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Investigation for Anti Diabetic properties of Methanol Extracts prepared from Dried Apricot *Kernel*s in Rats.

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ABSTRACT

Apricots are a sweet summer-fruit staple and a wonderful addition to your diabetes meal plan. One apricot has just 17 calories and 4 g of carbohydrates. Four fresh apricots equal one serving and provide more than 50 percent of your daily vitamin A requirement. These fruity jewels are also a good source of fiber. Modern medicine is best for treating any disease in the same way traditional medicine should also be encouraged cause the traditional medicine is available at cheaper cost and said to be without any side effects. To support this anti diabetic activity of apricot kernels is performed. In this study alloxan induced diabetic activity model rat was used. First the kernels are authenticated then extracted using methanol. The methanol extracts showed the presence of alkaloids, tannins, flavonoids etc. which maybe the reason for anti-diabetic activity. Alloxan forms an increased glucose levels that generates diabetes. Post treatment with *apricot kernel* extract produced significant decrease in glucose levels indicating the therapeutic effect of extract. The method for testing glucose levels in blood is done by glucometer On treatment a dose dependent (low dose-100mg/kg, high dose-200mg/kg.) decrease in glucose levels were observed. For the low dose, the glucose level decreased to 150.1±17mg/dl by 7th day from 293.1±9.83mg/dl. For the high dose, the glucose level decreased to 125.7±20.7mg/dl by 7th day from 280.5±42.4mg/dl. However, the standard medicine metformin at the dose of 450mg/kg showed better results with 112.3±2.4mg/dl. Even though the extracts results are lower than the standard with further research and purification of extracts better results can be achieved.

Keywords: Apricot kernel, diabetic activity, wistar rats etc.

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INTRODUCTION

Diabetes mellitus is one of the most common endocrine diseases in all populations and all age groups. It is a syndrome of disturbed intermediary metabolism caused by inadequate insulin secretion or impaired insulin action, or both. Diabetes mellitus comprises of heterogeneous group of disorders characterized by hyperglycemia, altered metabolism of carbohydrates, lipids and proteins. Diabetes mellitus is associated with complications such as nephropathy, retinopathy, neuropathy and cardiovascular disease.[1]

Long term complications

Diabetic Neuropathy:

Diabetic neuropathy may involve either the periphery, gastrointestinal, genitourinary, or all systems. Diabetic neuropathy produces symptoms in 60-70% of all diabetic persons. Neuropathic complications are divided into autonomic dysfunction and sensory dysfunction. Sensory complications include paresthesias and the loss of sensation in the extremities, leading to an increase in serious foot problems in diabetics. Autonomic complications include sexual dysfunction, and postural hypotension.[2]

Diabetic nephropathy:

It is a serious microvascular complication of diabetes. Diabetes mellitus is the most common cause of end stage renal disease in the United States. The risk factors for nephropathy are older age, male sex, non-Caucasian race, and poor blood pressure, glycemic, and lipid control. The kidneys have several important functions: excreting waste, maintaining blood pressure through the regulation of fluid and salts, production of erythropoietin (a regulator of red blood cell mass), and activation of vitamin D (a co-factor for calcium absorption). Normal kidney function involves the filtration of fluid from the blood and formation of urine. The early pathogenesis of diabetic nephropathy begins with hyperglycemia causing glomerular hyper filtration, which results in glomerular hypertrophy and glomerular basement membrane thickening.[2] Early nephropathy also involves hemodynamic changes, including, decreased plasma flow, and a moderately increased glomerular capillary pressure leading to an increased glomerular filtration rate (GFR).

Apricot: [3, 4]

Apricots' sweet and delicate flavor, as well as their impressive nutrient content, makes them a worthwhile addition to your diet. They come loaded with beneficial vitamin A, and provide considerable amounts of vitamin C, potassium, copper and manganese. In addition, apricots can help satisfy your sweet tooth without wreaking havoc on your blood sugar levels, unlike sweets that contain sugar and processed carbohydrates. Apricots' low glycemic index and nutrient content

help them regulate your blood sugar levels. Fiber accounts for some of apricots' beneficial effect on blood sugar. Dietary fiber slows digestion to control the release of sugar into your bloodstream, and it improves your body's ability to respond to insulin, which helps further control blood sugar. A cup of sliced fresh apricot contains 3.3 grams of dietary fiber, while a half-cup of dried apricots boasts 4.7 grams. Each serving of dried apricots provides 18 percent of the daily fiber requirements for women and 12 percent for men, established by the Institute of Medicine.[3]

The vitamin E found in fresh and dried apricots also contributes to their effect on blood sugar for some individuals. Vitamin E functions as an antioxidant, and a diet rich in antioxidants helps improve blood sugar levels for people suffering from Type 2 diabetes, explains the University of Maryland Medical Center. Consuming a half-cup of dried apricots boosts your vitamin E intake by 2.8 milligrams and provides 19 percent of the daily intake recommended by the Institute of Medicine. A cup of fresh sliced apricots contains 1.5 milligrams of vitamin E, or 10 percent of the recommended daily intake.[4]

Consuming More Apricots

Dried apricots make for a filling snack consumed on their own and work well with nuts and seeds for homemade trail mix, while chopped dried apricots make welcome additions to salads. Try combining quinoa, chopped dried apricots, green onion, fresh mint and an orange juice vinaigrette for a high-fiber salad that helps regulate your blood sugar. Consume fresh apricots on their own, or use thinly sliced apricots and all-natural almond butter to top whole-grain toast for a healthful breakfast.[5]

MATERIALS AND METHOD

Sodium citrate (Virat labs, Hyd, India), Diethyl ether (Finar chemicals limited, Ahmadabad.), Methanol (E-Merk, Mumbai, India.), Normal saline (Claris life sciences, Ahmadabad, India.), Formaldehyde (Finar chemicals limited, Ahmadabad, India), Alloxan monohydrate (Sigma, St Louis, U.S.A.), Metformin (MSN Formulations, HYD, India.) etc.

Collection and Authentication of Material

The fruits of *apricot* collected from local fruit market of jaggayypet. And authenticated by professor dr. k. Madhava chetty, department of botany, Sri Venkateswara University, Tirupati.

Figure 1: Apricot and Apricot Kernel

Approval of the study:

The institutional animal ethics committee under the Central Animal House Registration No: 1688/PO/E/2013/CPCSEA approves the animal testing of current animal testing application number: 17-004 from September 20th 2017 to December 20th 2017.

Extraction of Material

The seeds separated from the apricot fruits and dried in shade. After drying, seeds are placed in a conventional grinder to prepare powder of the seeds.

Cold Extraction using Methanol

In this work, the cold extraction process done with the help of methanol. About 200gms of powdered material taken in a clean, flat-bottomed glass container and soaked in 750 ml of Methanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.[6,7]

Evaporation of Solvent

The filtrates (Methanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract kept in vacuum desiccator for 7 days. The extract yield noted to be 12.7%.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the *apricot kernel* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids. as per the standard methods.

Animals

Healthy Adult Male wistar rats of 8-10 weeks old with Average weight in the range of 150-180gms were selected. Animals are housed 4 per cage in temperature controlled (27 ^{0}C ±3 ^{0}c) room with light/dark cycle in a ratio of 12:12 hrs is maintained. The Animals allowed to acclimatize to the environment for seven days and are supplied with a standard diet (food pellets) and water *ad libitum*. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

After conduction of the experiments/testing's. The animals moved to rehabilitation rooms never to be used for another procedure.

Acute toxicity studies

The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organization for Economic Co-operation and Development) guidelines no 423. Female Albino wistar rats (150-180g) were taken for the study and dosed once with 2000 mg/kg of the extract. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, 1/10 and 1/20 doses of 2000 mg/kg i.e. 100 mg/kg and 200 mg/kg were chosen for subsequent experimentation[8].

Induction procedure

Diabetes mellitus or hyperglycemia was induced in rats by administration of alloxan monohydrate (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-primidinetetrone) at dose of 120mg/kg intraperitoneally in normal saline. After one hour of alloxan administration, the animals given feed ad libitum. The animals kept fasting overnight and blood glucose levels estimated before and after 72hrs of alloxan treatment. Animals showing blood glucose levels of >200mg/dl is considered as diabetic and were used for study.[8]

Experimental Study Design

Diabetic rats divided in to five groups with each group four animals.

Group-I: Rats served as normal control group.

Group-II: served as diabetic/disease control. (Given alloxan monohydrate at dose of 120mg/kg. i.p.)

Group-III: Diabetic rats treated with *apricot kernel* extract at a dose 100mg/kg (low dose).

Group-IV: Diabetic rats treated with *apricot kernel* extract at a dose of 200mg/kg (high dose).

Group V: Diabetic rats treated with Metformin (standard drug) at 450mg/kg.

The treatment is given for 14 days and blood samples were collected at different intervals.

Statistical Analysis

All the values will be expressed as mean ±standard deviation (S.D). Statistical comparisons between different groups will be done by using one way analysis of variance. P value <0.05 will be considered as statistically significant.

Evaluation parameter

GLUCOSE

From the literature a simple and effective method for the evaluation of blood glucose levels is selected. Glucose levels are measured by electric glucometer (accu-check active) which gave the accurate glucose levels as compared to any other standard methods.[9,10] The rats to be tested is separated and gently placed in the animal holder. Then the tail is pricked to collect the blood drop. The blood drop collected from pricking followed slight pressure is placed on the testing strip carefully. Then the strip is placed in the glucometer to get the reading. The procedure must be done carefully otherwise it may lead to unnecessary damage to the animals.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Investigation revealed the presence of steroid, Alkaloid, Tannins & Flavonoid in Methanolic Extract of *apricot kernel*

Table 1: Preliminary Phytochemical Screening

Phytochemical	Results
Steroid	+
Alkaloid	+
Tannin	+
Carbohydrate	-
Phenol	-
Flavonoid	+
Saponin	-

(+) Present **(-)** Absent

Acute toxicity studies

As per (OECD) draft guidelines 423 Female albino rats were administered *apricot kernel* and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma. Overall results suggested the LD_{50} value as 2000 mg/kg. Hence therapeutic dose was calculated as $1/10^{th}$ and $1/20^{th}$ i.e. 100mg/kg and 200 mg/kg of the lethal dose for the purpose of antidiabetic investigations.

Glucose

Table 2: Effect of *apricot kernel* extract on serum glucose levels (mg/dl) in diabetic rats

Groups/Interval	0th Day	7th Day	15th Day
Normal	123.3±4.23	129.1±5.36	127.7±5.62
Diabetic control	283.8±5.01	286.4±12.4	300.3±8.64
MEAK(100mg/kg)	293.1±9.83	182.9±6.91**	150.1±17.1**
MEAK(200mg/kg)	280.5±42.4	155.5±7.20***	125.7±20.7***
Metformin(450mg/kg)	281.0±34.7	129.7±10.2***	112.3±2.4**

*All the values of mean±SD; n=6; ** indicates p<0.01, *** indicates [a]p<0.001 vs diabetic control.*

MEAK-Methanolic Extract of Apricot Kernel

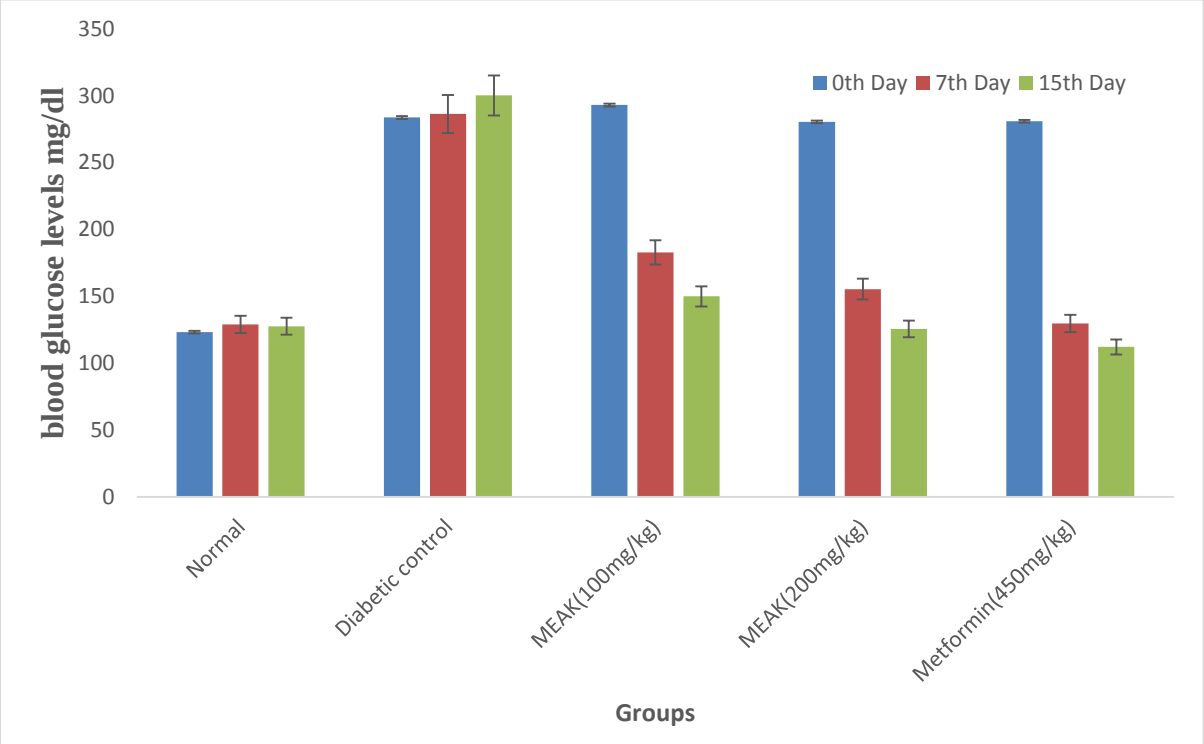

Figure 2: Effect of apricot *kernel* methanolic extract on serum glucose levels (mg/dl) in diabetic rats

On induction of toxicity with alloxan monohydrate, the glucose levels were elevated than normal in all groups. Extract of *apricot kernel* was administered to group-3 (100mg/kg) and group-4 (200mg/kg) and standard drug (metformin) was administered to group-5 (450mg/kg). on treatment with extract and metformin the glucose levels were reduced significantly.

CONCLUSION:

apricot kernel have many medicinal properties useful to cure anorexia, ulcers etc. *apricot kernel* have different medicinal properties due to its active phytochemical constituents and may able to treat diabetes & diabetics complications. Methanolic extract of *apricot kernel* was prepared from whole plant are subjected to acute oral toxicity studies and found that the Methanolic extract of

apricot kernel is safe to use up to the dose of 2000mg/kg. The methanolic extract of *apricot kernel* was found to be in dose dependent way against alloxan induced diabetes in rats. The reduction of the elevated blood glucose levels in diabetic rats on treatment with the extract at two different concentrations confirmed that methanolic extract of *apricot kernel* possesses Antidiabetic activity & has shown significant effect when compared to Alloxan administration. It needs comprehensive investigations for developing a safe and effective herbal drug. Further research is required to isolate the biomolecules responsible for the antidiabetic and antidiabetic complications.

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