

Evaluation of hypoglycaemic and antidiabetic effect of *Melia dubia* CAV fruits in mice

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Extracts of total fruits of *Melia dubia* in water, ether, alcohol and chloroform were tested on mice to evaluate its efficacy as a hypoglycaemic agent. The extract in alcohol was found to be most effective as a hypoglycaemic agent. Varying doses of ethanol extract of total fruits injected intraperitoneally into mice reduced blood glucose level, but maximum reduction of 52.14% was observed at a dose of 300 mg/kg body wt by the sixth hour. After establishing the hypoglycaemic activity of the fruit in normal mice, the antidiabetic activity of the fruit extract in streptozotocin-induced diabetic mice was studied. There was a gradual decrease in blood sugar level from the second hour onwards in the induced diabetic mice and the low sugar level was maintained up to 8 h. The glucose level started increasing gradually by the 12th hour; the blood sugar level was normal and a significant increase was observed at the end of 24 h. In mice that were administered fruit extract, gradual reduction of glucose level was seen by the second to the eighth hour and it was maintained up to 24 h. The untreated diabetic mice had increased glucose level by the end of the second hour and there was a continued increase up to 48 h. Glucose tolerance test studies indicate reduction of blood glucose level to 35%, in mice that were administered total fruit extract and this reduced level was maintained up to 3 h, with a maximum effect seen at the second hour. LD₅₀ studies with varying doses indicate that 50% mortality was induced at dose of 500 mg/kg body wt and sublethal doses varying from 50 to 300 mg/kg wt. The therapeutic index value of 2.5 of the total fruit extract suggests that the extract is not only safe but an effective, natural and novel hypoglycaemic agent, as indicated from the evaluation on mice.

Keywords: Antidiabetic activity, fruit extract, hypoglycaemia, *Melia dubia*, mice.

DIABETES is a metabolic disorder characterized by impaired glucose utilization and is the underlying factor for both hypoglycaemia and hyperglycaemia. Chronic hyperglycaemia results in impaired function or failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Despite advances in medicine, diabetes as

a major health complication seem to be growing at an alarming proportion world over and in India, in particular. By the end of 2030, 79.4 million Indians are expected to be affected by this metabolic disorder¹ and this accounts for nearly one-sixth of the world's diabetics. Treatment of diabetes is either using hypoglycaemic agents like biguanides and sulfonylureas, or using insulin. Prolonged use of these for treatment of diabetes is associated with side effects^{2,3}. There is growing awareness about effective herbal remedies because of the minimal side effects and more so because of their relatively low cost in comparison to non-traditional remedies. Demand for plant products that are hypoglycaemic agents, sans the side effects, is on the rise. Even though the bioactivity of various plant products is unknown, these are prescribed and used widely because of underlying faith in folk medicine based on time-tested traditional practices. Fruits of *Melia dubia* Cav, a member of the Meliaceae family are used in folk medicine as an antihelmintic, astringent and in the treatment of colic⁴. Paste made out of the green fruits is used to treat scabies and maggot-infested sores. Two tetranortriterpenoids, compositin and compositolide, have been isolated from the seeds and leaves of *M. dubia*⁵. There are undocumented and anecdotal evidences of the fruits of *M. dubia* being used for treating diabetes.

Since there were no documented reports available on the hypoglycaemic activity of *M. dubia* fruits either in experimental or clinical studies, the present investigation was envisaged to ascertain the hypoglycaemic and antidiabetic activities in a mice model.

Seeds of *M. dubia* Cav were collected from Nallamalai forests, Andhra Pradesh, identified by the Department of Botany, Osmania University, Hyderabad dried in the shade and pulverized. Four different solvents, namely alcohol, water, chloroform and petroleum ether were used for total fruit extracts and separated using Soxhlet apparatus.

Albino mice weighing 30–40 g were obtained from the Institute of Preventive Medicine, Nacharam, and kept at ambient temperature and humidity. Food and water were provided *ad libitum*.

Initial evaluation of hypoglycaemic activity of total fruit extracts was done by injecting the extracts obtained with four different solvents, namely water, ether, alcohol and chloroform, intraperitoneally. For each solvent extract, six mice were used and a dose of 250 mg/kg body wt was administered. Blood sugar level (BSL) was monitored every 2 h, up to 8 h. The most effective dose for hypoglycaemic activity was determined for eight batches of ten mice each and for each batch, varying doses from 50 mg/kg body wt to a maximum dose of 800 mg/kg body wt were injected intraperitoneally.

Since results showed maximum hypoglycaemic activity with total fruit extract in ethanol at a dose level of 300 mg/kg body wt, experiments were conducted with this dose on five groups of mice with six mice each and the BSL was monitored after 2, 4, 6 and 8 h. Since no sig-

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nificant changes were observed beyond 8 h, observations were not recorded beyond 8 h. Blood from the orbital sinuses was drawn using heparinized capillary tubes and the BSL was estimated using Ames Glucometer II by the glucose oxidase method.

In order to assess the antidiabetic activity of the fruit extract, investigations were made on induced diabetic mice. A single dose of 100 mg/kg body wt of streptozotocin was administered intraperitoneally to mice that were kept fasting overnight and were divided into two groups of six mice each. The first group served as the diabetic, untreated control. The second group was given a single dose of 300 mg/kg body wt of fruit extract intraperitoneally. Another group of six mice (third group) that were kept fasting overnight was injected with a fruit dose of 300 mg/kg body wt. This group served as the non-diabetic control. The BSL was recorded at regular intervals up to 48 h (Table 1).

Oral glucose tolerance test (GTT) was carried out to ascertain the activity of total fruit extract in the presence of high glucose load. The test was performed according to the method of McDonald *et al.*⁶. Two groups of six mice each, that were kept fasting overnight were administered 1.5 g/kg body wt of glucose, orally. Group I served as control and group II received total fruit extract of 300 mg/kg body wt intraperitoneally, prior to the administration of glucose. The BSL was estimated from 30 min to 3 h.

Screening for LD₅₀ was conducted on ten batches of 20 mice each. Different doses of total fruit extract in ethanol, ranging from 50 to 900 mg/kg body wt were administered intraperitoneally. Observations were recorded at 2 h and thereafter at regular intervals up to one week.

Figure 1 shows the hypoglycaemic activity of total fruit extract in different solvents. Water and petroleum ether extracts showed mild hypoglycaemic effect, while

the batch of mice treated with chloroform died after 30 min. The maximum hypoglycaemic effect of 45–50% was observed with total fruit extract in ethanol. Hence, this extract alone was used for further evaluation.

Figure 2 shows the effect of varying doses of total fruit extract on the BSL in mice. A dose of 50 mg/kg body wt of total fruit extract brought about 12.23% reduction in the BSL and with doses of 100 and 200 mg/kg body wt, the reduction was 17.39 and 43.5% respectively. Higher dose of 300 mg/kg body wt brought about a reduction of 52.14%. Higher doses of 400 and 500 mg/kg body wt had more or less the same effect as that with 300 mg/kg body wt. It therefore appears that 300 mg/kg body wt of total fruit extract in ethanol is the effective dose which elicits maximum response.

The time-dependent hypoglycaemic activity depicted in Figure 3, shows 20.5% and 35.04% reduction in the BSL at the second and fourth hour respectively. Maximum reduc-

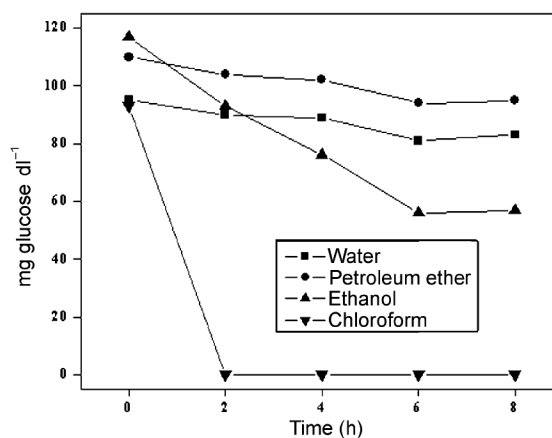


Figure 1. Hypoglycaemic activity of *Melia dubia* fruit extracts in different solvents.

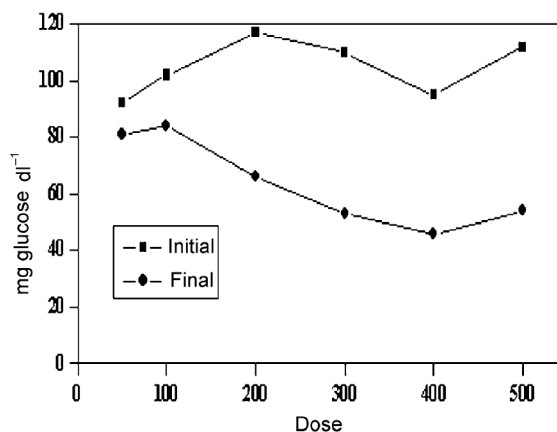


Figure 2. Dose-dependent effect of ethanolic extract of *M. dubia* fruit.

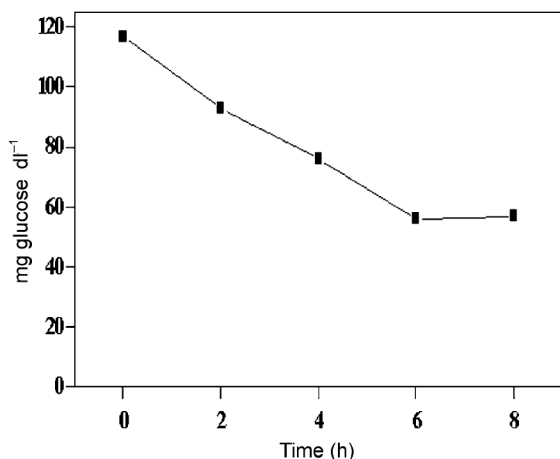
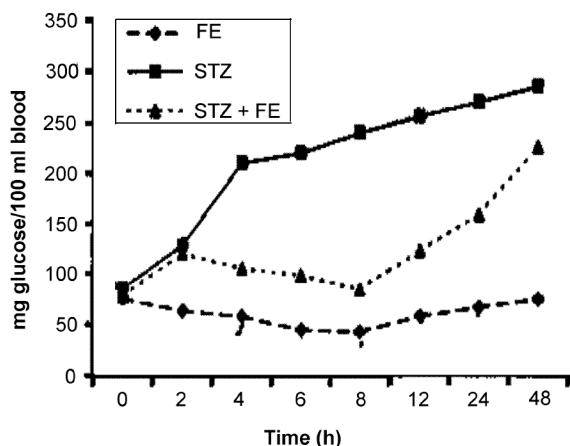
Table 1. Hypoglycaemic and antidiabetic effect of fruit extract (FE) in normal, streptozotocin (STZ)-induced diabetic and FE-treated mice

Time	FE	STZ	STZ + FE
0 h	75 ± 2.68	85 ± 1.78	78 ± 2.28
Second hour	63 ± 0.53	128 ± 1.78	120 ± 2.28
% Variation	-16	50.58	53.84
Fourth hour	54 ± 0.17	210 ± 1.78	105 ± 1.78
% Variation	-28	147.05	34.61
Sixth hour	44 ± 0.17	220 ± 1.78	98 ± 0.89
% Variation	-41.33	158.82	25.64
Eighth hour	42 ± 1.78	240 ± 2.28	84 ± 1.41
% Variation	-44	182.35	7.69
12th hour	57 ± 0.89	256 ± 1.41	122 ± 1.78
% Variation	-24	201.17	56.41
24th hour	66 ± 0.89	270 ± 2.82	158 ± 2.28
% Variation	-12	217.64	102.56
48th hour	74 ± 1.78	285 ± 2.28	195 ± 1.41
% Variation	-1.33	235.29	150

Values are given as mean ± SE.

Table 2. Dose-dependent hypoglycaemic effect of FE in ethanol on mice

Dose of FE (mg/kg body wt)	Initial glucose level (mg glucose per dl)	Final glucose level (mg glucose per dl)	Percentage reduction
50	92	81	12.23
100	102	84	17.39
200	117	66	43.5
300	110	53	52.14
400	95	46	50.03
500	112	54	51.72

**Figure 3.** Time course effect of ethanolic extract of *M. dubia* fruit.**Figure 4.** Hypoglycaemic and antidiabetic effect of fruit extract (FE) in ethanol on mice. STZ, Streptozotocin.

tion of 52.14% was observed in the sixth hour, and at the eighth hour the decrease was more or less equal to that of the sixth hour.

The antidiabetic activity of total fruit extract in ethanol on the mice model is shown in Table 2 and Figure 4. The diabetic mice had increased BSL of about 50% from the

second hour onwards, achieving a maximum increase of 235% by 48 h. Mice that were administered streptozotocin and total fruit extract showed an initial increase in the BSL at the end of the second hour, which started decreasing from the third hour and by the eighth hour the BSL was almost near the normal level. There was a gradual increase in the BSL from 12th hour onwards, but percentage variation was only half compared to the BSL of the first batch of mice that were administered only total fruit extract. Mice injected with total fruit extract had a slight fall in the sugar level by the second hour and the sugar level decreased gradually till the 12th hour. Maximum reduction in sugar level was observed at the eighth hour (-44%). From the 24th hour onwards, the BSL increased and by the 48th hour the BSL was near the normal sugar level.

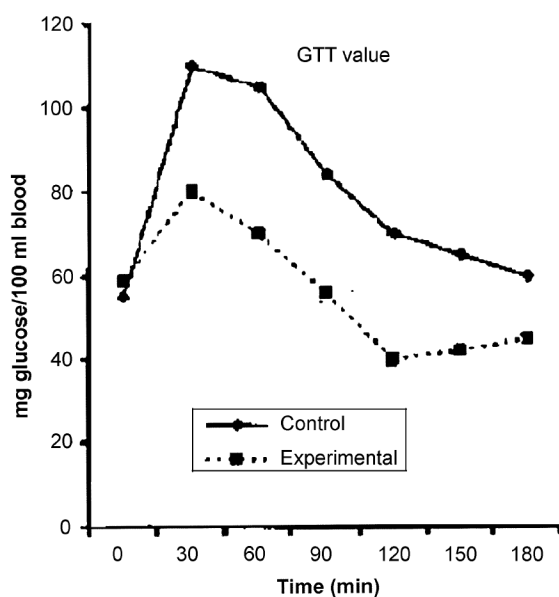
The results obtained using GTT are shown in Figure 5. These are in conformity with those of other hypoglycaemic agents. Within the first 30 min of glucose administration in control animals the peak is elevated, showing an increase in the BSL by 100%, but gradually decreasing to normal level by the end of 3 h. In experimental mice that were administered total fruit extract, prior to glucose load there was elevation of peak in the first 30 min, showing an increase of 35%, but the levels were considerably lower than that of the control animals. During the remaining period also, the BSL remained lower than that of the control group of mice.

Experiments carried out to evaluate LD₅₀ showed no toxic effects on mice given doses ranging from 50 to 300 mg/kg body wt, with no mortality being observed till one week and thereafter. Experiments carried out with higher doses of 400 mg/kg body wt induced 25% mortality on the sixth day and dose of 500 mg/kg body wt brought about 25% mortality on the fifth day. With administration of total fruit extract at doses ranging beyond 400 mg/kg body wt, the percentage of mortality increased proportionately to the dose, and a dose of 900 mg/kg body wt induced 100% mortality within 24 h (Table 3).

M. dubia and *Melia azadirachta* plants belong to the Meliaceae family. Both are important medicinal plants that are used in traditional medicine as a cure for many diseases. Both are also known to help in pest management^{7,8}. However, there are no earlier reports about the hypoglycaemic and antidiabetic activity of *M. dubia*

Table 3. Evaluation of toxicity of *Melia dubia* fruit extract

Group	Dose	Log dose	No. of mice taken	No. of mice dead	Per cent kill
1	50	1.69	20	0	0
2	100	2.00	20	0	0
3	200	2.30	20	0	0
4	300	2.47	20	0	0
5	400	2.60	20	5	25
6	500	2.69	20	10	50
7	600	2.77	20	15	75
8	700	2.84	20	16	80
9	800	2.90	20	18	90
10	900	2.95	20	20	100

**Figure 5.** Glucose tolerance test (GTT) curve after administering *M. dubia* fruit extract in ethanol to mice.

plant. Our investigation reports the hypoglycaemic and antidiabetic effect of ethanol extract of total fruits, and as such the plant product has potential use in the treatment of diabetes mellitus.

Experiments conducted to assess the dose- and time-dependent activity of the total fruit extract in ethanol show that 300 mg/kg body wt elicits maximum hypoglycaemic effect, indicating this to be the optimum dose. Beyond 300 mg/kg body wt, the hypoglycaemic activity is maintained for a short time. It is possible that the fruit extract may stimulate a biochemical response in the target organs gradually and elicit maximum response at a dose level of 300 mg/kg body wt. Thereafter, an increase in dose does not stimulate further changes, as indicated by no changes in the BSL.

Time-dependent hypoglycaemic effect of the fruit extract shows 20.5% reduction in the BSL level by 2 h and

to 52.1% by 6 h. The study indicates that the blood sugar lowering effect persists from 2 to 6 h, and thereafter the effect decreases and the net changes up to 8 h are marginal. These findings are in concurrence with the time-dependent changes of other established hypoglycaemic agents.

Like *M. azadirachta*⁹ and other hypoglycaemic agents, total fruit extract of *M. dubia* also shows significant reduction in the BSL in the mice model. The optimum duration of activity of the agents was up to 8 h and from the eighth hour onwards, a gradual enhancement of glucose level was observed in all the sets of animals. Similar trend in the BSL was reported when some of the natural and synthetic antidiabetic agents such as extract of fenugreek seeds¹⁰, *Mormodica charantia*¹¹, glibenclimide¹² and tolbutamide¹³ were administered to animals.

In streptozotocin-induced diabetic mice, the BSL increased gradually reaching 235% increase by the 48th hour, whereas total fruit extract-treated diabetic mice had a reduced sugar level from the third hour onwards; this state being maintained up to 8–12 h. Our studies indicate that total fruit extract of *M. dubia* has antidiabetic activity and maybe useful in controlling the elevated sugar level.

GTT reveals that the hypoglycaemic effect is seen by the end of the first hour and this level is maintained up to 3 h. It is possible that the active principal in the total fruit extract takes time to reach and act on the target tissues, eliciting a response to maintain the hypoglycaemic level. Though the exact mechanism of action cannot be ascertained through these investigations, there is a strong reason to support the fact that ethanol extract of total fruit of *M. dubia* acts as a hypoglycaemic agent. Our investigations indicate LD₅₀ value as 500 mg/kg body wt and 200 mg/kg wt as effective dose. The therapeutic index of 2.5, indicates the safety of the fruit extract as an antidiabetic agent. Fruits of *M. dubia* contain salain as a principal liminoid constituent¹⁴. Ethanol extracts of fruit of *M. dubia* are reported to show anti-viral activity¹⁵, and the plant yields meliastatins which show anti-neoplastic activity¹⁶. Compositolide and compositin were also isolated from the seeds. Fruit extract of *M. dubia* has been tested

on mice and found to be an effective hypoglycaemic agent. Further research is needed to elucidate the active constituent, which has an antidiabetic effect.

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Biochemical changes in neutrophils of cervical cancer patients treated with ⁶⁰Co

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Cervical carcinoma is the second most common malignancy of the female genital tract in India. The highest incidence occurs at Chennai. This study was conducted on 30 women with biopsy-proved squamous cell carcinoma of the cervix of stage IIb. The neutrophil count increased significantly in cancer patients compared to control subjects. Total protein, glycogen and total lipid increased in neutrophils of cervical cancer patients. The level of cholesterol, triglycerides and fatty acids increased significantly in neutrophils of such patients compared to control subjects. The activity of alkaline phosphatase increased significantly in cervical cancer patients. Upon treatment with cobalt-60, these changes were brought to near-normal levels. This study highlights the impairment in the neutrophil function in cervical cancer patients, which may lead to reduced immune status.

Keywords: Absolute neutrophil count, cervical cancer, glycogen, lipids, neutrophil alkaline phosphatase.

CERVICAL cancer is one of the most common malignancies among women¹. In India, cervical cancer ranks first among different forms of cancers². Incidence of cervical cancer is high among the rural population³. This could be due to difference in lifestyle, economic status, education, personal hygiene and healthcare. Cervical cancer is generally associated with HPV infection⁴. Radiotherapy is the primary local treatment for most patients with loco regionally advanced cervical carcinoma. Radioactive isotopes of cobalt-60 (⁶⁰Co) and gold-98 (⁹⁸Au) are used in the treatment of cervical cancer. Cobalt-60 is preferred because it is easy to handle and less expensive than gold.

Neutrophils (polymorpho nuclear leucocytes; PMNs) constitute the 'first line of defence' against infectious agents or 'non-self' substances that penetrate the body's physical barriers. This is the most abundant leukocyte present in the blood with mean concentration of 4.4×10^6 cells/ml. Neutrophils are derived from the common hematopoietic stem cell. Their development in the bone marrow takes approximately 10–14 days. The bone marrow produces approximately 1×10^9 neutrophils/kg/day⁵. As the neutrophils mature, they develop the capacity to enter the blood through increasing formability and

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