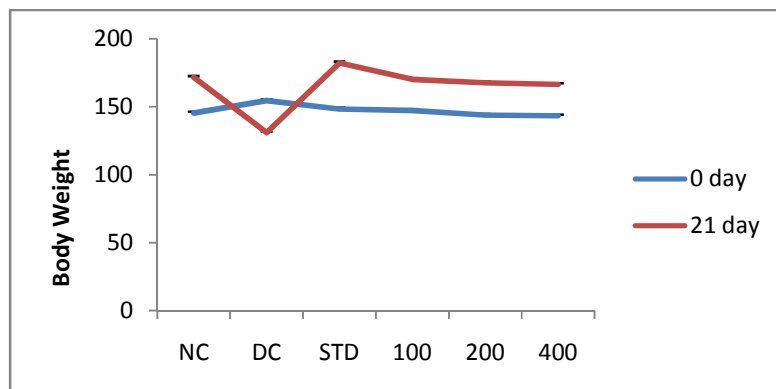


Fig.1: Effect of alcoholic seed extract of *Aegel marmelos* in change body weight of normal & diabetic rats. All values are expressed as mean  $\pm$  S.E.M.

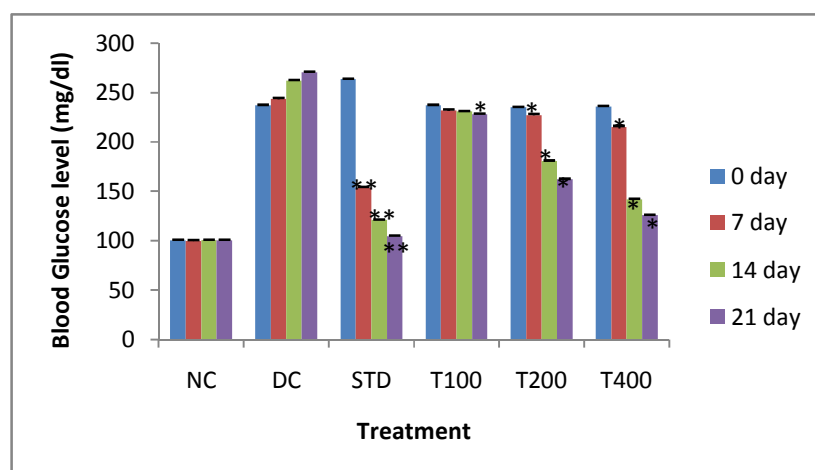


Fig no.2: Graph representing effect of alcoholic seed extract of *Aegel marmelos* on serum glucose in normal & diabetic rats

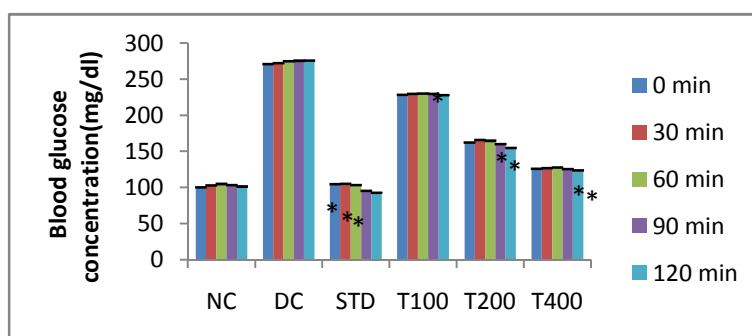
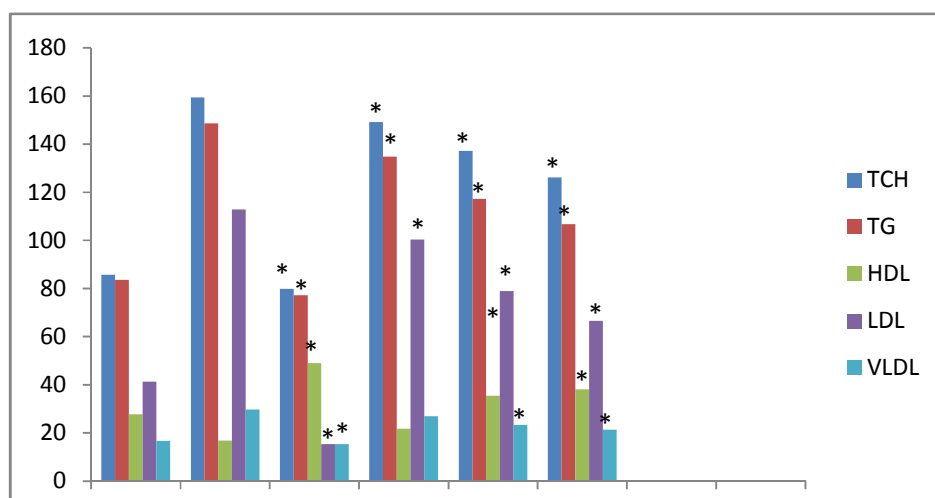


Fig no.3: Graph representing effect of alcoholic seed extract of *Aegel marmelos* on serum glucose in normal & diabetic rats in 0min, 30min, 60min, 90min, 120min



**Fig no .4 Graph representing effect of of alcoholic seed extract of *Aegel marmelos* on serum lipid profile in normal & diabetic rats**

#### Discussion

The anti hyperglycaemic activity of the alcoholic seed extract of *Aegel marmelos* on alloxan induced diabetic rats. Acute toxicity studies revealed the non toxic nature of the alcoholic seed extract of *Aegel marmelos*. There was no lethality or any toxic reactions found with the selected dose until the end of the study period. The fundamental mechanism in diabetic mellitus involves the over production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissue[20]. Alloxan has been shown to induce free radical production and cause tissue injury[21,22].Hence in the present study we observed on increased level of blood glucose in alloxane induced rats. The hyperglycaemic activity of alcoholic seed extract of *Aegel marmelos* has shown a significant ( $P < 0.005$ ) fall in blood glucose level from 7<sup>th</sup> day onwards in doses of 200mg/kg & 400 mg/kg per oral but fall in blood glucose levels from 14<sup>th</sup> day onwards the dose of 100 mg/kg per oral. Induction of diabetes with alloxane is associated with a characteristic loss of body weight which is due to increased muscle wasting and due to loss of tissue proteins[23].In our study a significant weight loss was observed in the diabetic group and significant improvement in weight was observed in the group treated with alcoholic seed extract of *Aegel marmelos* .This may due to the presence of proteins, alkaloids, flavanoids to reduce hyperglycemia[14].

Aegelin are one of the most numerous and wide spread group of alkaloids compounds in higher plant[24]. Some of them, due to their alkaloid structure are known to be involved in the healing process of free radical mediated diseases including diabetes.

The alcoholic seed extract of *Aegel marmelos* possesses aegelin as the anti diabetic principle agents. Hypoglycaemia is accompanied with dyslipidemia and represents a risk factors for coronary heart disease[25]. This abnormality high level of

serum lipids is mainly due to the uninhibited action of lipolytic hormones on the fat depots, mainly due to the action of insulin. Under normal circumstance, insulin activates the enzyme lipoprotein lipase, which hydrolysis triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceremia and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities[25].

In alloxanized animals there was an increase in the value of total cholesterol(TCH), triglycerides (TG),LDL, VLDL and decrease the value of HDL while the extract treated group showed an increased value of HDL and reduced VLDL, TC, TG in a significantly manner. This reduced the VLDL,TC and TG it may be presumed that the extract is responsible for the enhancement of the transcription of lipoprotein lipase similar to that of insulin.

From the above result we can confirm that the alcoholic seed extract of *Aegel marmelos* at dose of 100mg/kg, 200 mg/kg & 400 mg/kg per oral possesses significant antihyperglycemic activity with long term (21 day) treatment in rats.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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