# HYPOGLYCEMIC AND ANTIOXIDANT ACTIVITY OF AN ISOLATED COMPOUND FROM *Ficus arnottiana* BARK

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**Summary**

The hypoglycemic and antioxidant effect of an isolated compound (Ficanone) from *Ficus arnottiana* bark was investigated in normal and streptozotocin- diabetic rats. Administration of petroleum ether, chloroform, acetone and methanol extracts of *Ficus arnottiana* bark at a dose of 100 mg/kg, p.o. for 21 days caused a decrease in fasting blood sugar in diabetic rats (FBS). Among all the extracts, acetone extract was found to lower the FBS significantly in diabetic rats. In acute oral toxicity studies (OECD-425 guidelines), no mortality was observed up to the highest dose of acetone extract (2000 mg/kg, p.o). Acetone extract of the bark was subjected to column chromatography and Ficanone was isolated. Phytochemical studies indicated that Ficanone is a triterpenoidal compound. When administered to diabetic rats, Ficanone (50 mg/kg, p.o.) caused a significant ($p<0.01$) reduction in FBS. Ficanone also caused a considerable decrease in lipid peroxidation and improvement in the antioxidant enzymes (reduced glutathione, superoxide dismutase and catalase) levels in diabetic rats. Histopathology of pancreas also indicated improvement in the condition of β cells after treatment with Ficanone. The results of the present study indicated that Ficanone has antioxidant potential with antidiabetic activity and provides a scientific rationale for the use of Ficanone as an antidiabetic agent.

**Key words:** Antioxidants, *Ficus arnottiana*, Ficanone diabetes, lipid peroxidation, streptozotocin.

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## Introduction

Diabetes is a metabolic group of diseases characterized by hyperglycemia resulting form defects in insulin secretion, insulin action or both. Diabetes mellitus occurs in several forms, approximately 10% of diabetic patients have type 1 diabetes mellitus, and the remainder have type 2 (Non insulin dependent diabetes mellitus). Type 2 diabetes is a metabolic disorder characterized by a progressive decline in insulin action (insulin resistance, followed by the inability of pancreatic β cells to compensate for insulin resistance (1-2-3). Hyperglycemia is closely associated with increased production of free radical species and increased oxidative stress (4).

It is believed that oxidative stress due to chronic hyperglycemia plays an important role in the etiology of diabetic complications (5) and aggravate many diseases including various neurodegenerative diseases and diabetes mellitus. Lipid peroxide mediated tissue damage has been observed in the development of both types of diabetes. Increased concentration of TBARS (Thiobarbituric acid reactive substances) and the simultaneous decline in antioxidative defense mechanisms observed in diabetic patients promotes the development of late complications (6).

Since insulin is the mainstay of treatment for diabetes but causes severe hypoglycemia. Now a day oral hypoglycemic are the most widely used drugs for diabetes but they also have various side effects.

*Ficus arnottiana* miq. is a glabrous tree or shrub from Moraceae family. Commonly it is known as Paras Pipal. It is distributed throughout India; mostly in rocky hills 1,350 m elevations (7). The leaves of the plant are used for controlling fertility. Bark of the plant is used as astringent, aphrodisiac, demulcent, depurative, emollient. It is also useful in inflammation, diarrhea, diabetes, burning sensation, leprosy, scabies, wounds and skin diseases (8). The fruits of the plant contain β sitosterol, gluanol acetate, glucose, friedelin (9).

Therefore in the present study we are evaluating the hypoglycemic activity of different extracts of *Ficus arnottiana* in order to isolate the component responsible for the antidiabetic activity of the plant. Study is further carried out to evaluate the antidiabetic and antioxidant effect of active component (Ficanone) from *Ficus arnottiana* bark extracts on normal and NIDDM rats. The commonly used hypoglycemic agent glibenclamide was used as a standard drug for diabetes.

## Materials and Methods

### Materials

### Plant Material

*Ficus arnottiana* bark was collected from the forest of Dehradun in the month of April. The plant was identified, authenticated by Dr. G.S. Bisht (M.Sc., PhD in Botany) and the voucher specimen (A-32) has been kept at the herbarium of Sardar Bhagwan Singh (PG) Institute of Biomedical Sciences, Dehradun.

**Drugs and Chemicals:**
Streptozotocin was purchased from Calbiochem, Germany and standard antidiabetic drug glibenclamide was obtained from Ranbaxy Research Laboratories, Gurgaon, India. Analytical grade chemicals including various organic solvents from E. Merck India Ltd and Ranbaxy laboratories, India were used for the extraction and phytochemical study of the constituents

**Preparation of different plant extracts**
Bark was collected from the plant and shade dried at room temperature, ground into fine powder, and then extracted (amount 450 gm) with solvents of increasing polarity such as petroleum ether, chloroform, acetone and methanol, for 24 h with each solvent, by hot extraction using soxhlet apparatus at a temperature of $60^0$C. The extracts were concentrated under reduced pressure using a rotary evaporator to constant weight. The extracts were collected and preserved in a desiccator until used for further studies.

**Preliminary Phytochemical study**
A portion of residue from each extract was subjected to phytochemical analysis in order to identify the presence of sterols, alkaloids, carbohydrates, tannins, phenols etc in the bark extracts (10-11).

**Isolation of active principle (Ficanone) from the active extract of *Ficus arnottiana* bark**
All the extracts were screened for antidiabetic activity in diabetic rats. Acetone extract was found to show maximum reduction in blood sugar level, therefore attempts were made to isolate the active principle from the active acetone extract. The active extract was subjected to column chromatography using silica gel mesh (200-400 size) as adsorbent and $CHCl_3$: MeOH in different ratio as mobile phase which led to isolation of some compounds based on thin layer chromatography ($SiO_2$ and $CHCl_3$: MeOH). All of these compounds were screened for hypoglycemic activity. Among the isolated compounds, one compound which was given trivial name Ficanone showed maximum hypoglycemic and antioxidant activity, and regarded as active component of *Ficus arnottiana* bark.

**Determination of blood glucose and oxidative stress in diabetic rats**

**Animals**
Wistar albino rats of either sex were randomly bred in the Institutional animal house. The animals were housed in standard polypropylene cages and maintained under controlled room temperature($22 \pm 2^0$C) and humidity (55+5%) with 12:12 hour light and dark cycle. All the animals were provided with commercially available rat normal pellet diet and water *ad libitum.* The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Govt. of India were followed and prior permission and clearance were granted from the institutional animal ethics committee for conducting the animal experimental studies.

**Acute toxicity studies**

Acute oral toxicity was performed in mice by following Organization for Economic Cooperation and Development (OECD) guidelines AOT No 425 [12].

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**Induction of Diabetes**

The method of Portha et al was followed for the induction of diabetes. Diabetes mellitus was induced in five day - old neonates (50 animals) by intraperitonial injection of streptozotocin (90 mg/kg in 0.1M citrate buffer pH 4.5). The control group received equivalent amount of citrate buffer. The animals were allowed to live with their respective mothers and weaned from breastfeeding at 4 weeks of age. Eight weeks after injection of streptozotocin, the rats were checked for fasting blood sugar (FBS) level by glucose oxidase-peroxidase method. Animals showing FBS more than 150 mg/dl were considered as diabetic (38 animals) and included for the study (13).

**Treatment protocol**

The diabetic animals were divided into five groups each containing six animals, and one group of normal non diabetic animals. All the extracts of *Ficus arnottiana* bark were given at a dose of 100 mg/kg, p.o in 1% v/v of Tween 80 (1ml/kg p.o.) for a period of 21 days to different groups of animals.

Group I: Normal animals received 1% v/v Tween 80 (1 ml/kg p.o.) as a suspension in distilled water.
Group II: Diabetic animals received 1% v/v Tween 80 (1 ml/kg p.o.) as a suspension in distilled water.
Group III: Diabetic animals received standard antidiabetic drug glibenclamide (5 mg/kg, p.o.)
Group IV: Diabetic animals received Petroleum ether extract (100 mg/kg, p.o.)
Group V: Diabetic animals received chloroform extract (100 mg/kg, p.o)
Group VI: Diabetic animals received acetone extract (100 mg/kg, p.o)
Group VII: Diabetic animals received methanol extract (100 mg/kg, p.o.)

At the end of the experimental period the animals were fasted overnight and blood was taken from the retro orbital plexus under mild chloroform anesthesia, serum was separated and blood sugar level was evaluated by the method of glucose oxidase- peroxides method using Span Diagnostic kits (14) Acetone extract showed maximum reduction in the fasting blood sugar of diabetic animals. It was subjected to column chromatography which led to isolation of Ficanone.

**Pharmacological screening of Ficanone for its effect on serum glucose, lipid per-oxidation and antioxidant enzymes level in diabetic rats**

Fresh diabetic animals were divided into three groups of six animals and a group of normal non diabetic animals and received the following treatment for 21 days.

Group I: Normal animals received 1% v/v Tween 80 (1 ml/kg p.o.) as a suspension in distilled water.
Group II: Diabetic animals received 1% v/v Tween 80 (1 ml/kg p.o.) as a suspension in distilled water.

Group III: Diabetic animals received standard antidiabetic drug glibenclamide (5 mg/kg,p.o.).
Group IV: Diabetic animals received Ficanone (50 mg/kg, p.o.).

After treatment period, serum was analyzed for FBS by glucose oxidase-peroxidase method. Liver was isolated from the rats receiving Ficanone, it was washed in tris buffer pH 7.8, and then homogenized. The homogenate was centrifuged and the supernatant was taken to study the effect of Ficanone on lipid peroxidation (15), reduced glutathione (16), superoxide dismutase (17) and catalase. Results of the test were compared with that of the standard antidiabetic drug glibenclamide.

**Statistical analysis**

The results were expressed as Mean ± SEM. The unpaired t-test was used for analyzing the data between two groups. Statistical analysis of data was initially performed by using analysis of variance (ANOVA), when the overall ANOVA was significant, unpaired t' test was applied to study the difference among the groups.

## Results

**Phytochemical study**

The preliminary phytochemical study on the extracts revealed presence of sterols in the petroleum ether extract, carbohydrates & alkaloids in Chloroform extract and Phenols, alkaloids and tannins in acetone and methanol extracts. On the phytochemical basis it was found that Ficanone was a triterpenoidal compound. It was obtained as a white solid with M.P. of $110^0$C. The IR analysis showed signals at 1389 and 1361 $cm^{-1}$ (gem dimethyl group) and 1477 $cm^{-1}$ (metgyl group). This confirms the triterpinoid structure of Ficanone.

**Acute toxicity studies**

Acute toxicity studies revealed that *Ficus arnottiana* extracts were not showing any toxic symptoms when administered orally to mice at a dose of 2000mg/kg. The lethal dose (LD50 value) was 3 gm/kg body weight.

**Effect of *Ficus arnottiana* bark extracts on fasting blood sugar of diabetic rats**

Table 1 illustrates the effect of different extracts on serum glucose level in the diabetic rats. Results showed that all the extracts caused reduction in blood glucose level but maximum reduction was found in the acetone extract. Acetone extract showed 51% reduction ($p<0.01$) as compared to glibenclamide which showed 67% reduction in fasting blood sugar.

**Effect of Ficanone on serum glucose, lipid peroxidation and antioxidant enzymes level in diabetic rats.**

Table 2 illustrates the effect of Ficanone on serum glucose level in the diabetic rats. Results showed that, Ficanone exhibited significant reduction (55%) in fasting blood sugar as compared to glibenclamide (67%) in streptozotocin - induced diabetic rats.

Table 3 illustrates the effect of Ficanone and glibenclamide on the level of antioxidant enzymes (GSH, SOD and catalase) and lipid peroxidation. Ficanone caused significant ($p<0.01$) reduction

in the level of lipid peroxidation in diabetic rats. Ficanone also caused significant increase ($p<0.01$) in the level of GSH, SOD and catalase in diabetic rats.

**Effect of Ficanone on the pancreatic β cells**

Histopathology of pancreas (Fig.1) illustrates the damage of pancreatic β cells in diabetes. Fig. 3 shows the protection of pancreatic β cells after treatment with Ficanone.

**Table 1. Effect of *Ficus arnottiana* bark extracts on fasting blood sugar of diabetic rats (n=6).**

| **Groups** | **Blood sugar (mg/dl) before treatment** | **Blood sugar (mg/dl) after treatment** | **% reduction in blood sugar** |
|---|---|---|---|
| Control (diabetic rats) | 253 ± 1.3 | 235 ± 0.8 | - |
| Normal (Tween 80, 1 ml/kg, p.o.) | 90 ± 0.8 | 96 ± 1.1 | - |
| Diabetic + Standard drug (5 mg/kg, p.o.) | 250± 1.1 | 90 ± 1.3*** | 67 |
| Diabetic + Pt. ether (100 mg/kg, p.o.) | 254± 1.2 | 143 ± 0.9 * | 43 |
| Diabetic + Chloroform (100 mg/kg, p.o.) | 260± 1.5 | 137 ± 1.2 * | 45 |
| Diabetic + Acetone (100 mg/kg, p.o.) | 240 ± 4.5 | 116 ± 1.35 ** | 51 |
| Diabetic + Methanol (100 mg/kg, p.o.) | 257 ± 2.4 | 132 ± 0.06* | 48 |

Results were expressed as Mean ± SEM.
Results of the test and standard groups were compared with the control group.
*p<0.05, ** p<0.01, ***p<0.001

**Table 2. Effect of Ficanone on fasting blood sugar of diabetic rats (n=6).**

| Groups | Blood sugar before treatment (mg/dl) | Blood sugar after treatment (mg/dl) | % reduction in blood sugar |
|---|---|---|---|
| Control (diabetic rats) | 214 ± 0.6 | 213 ± 0.8 | - |
| Normal (Tween 80, 1ml/kg, p.o.) | 95 ± 0.8 | 97 ± 1.1 | - |
| Diabetic + Standard drug (5 mg /kg, p.o.) | 236 ± 0.5 | 94 ± 0.9*** | 60 |
| Diabetic + Ficanone (50 mg/kg, p.o.) | 218 ± 0.9 | 96 ± 0.8*** | 55 |

Results were expressed as Mean ± SEM.

Results of the test and standard groups were compared with the control group.

*p<0.05, ** p<0.01, ***p<0.001

**Table 3. Effect of Ficanone on antioxidant status and lipid peroxidation level in diabetic rat (n=6).**

| **Groups** | **Reduced glutathione (µg of gsh/mg of tissue)** | **Superoxide dismutase (EU/L)** | **Catalase (µg of $H_2O_2$ / min/ml)** | **Lipid peroxidation (n mol/l)** |
|---|---|---|---|---|
| Normal (1ml/kg, p.o) | 116 ± 0.5 | 104 ± 0.5 | 405 ± 0.8 | 15 ± 0.11 |
| Control (diabetic rat) | 64 ± 0.47 | 75 ± 0.7 | 224 ± 0.2 | 37 ± 0.3 |
| Diabetic +Std. (5 mg /kg, p.o.) | 91 ± 0.8** | 90 ± 0.4** | 354 ± 0.42** | 17 ± 0.1*** |
| Diabetic + Ficanone (50 mg/kg, p.o.) | 99 ± 0.6** | 95 ± 0.6** | 372 ± 0.9** | 21 ± 0.7** |

Results were expressed as Mean ± SEM.

Results of the test and standard groups were compared with the control group.

*p<0.05, ** p<0.01, ***p<0.001

**HISTOPATHOLOGY OF PANCREAS**

**Figure 1. Histopathology of pancreas in diabetic rats (Streptozotcin treated)**

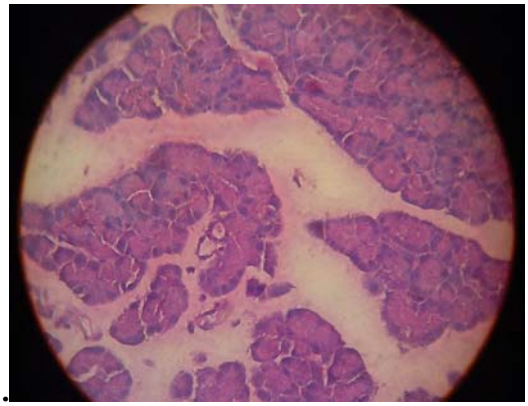


**Figure 2. Histopathology of pancreas after treatment with glibenclamide**

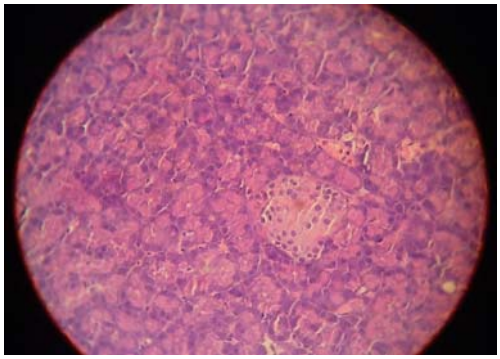
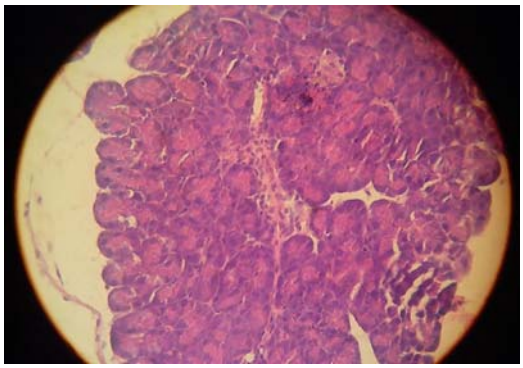


**Figure 3. Histopathology of pancreas after treatment with Ficanone**



## Discussion

The present study indicated that *Ficus arnottiana* bark has both hypoglycemic and antioxidant activity. Among all the extracts tested, acetone extract caused the only observed significant reduction in the serum blood glucose level as compared to diabetic control. Of the isolated compounds, Ficanone possesses significant hypoglycemic activity. The improvements in the level of antioxidant enzymes and reduction in lipid peroxidation in the diabetic rats after treatment with Ficanone indicates its antioxidant potential. Decrease in the oxidative stress in diabetic animals after treatment with Ficanone could be beneficial in preventing various diabetic complications, as well as improving glucose and lipid metabolism in the kidneys of diabetic patients (18). As figure shows treatment with Ficanone also improved the condition of pancreatic β cells in diabetic rats.

Since Ficanone has shown significant antidiabetic and antioxidant activity in rats, the authors have theorized that this mechanism of action of Ficanone is possibly due to decrease in the oxidative stress in diabetic pancreas and liver, increased peripheral glucose utilization, inhibition of carbohydrate intake from intestine, inhibition of peripheral glucose release, and increase in the secretion of insulin from the pancreatic β cells (insulinogenic action) or by any other mechanism which is not known.

Decrease in the fasting blood sugar level and improvement in the oxidative stress indicates that Ficanone is the principle component from *Ficus arnottiana* bark extract that is responsible for antidiabetic and antioxidant activity of the plant. Further study will give complete structure of Ficanone which will be a lead compound, on which structure activity relationship would be carried out, so that it could be an alternative cure for oral hypoglycemics.

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