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# *Ficus carica* Leaf Ethanolic Extract's Antidiabetic and Antioxidant Properties in Rats with Alloxan-Induced Diabetes

**Borse Sandhya Laxmikant[1], Ojha Abhijeet[2], Kaur Ajit Kiran[3], Naveenkumar M.[4], Kannan K.[5], Gupta Shiv Kumar[6], Aiya Avadhut Suresh[7] and Kumari Shanno[8]***

[1]School of Pharmaceutical Sciences, Sandip University, Nashik 422213, Maharashtra, India
[2]Amrapali Institute of Pharmacy and Sciences, Haldwani, Uttarakhand, India
[3]Accurate College of Pharmacy, Plot No. 49, Knowledge Park – III., Greater Noida 201310, Uttar Pradesh, India
[4]Vaageswari College of Pharmacy, Karimnagar 505481, Telangana, India
[5]Department of Agricultural Engineering, Mohammed Sathak College of Engineering, Sathak Nagar, Keelakarai 623806, Ramanathapuram, Tamil Nadu, India
[6]Saraswathi College of Pharmacy, NH24/09, Anwarpur, Pilkhuwa, Hapur 245304, Uttar Pradesh, India
[7]Department of Panchakarma, GAM and RC Shiroda, Goa University, Goa 403103, India
[8]Department of Pharmacy, Bansthali Vidyapith, Rajasthan, India

**Corresponding Author*

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**Abstract:** In this study, the livers of diabetic rats had higher amounts of hydroperoxides and lipid peroxides, which mean that more free radicals were being made. In people with diabetes mellitus, oxidative stress and lipid breakdown can get worse when antioxidant defenses inside the cells are shut down. The current study shows that rats that were given alloxan had much higher levels of lipid peroxidation. Treating the rats with Anjir (***Ficus carica***) leaves lowered these levels. This means that the flavonoids in Anjir leaves may help protect cells by scavenging harmful molecules like superoxide radicals and singlet oxygen. A lot of research on diabetes mellitus has shown that antioxidant levels drop and peroxidative state goes up. Less LPO, SOD, and CAT activity was found in the livers of the diabetic rats used in this study. It is very important to protect the pancreatic ß-cell's antioxidant potential so that it can live and keep making insulin when oxidative stress is high, according to these results. The liver may have less SOD and CAT activity when a person has diabetes mellitus because it makes reactive oxygen free radicals, which can also lower the activity of these enzymes.

**Keywords:** *Ficus carica,* Anjir, Ethanolic extract, Antidiabetic, Antioxidant, Diabetes

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## Introduction

Diabetes is a metabolic condition that is distinguished by heightened levels of glucose in the bloodstream and inadequate or inefficient synthesis of insulin (Arafa *et al*., 2020). Diabetes

affects 5.9% of the American population and ranks as the sixth most common cause of death in India (Dureshahwar *et al*., 2019). Diabetes cannot be cured, but with the correct care and treatment, a person with the disease can lead a normal, healthy life. Type I and Type II diabetes, which are the two primary forms are discussed (Surana and Mahajan, 2022). Active components such as glycosides, alkaloids, flavonoids, tannins, vitamins, and coumarin chemicals that have the ability to alter physiological processes in humans and animals or that interact biologically with pathogens are necessary for plants to be considered medicinal (Petchi *et al*., 2015). Antibiotics are the only products made from certain plants. After being harvested, medicinal herbs are typically dried in lofts, under trees, or in dryers designed specifically for that use (Ahire *et al*., 2023). Certain recently acquired medical plants have sections from which essential oils and juices can be extracted. In addition to being employed in medicine and the production of galenics, dried medicinal herbs are also used in the chemical and pharmaceutical industries. Because of environmental influences and plant age, the kinds and concentrations of active substances in the various portions of medicinal plants vary throughout the year (Singh *et al*., 2011). The plant portions with the highest concentration of these compounds are the ones that are first gathered. Since more potent medications have been developed, many traditional herbal remedies have been abandoned. Yet, plants account for more than 30% of all pharmaceuticals purchased in the USSR (Barua *et al*., 2017). Compared to synthetic medications, these are usually less harmful and have no negative side effects. However, those who wish to heal themselves with medicinal plants should do so under a doctor's supervision. The Ministry of Medicinal Industry oversees 23 sovkhozes (a form of state-owned farm in the Soviet Union) where the remaining crops are cultivated (Keservani and Sharma, 2014). On kolkhozes (typically created by combining small individual farms together in a cooperative structure), peppermint and poppies are also grown. Numerous therapeutic plants are grown and collected in their indigenous habitats. The aforementioned plants encompass burmari-gold, plantain, motherwort, henbane, valerian, ginseng, and marshmallow. When collecting the raw materials, it is advisable to extract only a little section of the plant or cut it off to allow it to naturally regrow. The importation of therapeutic herbs into the USSR is limited (Ahire *et al*., 2022). Each year, the Soviet Union exports a significant number of medical plant kinds. One of these is the presence of thousands of tons of licorice roots. Medicinal plants are widely utilized in several industries such as culinary, fragrance, and metallurgical sectors (Supalkar *et al*., 2023). Numerous scientific research and educational organizations, such as botanical gardens, pharmaceutical institutes (specifically pharmaceu-tical departments), the All-Union Scientific Research Institute of Medicinal Plants, various institutes affiliated with the Academy of Sciences of the USSR and the Academies of Sciences of the Soviet republics, among others, are actively engaged in the pursuit of novel plant-based medicinal remedies (Aswathy *et al.*, 2021). Furthermore, they engage in the cultivation of therapeutic plants, do study on their inherent features, and formulate a systematic schedule for their utilization (Ahire *et al*., 2023).

Gestational diabetes has a prevalence rate of approximately 4% among pregnant women, resulting in an annual incidence of 135,000 cases in the United States. Following childbirth, gestational diabetes often resolves, although recurrence is more probable in individuals who have already experienced it (Behera *et al*., 2010). When women diagnosed with Type 1 or Type 2 diabetes become pregnant, it is imperative for them to adhere to their prescribed medication regimen until the time of childbirth. Considering the high incidence of Type 2 diabetes among women with gestational diabetes, it seems that having gestational diabetes raises the likelihood of developing Type 2 diabetes in the future. Insulin

resistance is a common characteristic observed in both Type 2 diabetes and diabetes occurring during pregnancy (Khairnar *et al*., 2024). Implementing a few small lifestyle modifications will assist in preserving diabetes-free condition following the onset of gestational diabetes. Certain individuals exhibit elevated blood glucose levels above the average, however, fall short of meeting the diagnostic criteria for diabetes. This condition is commonly known as pre-diabetes. According to the American Diabetes Association, an estimated 41 million additional individuals in the United States are affected by pre-diabetes, while 18.2 million Americans get diabetes (Bansod, *et al.,* 2010). The aim of the present study was to evaluate the anti-diabetic and anti-oxidant properties of *Ficus carica* (Anjir) leaf ethanolic extract in alloxan treated rats.

## Materials and Methods

### *Preparing ethanolic leaf extracts from Anjir*:

The leaves were removed from the ***Ficus carica*** (Anjir) plant and subjected to a thorough cleaning process using 100% ethanol in order to inhibit microbial growth. The leaves were then mechanically ground into small pieces, left to dry in the shade, and mixed with 500 ml of ethanol in a Soxhlet apparatus over the course of 3 days to get 100 g of powdered Anjir leaves (Patel and Shah, 2013). Ethanol recovery made it possible to concentrate the material that was removed. To make an ethanolic extract from Anjir leaves, the concentrated result was used (Talele *et al.*, 2022).

### *Investigation of acute toxicity in accordance with OECD guideline 425*:

Finding out if the substance is acutely toxic when eaten is usually the first step in figuring out how dangerous it is. It goes into length about the possible health risks that come with a short oral exposure. It is called "acute oral toxicity" when bad things happen right after a single oral dose or several doses taken over the course of a day. Data from an acute study could be used to classify and name things. "Medium lethal 50 doses," or LD, is the name for a single dose of a drug that has been shown to kill 50% of animals when given orally. The lowest amount that can be found ($LD_{50}$) is recorded in milligrams per kilogram of test animal. This is the first step in making a dosage plan for subchronic and other studies. It could also give us a sneak peek into how a drug causes toxicity (Bhatt *et al*., 2022; Aher *et al*., 2023).

The dose given to the next animal is changed based on how the previous animal did, generally by a factor of 3.2. This process is continued until the first result is reversed, that is, until a higher dose kills instead of saves lives or until a lower dose saves lives instead of killing them (Kumari and Satyanarayana, 2019). Subsequently, the up-down methodology is employed to provide doses to additional animals until a predetermined cessation threshold is achieved. In the event that no alternative dose is available prior to the selected upper limit of 2000 or 5000 mg/kg, a predetermined quantity is administered to limit the dose. The utilization of an upper limit dose of 5000 mg/kg should be limited to instances when a specific regulatory rule mandates its application (Soni *et al.*, 2013).

Acute oral toxicity tests were done on healthy Wistar rats (180–220 g) using the "staircase" method. Animals in experiments were given increasing amounts of Anjir leaf extract in 0.5% tween 80 by mouth through gavage. Each group only got one dose of the extract. Subsequently, the impact of any fatalities was documented at 0 h, 1 h, 2 h, 4 h, and 24 h (Keservani *et al*., 2017; Supalkar *et al.*, 2023).

### *Biological Activity Study*:

### *Alloxan-induced diabetic model for Anti-oxidant and Anti-diabetic activity of Anjir extract*:

Before giving each animal an injection of alloxan monohydrate, the weight was measured. The amount given was 150 mg/kg of body weight inside the peritoneum to make the rats diabetic. The animals were fed whatever they wanted and a 5% dextrose solution in a feeding bottle for a day to help them get through the first hour of low blood sugar after being given alloxan. A

glucometer was used to check the animals' blood glucose levels 48 h after they were first seen. As part of the experiment, the diabetic rats were split into five groups of six animals each (Rane *et al*., 2023).

The blood sugar level of each animal was checked from day 0 to day 20. Rats in groups I, II, and III were treated with a normal drug. The results of groups IV and V, which were treated with an Anjir leaf extract, were then compared. Tests are also done on blood samples from each animal to find out the lipid makeup, how much glycogen is in the liver, and how much LPO, SOD, and CAT are in the tissues (Sonawane *et al*., 2023). We compared the results of the groups that were given Anjir leaf extract to those of the control group and the standard drug treatment group (Keservani *et al.*, 2016).

## Results and Discussion

### *Investigation of acute toxicity in accordance with OECD guideline 425*:

There were no deaths in an acute toxicity test with 3000 mg/kg of Anjir leaf extracts after 24 h of monitoring. $LD_{50}$ for the ethanolic leaf extract is more than 3000 mg/kg body weight. The extract did not cause any significant adverse changes in behavior, like anxiety, restlessness, breathing problems, seizures, or coma. Still, it was seen that the rats' body weights were decreasing. The drop in body weight might be because the rats drank less water and other fluids. This could be because the extract made them feel full and slowed down their hunger. The very high $LD_{50}$ value suggested that the Anjir leaf extract is not really harmful.

### *Alloxan-induced diabetic model for Anti-diabetic and Anti-oxidant activity of Anjir extract*:

Using an alloxan-induced model to test the experimental design of antidiabetic activity was decided to provide a scientific base for the plant's possible use in the treatment of diabetes. As expected, the diabetic control group had high blood sugar levels compared to the healthy animals. The blood glucose levels of the diabetic control rat were compared to those of the diabetic rat. The usual drug Glibenclamide was found to lower the blood glucose level bringing it almost back to normal. But on days five, ten, fifteen, and twenty, the diabetic rats' blood serum glucose levels dropped a lot after the administration of ethanolic extract of Anjir leaves.

The finding that alloxan can cause diabetes was supported by the fact that the diabetic rats had higher blood sugar levels and lower body weights than the normal rats. Even though diabetics eat more, they often lose weight because their bodies do not process glucose properly and cause too many proteins in their tissues. The average body weights of the rats were increased after administration of ethanolic extract of Anjir leaves. This shows that the hyperglycemic state was able to limit the rats' overeating and muscle loss.

The β-cells in the islets of Langerhans are killed by alloxan, which decrease insulin production. In our study, we discovered that giving Anjir leaves to rats that had alloxan-induced diabetes might have caused a statistically significant rise in plasma insulin levels. This could be because the pancreas is secreting more insulin from its β-cells or because bound insulin is releasing it, which makes insulin work better in the blood. An extra possible reason for the strong and long-lasting effect of Anjir leaves on alloxan-diabetic rats is that they increase the uptake of glucose by peripheral tissues. Table 1 shows blood glucose levels of Alloxan-induced diabetic rats treated with an ethanolic extract of Anjir leaves.

When rats were given Anjir leaf extract, their serum lipid levels returned near to the control groups. Anjir leaves to diabetic rats made their lipid profiles much better and reduced the amount of glycogen in their liver. Diabetic rats exhibited significantly elevated levels of tissue lipid peroxidation and hydroperoxides. Anjir leaves were administered to rats afflicted with diabetes, resulting in a restoration of tissue lipid peroxidation and hydroperoxide levels to their baseline levels. The levels of LPO, SOD, and CAT in the tissues of diabetic rats were significantly

**Table 1:** Blood glucose levels of Alloxan-induced diabetic rats treated with Anjir leaves ethanolic extract

| Group | Treatment | Day 0 | Day 5 | Day 10 | Day 15 | Day 20 |
|---|---|---|---|---|---|---|
| I | Normal control | 99.77±4.11 | 93.68±7.33 | 86.97±9.55 | 92.25±6.90 | 88.50±7.84 |
| II | Diabetic control | 376.41±14.12 | 397.65±19.88 | 406.87±14.11 | 437.89±16.51 | 453.76±19.41 |
| III | Diabetic with Glibenclamide | 369.81±11.35 | 305.93±15.41 | 251.81±16.17 | 235.46±12.55 | 209.22±12.69 |
| IV | Diabetic with Extract 200 mg/kg | 373.21±13.24 | 365.42±07.11 | 337.16±05.89 | 344.85±13.11 | 296.52±07.82 |
| V | Diabetic with Extract 400 mg/kg | 378.73±13.52 | 353.41±16.52 | 317.71±17.85 | 285.81±11.22 | 254.72±13.93 |

Table 2: The ethanolic extract of Anjir leaves has antioxidant effects on rats that have been given alloxan to make them diabetic

| Group | Treatment | No. of moles MDA formed/mg of protein | Protein Amount required for 50% inhibition | $H_2O_2$ Moles decomposed/minute/ mg of protein |
|---|---|---|---|---|
| **I** | Normal Control | 3.71 | 8.37 | 94.54 |
| **II** | Alloxan induced Diabetic control | 5.37 | 4.91 | 50.41 |
| **III** | Alloxan induced with Glibenclamide | 4.25 | 7.58 | 82.45 |
| **IV** | Alloxan induced Diabetic with Extract 200 mg/kg | 5.25 | 5.30 | 56.37 |
| **V** | Alloxan induced Diabetic with Extract 400 mg/kg | 4.61 | 6.96 | 73.91 |

reduced compared to the normal group. Some enzymes in diabetic rats get back to almost normal function when they are given Anjir leaf extract (Table 2).

There are certain issues with the present diabetic mellitus medication regimens. We therefore require improved and more potent diabetes medications. Finding out if Anjir leaves can benefit diabetics was the aim of this investigation. In this study, the blood sugar levels of diabetic rats were lowered by oral administration of Anjir leaf extract. According to reports, insulinogenic effects and ß-cell stimulation result from treating diabetic rats fed alloxan. The extract is a medicinal herb. By instructing the remaining ß-cells in the islets of Langerhans to produce more insulin, Anjir leaves may have reduced blood sugar. The fact that diabetic rats receiving Anjir leaves might have increased blood insulin levels. varying concentrations of hyperglycemia in the models resulted in varying percentage drops in plasma glucose levels. Thus, the amount of the plant's diabetogenic agent-- which kills ß-cells-- determines how effective the plant is at lowering

blood sugar. Numerous other plants have also been shown by researchers to reduce blood sugar by inducing the release of insulin. In comparison to the control group, the diabetic rats had significantly higher concentrations of lipids, such as blood cholesterol. Low insulin levels lead to a variety of issues with the metabolic and regulatory systems, which in turn lead to the accumulation of fat. When insulin release is slowed down, lipids are broken down from adipose tissue to plasma more quickly. Furthermore, it has been shown that Glibenclamide-treated diabetic rats' fat levels have returned to normal. Anjir leaf reduces cholesterol levels, which demonstrate that it functions similarly to insulin. Diabetes mellitus development has been connected to oxidative stress. Research has demonstrated over time that antioxidants can benefit diabetics. Oxygen free radicals (OFRs) are produced in diabetes mellitus when $H_2O_2$ is increased. In this study, we discovered that lipid peroxides and hydroperoxides were present in greater amounts in the livers of diabetic rats. This indicates that the production of free radicals increased. Because of their weakened antioxidant defenses, cells with diabetes mellitus are more reactively stressed, which can lead to increased lipid peroxidation. According to the current findings, rats treated with Anjir leaves showed much lower levels of lipid peroxidation than rats subjected to alloxan. This suggests that the flavonoids present in the leaf may be the cause of the protective action of Anjir leaf extracts. These flavonoids have potent anti-superoxide and anti-singlet oxygen properties. Numerous studies have demonstrated that individuals with diabetes mellitus have increased amounts of peroxidative chemicals and decreased levels of antioxidants. In this study, the livers of diabetic rats had significantly reduced levels of LPO, SOD, and CAT activities. The significance of maintaining the high antioxidant potential of pancreatic ß-cells to enable their survival and continued secretion of insulin during elevated levels of oxidative stress is evident from these findings. Reactive oxygen free radical generation may be the reason for the liver's reduced SOD and CAT levels because these free radicals can decrease the action of these enzymes.

## Conclusion

When test rats were given an ethanolic extract of Anjir leaves (200 and 400 mg/kg) instead of the control drug Glibenclamide (5 mg/kg), the aberrant enzyme profile in the rats was significantly reduced. Anjir leaf extract helps regulate blood sugar levels, enhances lipid metabolism, and protects against lipid peroxidation and antioxidant system issues associated with diabetes. We discovered that when diabetic rats were administered alloxan, Anjir leaves had significant anti-oxidant and anti-diabetic benefits. Additionally, the current study has opened up new research avenues, particularly in relation to the use of Anjir leaves in the preparation of a potent mixture for diabetes mellitus. Activity-based formulation, measurement, and fractionation are under development and should be accessible soon. It is imperative to ascertain the immediate and long-term adverse consequences of consuming Anjir leaves.

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