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RESEARCH ARTICLE

STUDIES ON ANTIDIABETIC ACTIVITY OF AEGLE MARMELOS (L.) CORR. SERR.IN NORMAL AND ALLOXAN INDUCED DIABETIC RATS

***[1]Beena, V. L. [1]Subhash, C. P. and [2]Brijith Lal,**

[1]Department of Botany, University of Kerala, TVPM
[2]Department of Botany, Bishop Moor College, Mavelikkara.

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ABSTRACT

Medicinal plants have been used in the Ayurvedic system of medicine to maintain health and treat diseases. *Aegle marmelos* is one such plant the leaves and fruit of which have been reported to have varying medicinal properties. The main objective of the study was to evaluate the antidiabetic principle present in the aqueous extract of the leaves of *Aegle marmelos* in Alloxan diabetic animal model. Albino rates were selected for the study. Diabetes was introduced by a single intra peritoneal injection of Alloxan monohydrate. The diabetic rates were divided into groups were treated with the aqueous extract of *Aegle marmelos* of two different concentrations (con: 200mg/kg, 400mg/kg). One diabetic group was treated with Daonil. Another group was left untreated the kept as diabetic control. A group of rates were taken as normal control. The period of study was six weeks. The biochemical parameter, were estimated at periodic intervals of 1st 2nd 4th and 6th weeks of dry feeding and the results evaluated with respect to the hypoglycemic activity and toxicity of the drug.

INTRODUCTION

Herbal medicines have been used by man since the dawn of a civilization to maintain health and to treat various diseases. In the last few decades search for newer antidiabetic drug from natural resources has been intensified. A number of herbal preparations and plant extracts have been used with varying degree of success management of diabetes mellitus. Diabetes is essentially a metabolic disorder caused by little or no ability of the pancreas to produce insulin. Insulin, the hormone that allows glucose to be released in to the blood is taken up by the cells where it is used for energy. Insulin acts by binding to a plasma membrane receptor on the target cell. Human insulin receptor gene is located on the chromosome 19. Insulin receptor is a glycoprotein with four subunits, two alpha and two Beta sub units. The alpha units are located on the extra cellular side, to which insulin bind. The beta subunits traverse the membrane and are exposed on the cytoplasmic side Beta subunits has tyrosine kianase activity (Vasudevan *et al.,* 2001). In humans, diabetes mellitus is caused by the deficiency of insulin. In animals it can be induced by the administration of alloxan, an anti insulin antibody. This drug will destroy the pancreas (William *et al*., 2001). Most of the commercially available insulin is prepared from pancreas of beef or pig (Jain *et al*., 2000).

Corresponding author: Beena, V. L.
Department of Botany, University of Kerala, TVPM

Artificially human insulin is produce through genetic engineering technology (Dubey, 2000). Many plants reported to be useful for the treatment of diabetes mellitus in ayurvedic medicine are being tested for their hypoglycemic activity in experimental animals. *Aegle marmelos* is one such plant commonly used in ayurvedic system of medicine. The present investigation is part of continuing program related to biochemical screening of the plant *Aegle marmelos* used in ancient Indian medicine. The main objective of this study was to assess the antidiabetic effect of leaf juice of *Aegle marmelos* in Alloxan induced diabetic rats in terms of controlling blood glucose level, lipid profile bilirubin and lipid peroxides of experimental animals. The activity of the active constituents of *Aegle marmelos* leaf juice was compared with Daonil- a standard drug.

MATERIALS AND METHODS

Plant materials: The fresh leaves of *Aegle marmelos* were collected from the different localities of Kerala.

Preparation of Plant Extracts: Take 20 leaves for preparing 100ml juice. The leaves were ground in the mixie and filtered ground through fine cheesecloth then take the juice and administered to the rats by using a glass syringe orally in various dosages.

Animals: Albino rats of either sex (200-350 g) maintained on Hindustan pellet diet and distilled water adlib in biochemistry department in Bishop Moor College. The rats were made diabetic by injecting Alloxan monohydrate solution (150mg/kg body weight) intraperitonially. Blood samples are collected periodically 1st day, 7th day, 14th day, 28th day and 42nd day from the retinobulbular venous plexus capillary after the drug treatment. Blood sugar was estimated in semi auto analyzer by the glucose oxidase method using Auto span kit. Other biochemical parameters such as cholesterol, triglycerides, SGOT, SGPT, Alkaline phosphatase were also determined in the semi auto analyzer using Autospan kit.

The rats with blood sugar 200-350 mg were randomly divided in to five group of six each. The groups were as follows:

Group I Normal → distilled water
Group II Control → Alloxan
Group III Test drug → 200mg/kg
Group IV Test drug → 400mg/kg
Group V Standard drug → Daonil 10mg/kg

RESULTS

Effect of *Aegle marmelos* leaf juice on body weight in Alloxan induced diabetic rats: Table I and Fig I shows the change in the body weight or the rats under study, 16% increase in weight was seen in untreated rats after 42 days after 42 days of feeding. On the other hand the diabetic rat showed a decrease in weight by 15%. But in the test drug treated diabetic rat (Group 3rd & 4th) there was an increase in weight was 15% and 20% respectively, where as in the reference drug daonil treated diabetic group the increase in weight was 16%.

Effect of *Aegle marmelos* leaf juice on blood sugar level in Alloxan induced diabetic rats: Table II and Fig II shows Blood sugar reduction at the periodic stages is represented in the table II. The blood sugar was significantly increased in rats of group II when compared to normal rats. On 1st, 2nd, 4th and 5th weeks of expected study. A significant decrease in blood glucose level was observed in rats treated with drug at both the concentrations (group 3rd and group 4th) similar decrease in blood sugar was also observed in the Daonil group (group 5th) when compared to the diabetic control group. At the end of the 42nd day the increase in blood sugar in the diabetic group was 39% the decrease in blood sugar was 63%, 58%, 70%, in groups 3rd, 4th and 5th respectively.

Effect of *Aegle marmelos* leaf juice on liver function test in Alloxan induced diabetic rats: Table III and Fig III shows the liver function test and cholesterol level of the rats under study are presented in table III. The level of serum bilirubin and protein showed no significant change. The level of SGOT, SGPT, and alkaline phosphatase were not significantly altered in the group 3rd and 4th when compared to others. But the level of cholesterol was significantly increased in the diabetic group 2nd. The raised cholesterol was significantly decreased in group 3rd, 4th, and 5th.

Effect of *Aegle marmelos* leaf juice on lipid peroxide and glutathione in liver of Alloxan induced diabetic rats: Table IV and Fig IV shows the antioxidant status of the drug. The level of lipid peroxides was significantly increased in the diabetic groups of rats. The increase level was significantly decreased in the drug treated and Daonil group. The glutathione content was significantly decreased in group 2nd. The decreased glutathione was increased in group 3rd, 4th, and 5th.

Table 1. Value mean ± standard deviation of six rats in each group

Sl. No.	group	Body weight	in grams	% of change
		initial	final	
1	Normal(only distilled water)	180±20	210±25	16.87
2	Diabetic (Alloxan monohydrate)	165±15	140±20	15
3	Diabetic +Drug (200mg/kg)	150±15	180±10	20
4	Diabetic +Drug (400mg/kg)	185±25	205±15	10.8
5	Diabetic +Daonil (10mg/kg)	145±10	160±15	16

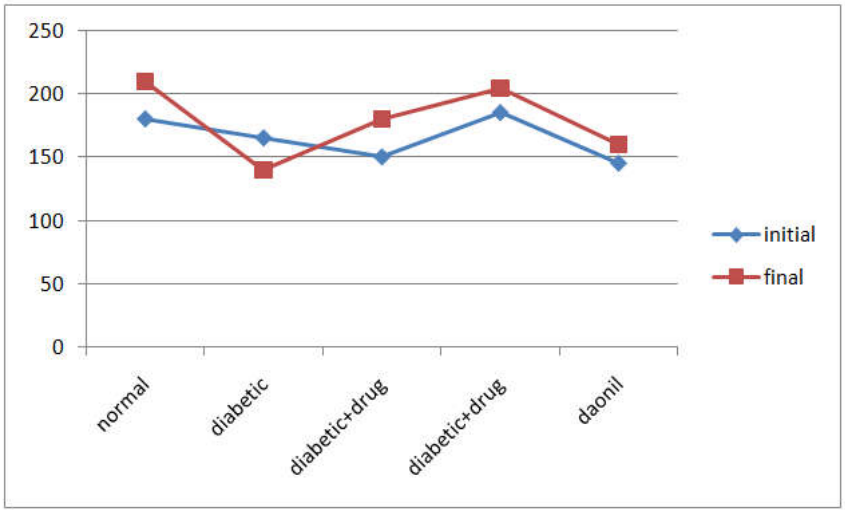


Fig. 1.

Table 2. Value mean ± standard deviation of six rats in each group

Sl.no	Groups	Initial	7th day	14th day	28th day	42nd day
1	Normal(only distilled water)	74±20.1	84±10.6	89±2	80±2.1	76±1.3
2	Diabetic (Alloxan monohydrate)	281±10	299±11.5	315±18.8	333±15.8	391±15.2
3	Diabetic +Drug (200mg/kg)	275±11	202±16.3	148±18.6	121±12.4	99.6±18.6
4	Diabetic +Drug (400mg/kg)	263±11	194±10.1	166±20.1	133±16.6	110±11.0
5	Diabetic +Daonil (10mg/kg)	294±12	193±16.1	160±5	110±2.6	86±12.3

Fig. 2.

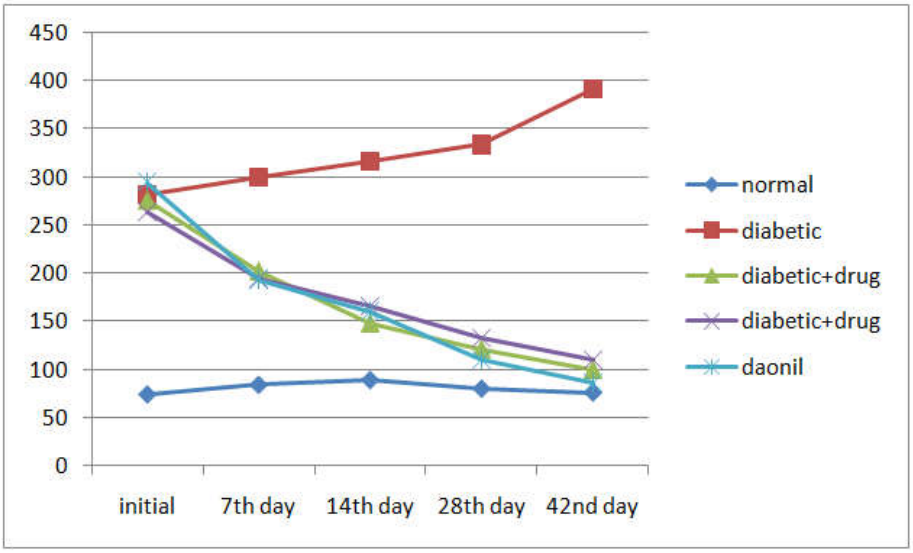


Table 3. Value mean ± standard deviation of six rats in each group

no	Group	S.b mg%	SGOT	SGPT	AP	Al	TP	G	Ch
1	Normal(only distilled water)	.6±0.001	63±2.1	41±1.6	97±3.9	3.5±.08	6.5±.8	2	66±2
2	Diabetic(Alloxan monohydrate)	.8±0.002	81±3.6	44±1.6	1.10±4	13.4±.09	5.5±.1	2.1	207±3.1
3	Diabetic +Drug (200mg/kg)	.6±0.001	201±6	63±1.3	251±7	2.2±.05	5±.8	2.9	62±1.3
4	Diabetic +Drug (400mg/kg)	.8±0.02	230±9	99±2	312±2	2±.05	44.8±.8	2.8	69±2
5	Diabetic +Daonil (10mg/kg)	.7±0.01	77±2.8	39±2.1	126±2.8	3.8±.1	6±.3	2.2	85±2

Sb:Bilirubin, AP: alkaline phosphatase, Al:albumin, Al:total protein, G:glucose, Ch: cholesterol

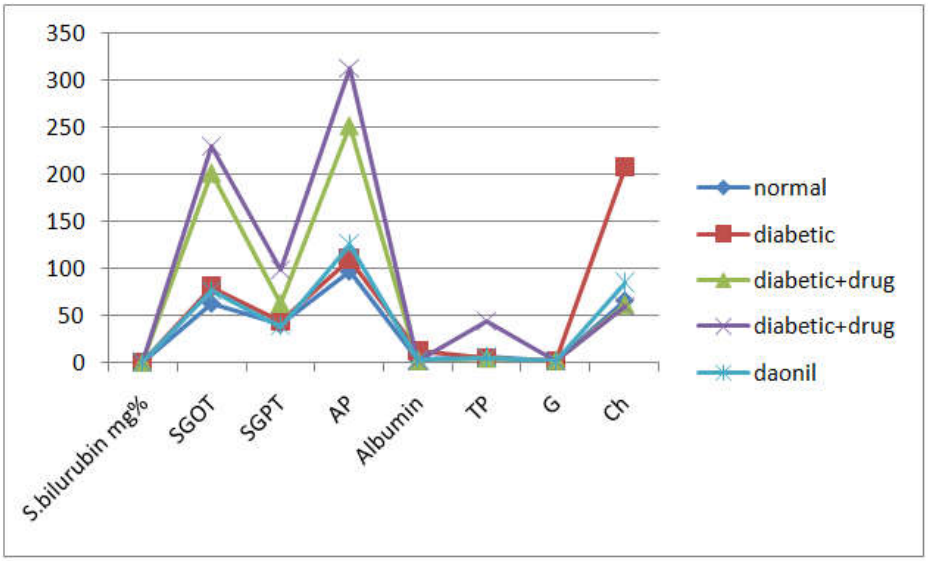


Fig. 3.

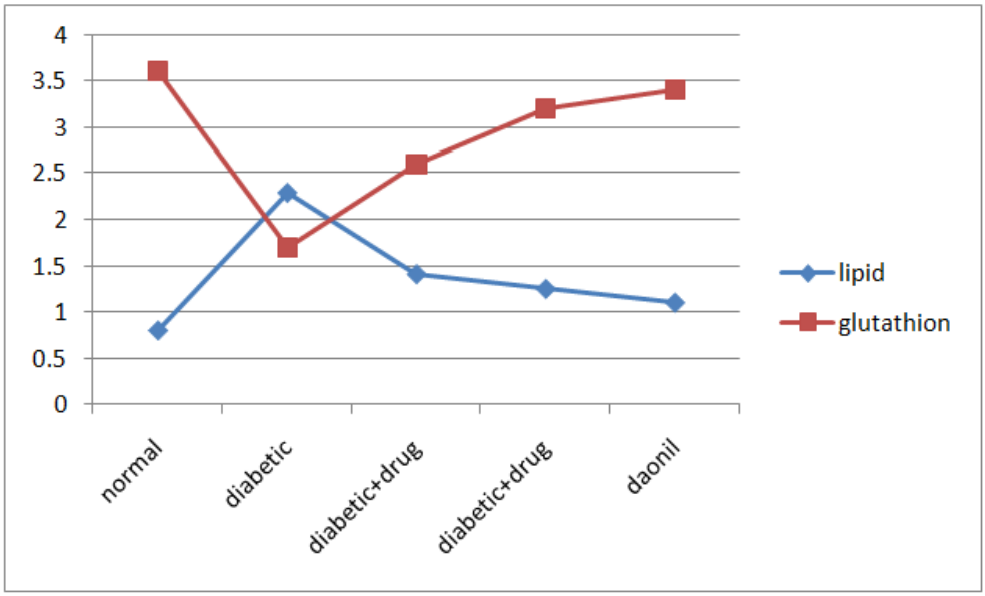
DISCUSSION

Diabetes mellitus has been found to affect a large number of people throughout the world. Basically this disease of associated with the defective utilization of glucose by the cells and derangements to varying degree in the metabolism of protein and lipids. Clinically this disease is associated with a number of chronic complication affecting the eyes, kidneys, and nerves of the arteries.

Table IV (Value mean ± standard deviation of six rats in each group)

Sl.no	Groups	LP moles/mg/ptn	G mg/gm/ptn
1	Normal(only distilled water)	0.8±0.19	3.6±0.53
2	Diabetic (Alloxan monohydrate)	2.2999±0.22	1.7±0.45
3	Diabetic +Drug (200mg/kg)	1.41±0.21	2.6±0.54
4	Diabetic +Drug (400mg/kg)	1.21±0.18	3.2±0.3
5	Diabetic +Daonil (10mg/kg)	1.1±0.17	3.4±0.28

LP:lipid peroxides, G:glutathione.



Plant based drugs have been in use against various diseases since time immemorial. The primitive man used herbs ask therapeutic agents, which they were able to procure easily. The essential values of some plants have been published, but a large number remain unexplained as yet. There are many hypoglycemic plants know through the folk fore, but their introduction to modern therapy await the discovery of animal test system that closely parallel to the pathological course of diabetes in humans. One such plant *Aegle marmelos*, which has been reported to have hypoglycemic principles and used for treatment in the Ayurvedic system of medicine Ravishankar,B &Shukla, V J (2007). Hence our study was aimed out to evaluating the antidiabetic and antihyperlipidemic and antioxidant potential of leaves of Aegle *marmelos* on Alloxan diabetic rats.An observation of the body weight gain in the normal and *Aegle marmelos* treated diabetic rats suggested the positive effect of the drug on the diabetic rats suggested the positive effect of the drug on the diabetic rats, since similar weight gain was also observed in the Daonil treated groups. The insignificant weight gain may be due to the normal food intake of the animals. As evidenced in the Table II, the significantly increased blood glucose in the Alloxan treated rats is due to the distraction of beta cells o the islands of langerhans leading to the decrease secretion of insulin. In this group of rats the blood sugar level remained significantly high at the end of the 40 day study. On the other hand leaf juice of Aegle marmelos treated diabetic rats showed a significant decrease in the blood sugar at the end of the 40 days study the decrease being 60%, which is a remarkable change. The reduction was significant at both the dose level and the results are comparable to that of the Daonil treated diabetic group of rats, which is standard drug in clinical practice and Daonil, was used as a reference drug for comparison.

The decrease in the blood sugar was observed from the 7th day of feeding the leaf juice of *Aegle marmelos* and the significant decreases in the blood sugar was observed the end of the study. Gopalsamy *et al*., (2012), in their study on the methanolic extract of the bark of *Aegle marmelos* observed a decreased in blood sugar from the sixth day on wards in Alloxan diabetic rats and continues administration of the extract lead to the finding of a 54% decreased in the blood sugar at the end of the 12th day and their study thus correlated with the result of our study. The exact mechanism of action is not known, the reduction in blood sugar can be due to the capacity of the drug to induce the pancreatic secretion of insulin from the beta cells by increasing the phosphorylation of the insulin receptors and this aid in triggering the insulin cascade system. The decrease in blood sugar may also be due to an enhancement in the transport of blood glucose to the peripheral tissues or to the reduced glucose absorption from gastro-intestinal tract. Narayan P. Yadav and Chanotia C S (2009), in their attempt to evaluate the potential antidiabetic effect of *Aegle marmelos* leaves extract in Alloxan, diabetes noted a significantly increased glucose tolerance in animals orally given *Aegle marmelos* leaf extract prior to the experiment.

They have also observed the reversal of liver glycogen to almost normal. Narayan P. Yadav and Chanotia C S (2009), from their observations have concluded that the active principle present in *Aegle marmelos* leaf extract has similar hypoglycemic action as that of insulin. Sivaraj *et al*., (2009), had designed their study to elucidate the protective effect of the aqueous extract of *Aegle marmelos* fruit on the pancreas of steptozotocin induced rats, and they have reported an increase in the insulin level associated with a decrease in blood sugar. The extract could thus improve the functional state of the

pancreatic B cells and partially reverse the damage caused steptozotocin as evidenced by their histopathological study and they have also concluded that the protective effect of *Aegle marmelos* fruit juice on the pancreas was graded than that glienclamide. The effect of the *Aegle marmelos* fruit juice was further confirmed by the observation of Stanley prince *et al*., (1998) on their finding that fruit extract could significantly decreased, the increased blood sugar and glycosyleted hemoglobin and increased the plasma insulin and liver glycogens in streptozotocin treated diabetic rats, on the other hand Padayatti *et al*., (1996) have reported that alteration in the qualitative and quantitative release of the enzyme malate dehydrogenace – an important of glucose metabolism has been suggested to contribute to the pathological state of diabetes and *Aegle marmelos* leaf extract could significantly reverse the km value but not V max or the enzyme malate dehydrogenase and thus being effective in restoring the blood sugar and body weight to normal value in steptozotocin diabetes rats.

On further extensive study the Padayatti *et al*., have found that *Aegle marmelos* leaf extract could significantly reversed the altered histopathological and ultra structural parameters in tissues of steptozotocin diabetic rats by light and electron microscopy to near normal and improve the functional state of pancreatic B-cells and attributing the hypoglycemic effect of the drug to be mediated through regeneration of damaged pancreas. An observed in table 3 the increased cholesterol level was decreased by the *Aegle marmelos* leaf extract in Alloxan diabetic rats there by denoting the hyperlipedimic action. Our results are confirmed by the study of Kmalakannan *et al*., (2005) who have observed that the *Aegle marmelos* fruit juice could exhibit anti hyperlipedemic effect by decreasing the serum and tissue lipids. Cholesterol, triglycrides, phospholipids and fattyacids. No significant change was observed in the liver function test the enzymes SGOT, SGPT and alkaline phosphatase and also the level of bilirubin and protein, which shows that the drug has no toxic effect on the liver. Oxidative stress is produced during normal metabolic process in the body as well as induced by a variety of factors and chemicals. Oxidative stress induced by alloxan has been shown to damage pancreatic B-cells and produce hyperglycemia in animals. In the present study we have observed an elevated lipid peroxide in the liver and a decreased liver glutathione, which is a clear manifestation of excess formation of free radicals and activation of liquid peroxide system resulting in tissue damage. In hyperglycemia glucose undergo auto oxidation and produce super oxides resulting in the release of free radicals which in turn leads to the lipid per oxidation in lipoproteins. on treatment with *Aegle marmelos* leaf juice the lipid peroxides were decreased the level of glutathione was increased which shows that *Aegle marmelos* can effectively increase the anti oxidant potential in vivo glutathione is a major non-protein thiol in living organisms which play a central role in coordinating the body's anti-oxidants defense process. Perturbation of glutathione status of a biological system can lead to serious consequences and there was correlated by the aqueous extract of the leaves of *Aegle marmelos* treated rats indicated that the oxidative stress elicited by Alloxan had been nullified due to the effect of the extract. Sabu *et al*., have reported that oxidative stress induced by Alloxan has been shown to damage pancreatic B-cell producing hyperglycemia in rats.

Aegle marmelos treated rats could significantly decrease the oxidative stress as evidenced by the decrease in lipid peroxidation in serum and liver and an increase in catalase, and glutathione peroxidase and their study further confirm our finding indicating that the leaf extract of *Aegle marmelos* could effectively reduce the oxidative stress of Alloxan resulting a decrease in Blood sugar. Kamala Kannan *et al*., (2003) have given similar reports on their work on *Aegle marmelos* fruit juice. *Aegle marmelos* fruit juice exhibited antidiabetic ant hyperlipedimic and anti-oxidant property in steptozotocin treated rats. A significant reduction in lipid per oxidation and an increase in superoxide dismutase, catalase and glutathione peroxidase in the hepatic and renel tissue of steptozotocin rats was noted. The exact role of oxidative stress in the etiology of human diabetes is however not known. Oxidative stress has been shown to produce glycation of protein in activation of enzymes, alteration in structural function collagen basement membrane and may have a significant effect in glucose transport protein or insulin receptors scavengers of oxidative stress may have an effect in reducing the increased glucose level in diabetes and may alleviate diabetes and reduce the secondary complication. Alloxan which is an accepted model for the induction of diabetes damage the B-cells by the liberation of oxygen radicals. The *Aegle marmelos* leaf extract could effectively counteract the stress on observed in the study.

Alternative medicine is used to treat diabetes for thousands of years. There is a growing interest in correlating phytochemical constituents of plants with its pharmacological activity. In future more coordinated multi dimensional research is aimed at in correlating botanical and phytochemical property to specific pharmacological property. Majority of the drug ar at the experimental stage and have to undergo well planned clinical trial being used in the therapy of diabetes. Today concurrent consumption of drugs from different discipline is a common finding. Interaction of plant drugs with modern medicine is important from the clinical point of view. Natural oxidants strengths the endogenous anti oxidant defense from reactive oxygen species and restore optimal balance by neutralizing the reactive species. There are gaining immense importance by virtue of their critical role in disease protection. In this context Aegle *marmelos* can rightly be mentioned as a plant of considerable interest in diabetes therapy. The antidiabetic effect and the non toxicity of the aqueous extract of *Aegle marmelos* was evaluated in alloxan diabetic rats. The blood sugar was significantly increased in alloxan diabetic rats. The aqueous extract of *Aegle marmelos* leaves at the two different concentrations could decrease the level of blood sugar at the different stages of the study. The decrease being significant at the end of the study. The extent of decrease being 60% thus exhibiting the hypoglycemic effect of the drug. The level of serum cholesterol was decreased in the treated group of rats compared to the diabetic rats which also show the hypolipidemic action of the drug. The liver function tests- serum bilirubin, SGOT, SGPY, ALP and serum proteins. Remained altered thus indicating the non toxicity of the drug. The increase in lipid peroxide and the decrease in glutathione in the liver of the alloxan diabetic was reversed on treatment with the aqueous extract of the leaves of *Aegle marmelos* further confirming the anti oxidant potential of the drug. All the above results were comparable to that shown by the reference drug daonil used in the study. The observed result in

the present study suggests that the leaves of *Aegle marmelos* may prove to be a potential therapy to treat diabetes induced by pancreatic dysfunction. However further extensive study is required before exploiting in human kind regarding their dosage and safe usage.

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