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Original research article

Antidiabetic effect of medicinal plant *in vitro* and *in vivo* in animal species

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Abstract
Aegle Marmelos is a traditional medicinal plant in India which belongs to Rutaceae family which possesses innumerable health benefits. The entire plant body including its leaves, stem, root, inflorescence and seed are proved to be significant medicinal value and hence it is one among the inevitable plant used in the preparation of various Ayurvedic pharmacological products. The plant is a rich source of various components including eugenol, Vicenin-2, linoleic acid, oleic acid, rosmarinic Calcium, Phosphorous and many more. Its Ethnopharmacological properties such as Anti-diabetic, Anti-cancerous, Analgesic, Anti-inflammatory, Radio-protective. *In vivo* toxicity study concluded that no mortality was find in toxicity study.
Keywords: Vicenin-2, chloroform, ethyl acetate, STZ, glibenclamide

Introduction

Bioprospecting refers to discovery of new or known chemical compounds from biological resources and it often draws from indigenous knowledge on the uses and characteristics of these resources. It is an effective and economical strategy for drug discovery. In spite of past success in New Chemical Entities (NCEs) generation from natural products for drug discovery and development, there has been a recession in the generation of NCEs by the pharmaceutical companies.

Human body is a complex network and the pathogenesis of any disease including diabetes mellitus involves multiple pathways. Modern medicines attempt to use a single compound to hit single target of a particular pathway for combating the related disease. This approach may be effective for an instantaneous relief but it may not prove to be a complete solution. Further, a risk of unwanted side effects almost always is a part and parcel of modern medication. On the contrary, traditional medicines exert synergistic effects due to multi-constituents and multi-targets. Thus the traditional formulations can be used as readily available way for investigative new drugs (IND) for the development of potent efficacious medicaments [1].

Diabetes is the condition in which the body does not properly process food for use as energy. Most of the food we eat is turned into glucose, or sugar, for our bodies to use for energy. The pancreas, an organ that lies near the stomach, makes a hormone called insulin to help glucose get into the cells of our bodies. When you have diabetes, your body either doesn't make enough insulin or can't use its own insulin as well as it should. This causes sugars to build up in your blood. This is why many people refer to diabetes as "sugar." Diabetes can cause serious health complications including heart disease, blindness, kidney failure and lower-extremity amputations. Diabetes is the seventh leading cause of death in the United States.

Material and Method

1. Method

1.1 Collection of plant material

Leaves of *Aegle marmelos* were collected from local area of Bhopal (M.P.) in the month of June, 2021.

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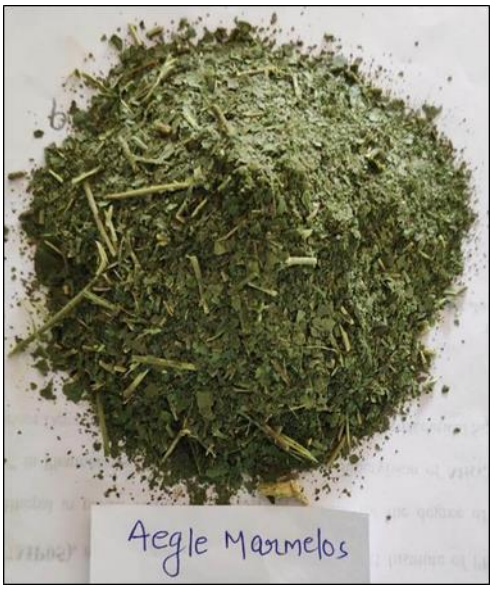

Fig 1: Collection of leaves of *Aegle marmelos* **Fig 2:** Shade dried powdered leaves

1.1.1 Drying

Drying of fresh plant parts was carried out in sun but under the shade.

1.2 Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered leaves [2].

1.2.1 Extraction by maceration method

The shade dried material was coarsely powdered and subjected to extraction with petroleum ether by maceration [64]. The extraction was continued till the defatting of the material had taken place. 128gm of dried leaves powder of *Aegle marmelos* were exhaustively extracted with successive solvent like chloroform, ethyl acetate, ethanol, aqueous using maceration method. The extracts were evaporated above their boiling points and stored in an airtight container free from any contamination until it was used. Finally, the percentage yields were calculated of the dried extracts.

Fig 3: Extraction by maceration method

DPPH method

DPPH scavenging activity was measured by the spectrophotometer [69]. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15

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minutes at 517 nm [3-5].

$$Calculation\ of\ \%\ Reduction \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

***In vitro* antioxidant activity of ethanolic extracts of *Aegle marmelos* using nitric oxide method**

Nitric oxide was produced from sodium nitroprusside and the Griess reagent was measured. Sodium nitroprusside spontaneously produces nitric oxide in aqueous solution at physiological pH, interacting with oxygen to generate nitric ions that can be estimated using Griess reagent. Nitric oxide scavengers compete with oxygen resulting in decreased nitric oxide manufacturing (Marcocci *et al*., 1994). Sodium nitroprusside (10 mmol/L) was mixed with various extract concentrations in phosphate buffer saline (PBS) and incubated at 25 °C for 150 min. Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthyl ethylenediamine dihydrochloride) was added to the specimens. The chromophore absorbance created during the diazotization of sulfanilamide nitrite and subsequent coupling with napthylethyleneediamine was read at 546 nm and referred to the absorption of conventional ascorbic acid solutions treated in the same manner with Griess reagent as a positive control. [6] All triplicate experiments were conducted and the chart was plotted with the mean values. The inhibition proportion was evaluated using the following formula:

Radical scavenging activity (%) = $(A_{control}-A_{test})/A_{control}\times 100$

Where a control is the absorption (without extract) of the control and where a test is the absorption in the presence of the extract was/standard.

Antidiabetic screening

Body weight: The body weight was measured weekly using calibrated weighing balance [7].

Blood glucose

Fasting blood glucose levels was monitored once in a week using a glucometer.

Result

DPPH method

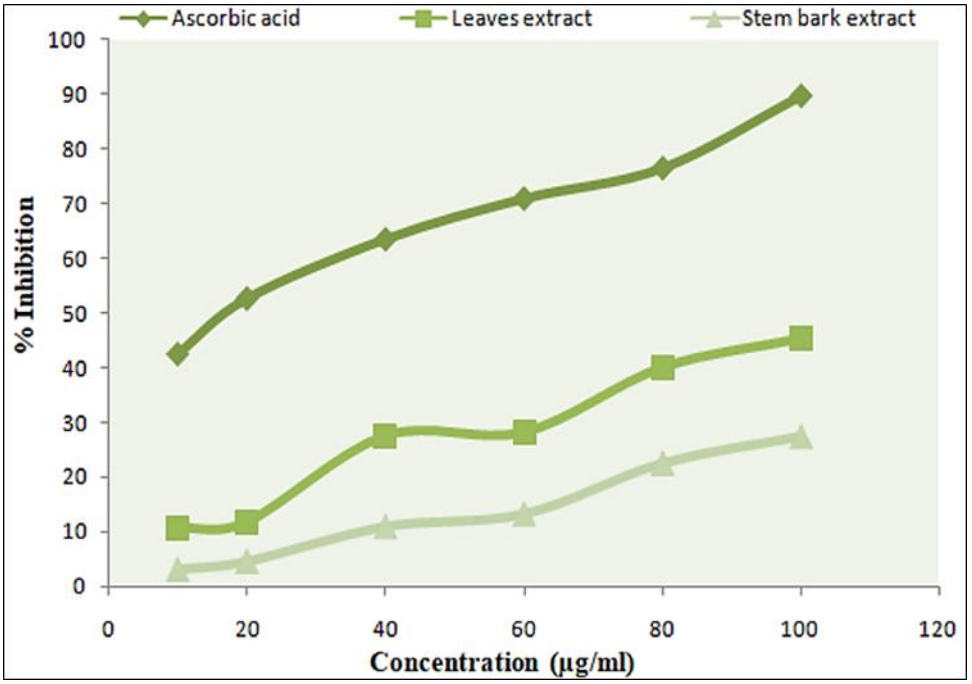

Fig 4: % Inhibition of ascorbic acid and extracts of *Aegle marmelos* using DPPH method

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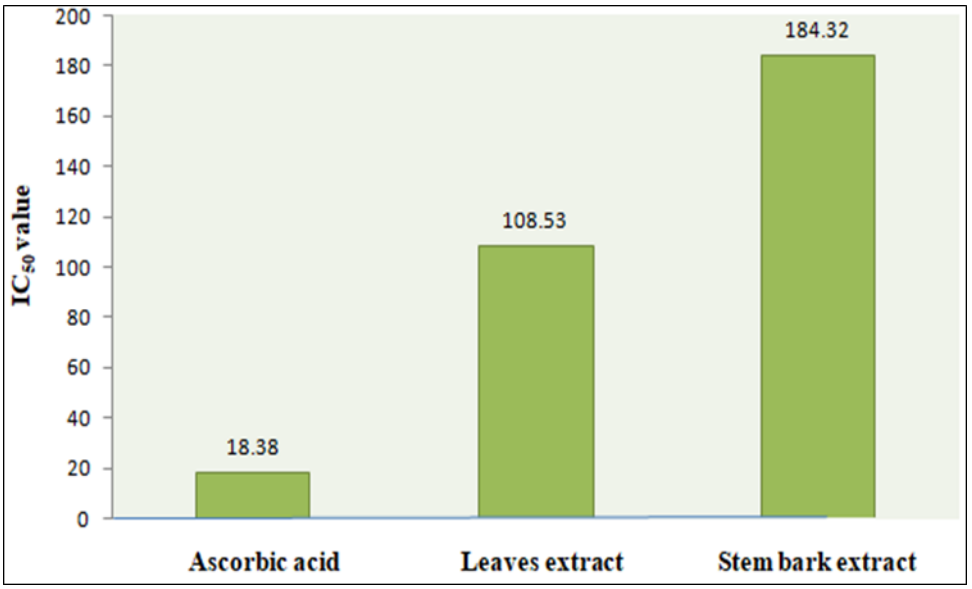

Fig 5: IC_{50} value of ascorbic acid and extracts of *Aegle marmelos*

1. No. Model

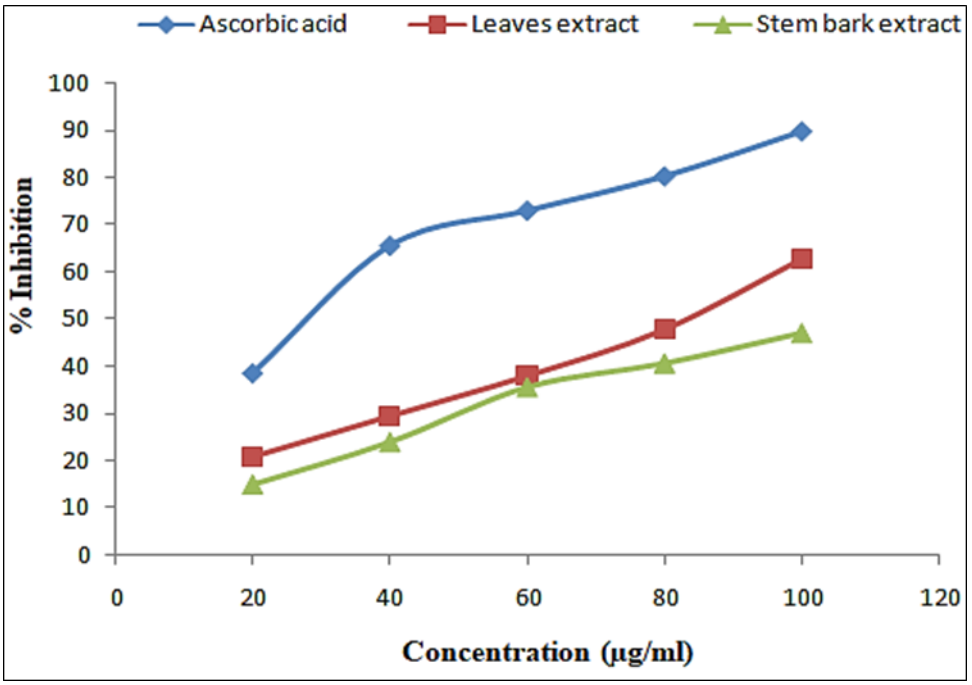

Fig 6: % Inhibition of ascorbic acid and extracts of *Aegle marmelos*

***In vivo* pharmacological study**

The various results obtained from different experiments carried out were compiled here under. Blood glucose level of animals in all groups was recorded at 0,8th and 21th day and change in % blood glucose was also mentioned as shown in Table 2. Progressive decrease in blood glucose level was found in all treatment groups during study. At the end of experiment, all treatment groups, with 100 mg/kg and 200 mg/kg of *Ethanolic extract of Aegle marmelos leaf* (132.40±8.3.) and (130.25±6.26), 100 mg/kg and 200 mg/kg of ethanolic extract of *Aegle marmelos* stem (138.2±9.43) and (125.89±8.32), was decrease significantly (p<0.001) serum glucose level, as represented in Table and Figure 1.

Table 1: Antidiabetic effect of *Aegle marmelos* extract on blood glucose (mg/dl) in STZ+HFD induced diabetic rats

Groups	Treatment	Day 0	Days 8	21 Days
I	Normal	80.3±5.88	98.32±5.32	118.23±8.32
II	STZ +HFD Control	280.45±9.31	405.3±9.1	430.2±9.32
III	STZ +HFD + Glibenclamide (600µg/kg)	267.41±9.32	150.31±5.87*	130.2±5.78*
IV	STZ +HFD+ ethanolic extract of Aegle marmelos leaf (100 mg/kg)	285.3±8.43	170.3±8.3*	132.40±8.3*
V	STZ +HFD+ ethanolic extract of Aegle marmelos leaf (200 mg/kg)	260.42±7.32	155.35±6.8*9	130.25±6.26*

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VI	STZ +HFD+ ethanolic extract of *Aegle marmelos stem bark* (100 mg/kg)	28.21±6.89	178.34±8.21*	138.2±9.43*
VII	STZ +HFD+ ethanolic extract of *Aegle marmelos stem bark* (200 mg/kg)	270.31±9.32	155.36±9.43*	125.89±8.32*

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at # $p<0.01$ vs. normal group; **$p<0.01$, *$p<0.05$ vs. diabetes control group respectively (One-way ANOVA followed by Tukey's post hoc test).

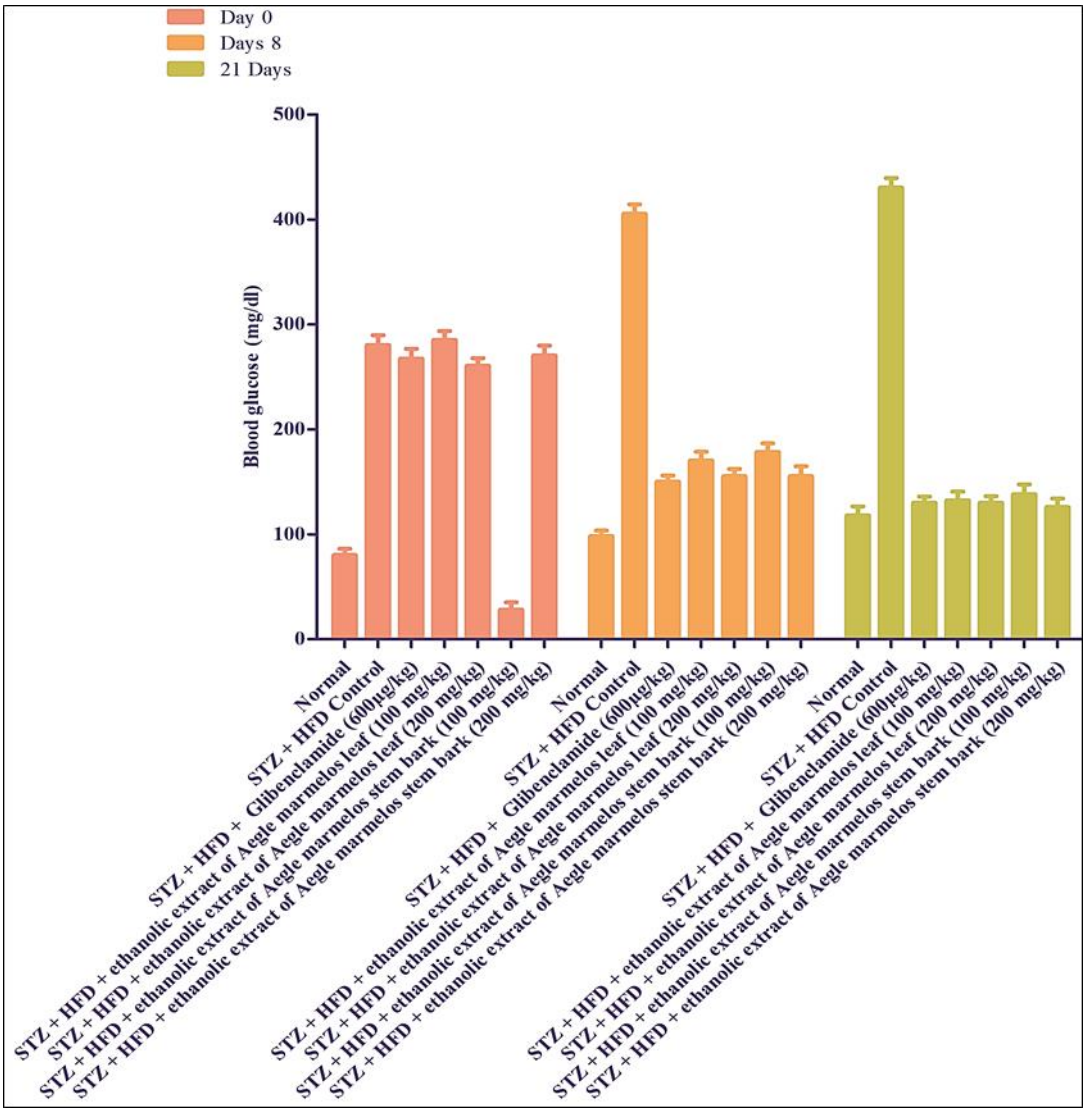

Fig 7: Antidiabetic effect of *Aegle marmelos* extraction blood glucose (mg/dl) in STZ+HFD+HFD induced diabetic rats

Body weight of animals in all groups was recorded at initial and final day weight was mentioned. In all treatment groups, with 100 mg/kg and 200 mg/kg of *Ethanolic extract of Aegle marmelos leaf* (198.3±8.45) and (205.37±11.46), 100 mg/kg and 200 mg/kg of ethanolic extract of *Aegle marmelos* stem (217.3±10.5) and (219.3±11.23) was not significant in body weight during study period as represented in Table and Figure 2.

Table 2: Antidiabetic effect *Aegle marmelos* extract on body weight in STZ+HFD induced diabetic rats

Groups	Treatment	Initial weight	Final weight
I	Normal	210.34±10.48	210.33±12.89
II	STZ + HFD Control	208.5±10.42	190.3±8.3
III	STZ + HFD + Glibenclamide (600µg/kg)	220.3±11.23	225.87±9.72*
IV	STZ + HFD + ethanolic extract of Aegle marmelos leaf (100 mg/kg)	208.3±7.88	198.3±8.45*
V	STZ + HFD + ethanolic extract of Aegle marmelos leaf (200 mg/kg)	180.3±9.31	205.37±11.46*
VI	STZ + HFD + ethanolic extract of *Aegle marmelos stem bark* (100 mg/kg)	222.3±9.3	217.3±10.5*
VII	STZ + HFD + ethanolic extract of *Aegle marmelos stem bark* (200 mg/kg)	223.4±10.43	219.3±11.23*

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at #$p<0.01$ vs. normal group;**$p<0.01$, *$p<0.05$ vs. diabetes control group respectively (One-way ANOVA followed by Tukey's post hoc test).

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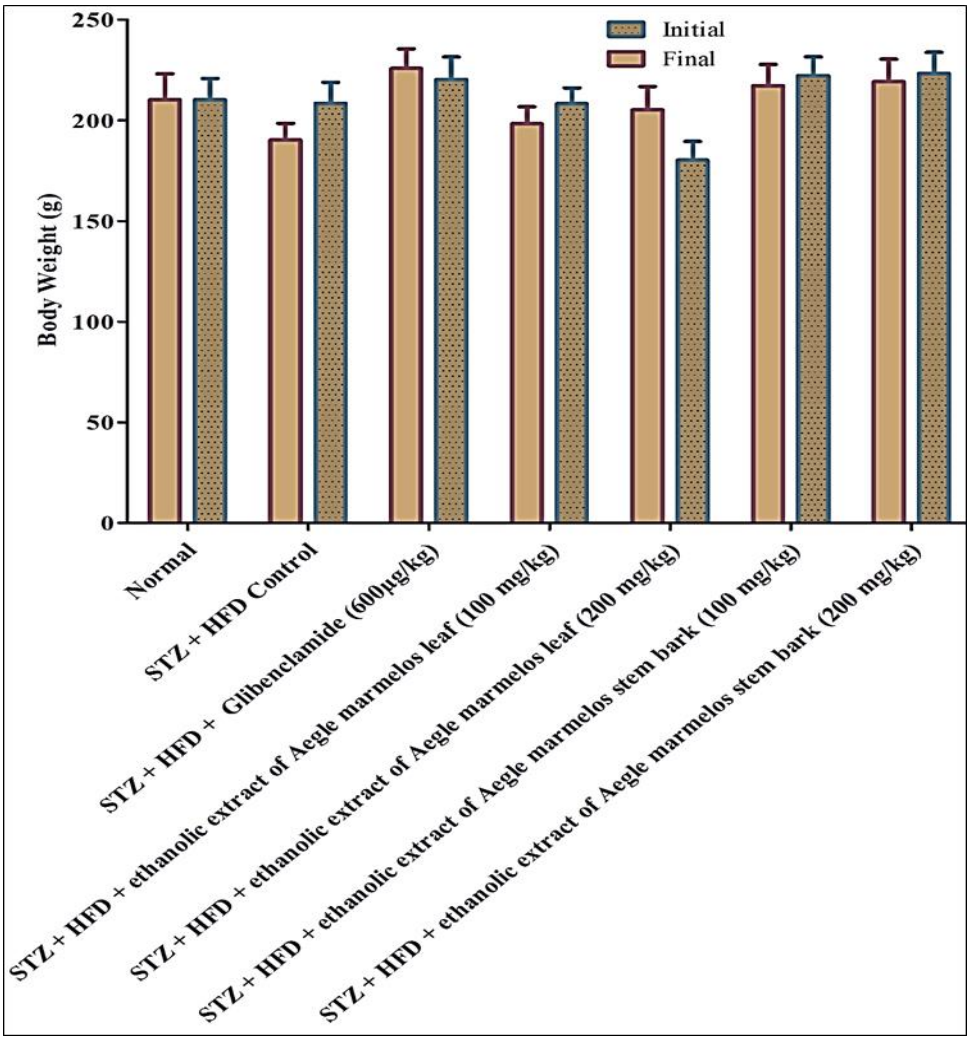

Fig 8: Antidiabetic effect of *Aegle marmelos* extract on body weight in STZ+HFD induced diabetic rats

Discussion

Diabetes is the situation in which our body does not accurately process food as energy. Food directly turned into glucose, sugar. Present time diabetes is 7th largest cause who is raised rate of mortality. Serious complication occurs due to diabetes it may be heart disease, blindness, kidney failure etc.

Plants have been used to treat many ailments since time immemorial. India has several plant species and thousands of them have claimed to possess medicinal properties. A number of plants have shown varying degree of hypoglycemic and anti-hyperglycemic activity. The active principles present in medicinal plants have been reported to be capable of pancreatic β-cell regeneration, insulin secretion and reversal of insulin resistance

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