***Original Research Article***

**Antidiabetic Properties of Medicago sativa (Alfalfa) Aqueous Leaf Extract on Alloxan-induced Diabetes in Albino Rats**

**ABSTRACT**

The study investigated the antidiabetic property of *Medicago sativa* (Alfalfa) aqueous leaf extract on alloxan-induced diabetes in Wistar Albino Rats. A total of 37 rats weighing 120-150g were used for the study. **Twelve (12) rats were acclimatized for five days and acute toxicity study of the extract was carried out,** while twenty five (25) rats were acclimatized for seven days and separated into five (5) groups of five (5) rats each; the normal control (NC) group, the diabetic control (DC) group, two (2) test groups and a standard group. **The rats were fed with commercial pelletized rat feed and water *ad libitum.***All groups, except Normal control, were induced with diabetes intraperitoneally **by a single dose of** 150mg/kgb.wt of alloxan monohydrate. The two test groups were respectively treated orally with 250mg/kg and 500mg/kg.bwt of aqueous leaf extract of *Medicago sativa* (Alfalfa) daily for 21 days. The standard group was treated with 250mg/kg.bwt of Glucophage (Metformin) daily for 21 days. Blood glucose level was measured at the end of the experiment, and **the results were statistically analyzed and expressed as mean and standard deviation**. The result revealed that the blood glucose levels of the treated rats were significantly (P<0.05) reduced by both the extract and the metformin. The 500mg/kg.bwt of the extract showed a higher efficacy than the 250mg/kg.bwt of the extract, but it (the 500mg/kg.bwt of the extract) has a comparable effect with metformin. Therefore, the result shows that *Medicago sativa* leaf aqueous extract has antidiabetic effect on alloxan-induced diabetes in albino rats, in a dose dependent manner.

**Key words**: Alloxan, Blood Glucose, Diabetes Mellitus, *Medicago sativa*, Metformin.

**INTRODUCTION**

“Diabetes Mellitus is a chronic metabolic malady associated with high blood glucose levels. It is generally caused by defects in insulin secretion and insulin action” **(Kononenko et al., 2020)**. “Diabetes Mellitus is one of the major public health challenges, affecting millions of people worldwide” (Shahed et al*.,* 2021). “In Nigeria, the prevalence of diabetes is estimated to be around 2.2% of the population, with a projected increase to 4.8% by 2030” (Davies et al*.,* 2017). “It occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces” (Kononenko et al., 2020). **“Insulin is a hormone that regulates blood glucose level. It is secreted by the beta-cells of the *islets of Langerhans* in the pancreas”** (Kononenko et al., 2020). “Diabetes is a prime risk factor for cardiovascular disease and also affects the heart muscle causing both systolic and diastolic heart failure” **(Ceriello et al., 2021)**. “Nearly 1 in 10 adults worldwide are now affected with diabetes” (Okoli et al.*,* 2010).

“Current treatments for diabetes include insulin therapy, oral hypoglycemic agents, and lifestyle modifications. However, these treatments have limitations, including high costs, side effects, and the need for lifelong management” (Prasannakumar et al., 2018). As a result, there is a growing interest in alternative therapies, including herbal remedies, for the management of diabetes.

“Herbal drugs are commonly used for the treatment of diabetes mellitus in some countries of the world, especially India and China” (Bamidele et al., 2014). “The importance of antidiabetic plants in the development of low-cost and effective treatment for diabetes has been recognized by the World Health Organization” (Bamidele et al., 2014). “Most of the antidiabetic plants have been found to contain phytochemicals like glycosides, alkaloids, terpenoids, flavonoids, etc” (Bamidele et al., 2014).

***“Medicago sativa* (Alfalfa) is a leguminous plant that has been used in traditional medicine for the treatment metabolic ailments like diabetes and digestive disorders” (Burhan and Kokaz, 2019;** [**Bora and Sharma, 2011**](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10582054/)**). “The leaves of the Alfalfa are rich in bioactive compounds like flavonoids, alkaloids and phenols, which have been shown to have antidiabetic and antioxidant properties” (Prasannakumar et al., 2018). “It has an extensive history of usage as a Homoeopathic and Ayurvedic medicine” (**[**Bora and Sharma, 2011**](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10582054/)**). “In China, North Africa, Russia and United States, *Medicago sativa* is used as a food ingredient due to its high concentration of vitamins and bioactive components” (**[**Liu *et al.*, 2016**](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10582054/#b0305)**). “Its contents include a variety of digestive-aiding enzymes like amylase, invertase, and pectinase” (Esmaiel et al*.,* 2015). “Phytochemicals found in *Medicago sativa* aqueous leaf extract include flavonoids, alkaloids, phytoestrogens, phytosterols, amino acids, silymarins, organic acids, phenolic compounds, proteins, vitamins, digestive enzymes, triterpenes and saponins” (Esmaiel et al*.,* 2015).** “Due to valuable chemical constituents which show phytobiotic action on humans, lucerne is used in folk medicine and phytotherapy” ([Farsani et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10582054/)). “Alfalfa tea is used to strengthen the digestive system” ([Farsani et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10582054/)). “Alfalfa is rich in minerals and is very useful for children who are growing up and do not have strong bones” ([Farsani et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10582054/)). “Even today, the powder of this plant is sold in pharmacies, which can be used for infants” ([Farsani et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10582054/)). “Many enzymes are found in alfalfa, including invertase and pectinase” ([Farsani et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10582054/)). “Alfalfa contains about 20% protein. In addition, it contains carbohydrates, diastases, a type of saponin with a sneezing effect, two pigments, phosphoric acid and various vitamins. According to literature evaluation, alfalfa leaves are believed to be used in treating diabetes but there isn't enough data to make a scientific determination about its effectiveness in treating diabetes” ([Farsani et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10582054/)). Objectively, this work was carried out to evaluate the anti-diabetic effect of aqueous extract of *Medicago sativa* leaves in alloxan-induced diabetic albino rats

**Materials and Methods**

**Collection of Plant Materials**

*Medicago sativa* leaf was collected from a local farm located at Umudo Obollo in Isiala Mbano Local Government Area of Imo State, Nigeria and was identified and authenticated by a Taxonomist, Dr. C. I. N. Unamba in the Department of Botany, Imo State University, Owerri, Imo State, Nigeria.

**Experimental Animals**

Twenty five (25) adult male and female Wistar strain albino rats weighing 120–150g were used for the study. The animals were acquired from Nano Laboratories Animal Farm House, Owerri. The rats were housed in stainless steel cages at standard laboratory conditions (temperature 24 ± 2oC, relative humidity 45–55% and cycle of 12 hour light and 12 hour dark) (Zymantience et al., 2016). They were provided with commercial pelletized rat feed and water *ad libitum*. The animals were acclimatized to laboratory conditions for 7 days prior to the studies (Zymantience et al., 2016).

**Preparation of Extract**

The collected leaves were washed with tap water carefully and completely dried under the shade (Esmaiel, 2015). The shade-dried leaves were ground into fine powder (Esmaiel, 2015). The aqueous extract was obtained by soaking about 100g of the powdered plant material in 500ml of boiled distilled water (80oC) for 24 hours (Esmaiel, 2015). The extract was filtered to remove debris using Muslin clothe and subsequently with Whiteman No. 1 Filter Paper to remove fine particles. The crude extract obtained was concentrated by evaporating in a water bath at 55oC to obtain a semi-solid slurry. The extract was stored in a refrigerator at -4oC using air-tight plastic bottles.

**Acute Toxicity Study (LD50) of the Extract**

Acute toxicity study of the aqueous leaf extract of *Medicago sativa* was carried out according to the Organization for Economic Co-operation and Development (OECD) test guideline 423 (Test No. 423 – Acute Toxic Class Method) **(Ouedraogo et al., 2024)**. The test animals were divided into four (4) groups of three (3) rats each. The animals were healthy, young adult rats weighing 120-150g. The rats were acclimatized for 5 days under standard laboratory conditions before the commencement of the toxicity study. The four groups were respectively administered orally with single doses of 200mg/kg, 500mg/kg, 1000mg/kg and 2000mg/kg body weight of the extract (Ouedraogo et al., 2024). The rats were observed daily for 14 days for a sign of toxicity or mortality (Pramod et al., 2017).

**Induction of Diabetes Mellitus**

In overnight fasted rats, diabetes was induced by single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight) dissolved in normal saline (Esmaiel et al*.,* 2015). After 72 hours of alloxan monohydrate injection, blood glucose levels of the rats were measured using a glucometer and rats with blood glucose levels above 200mg/dl were considered diabetic (Esmaiel et al.*,* 2015).

**Experimental Design**

The study was carried out for 21 days (Esmaiel et al*.,* 2015). The rats were divided into five (5) groups of five (5) animals each as follows:

Group 1: Normal control (no inducement and no treatment); fed with only normal feed and water *ad libitum*.

Group 2: Diabetic control; induced with Diabetes Mellitus using Alloxan (150mg/kg.bwt) and also fed with normal feed and water *ad libitum* (Pramod et al, 2017).

Group 3: Standard test; induced with Diabetes Mellitus using Alloxan (150mg/kg.bwt), and treated with Glucophage (Metformin, 250mg/kg body weight) daily for 21 days (Pramod et al, 2017) and also fed with feed and water *ad libitum*.

Group 4: *M. sativa test 1;* induced with Diabetes Mellitus using Alloxan (150mg/kg.bwt), and treated with 250mg/kg body weight of the aqueous extract of *M. sativa* and also fed with normal feed and water *ad libitum* (Esmaiel, 2015).

Group 5: *M. sativa test 2;* induced with Diabetes Mellitus using Alloxan (150mg/kg.bwt), and treated with 500mg/kg body weight of the aqueous extract of *M. sativa* and also fed with normal feed and water *ad libitum*.

**Blood Glucose Measurement**

Blood glucose was measured using a glucometer test kit at baseline (0 hour), 24 hours, 48hours, 7 days, 14 days and 21 days intervals. The analysis is based on glucose oxidase reaction (Frank et al., 2012). A drop of blood from the tip of the tail of each rat was dropped to a chemically treated, disposable glucose test-strip, which was then inserted into a glucometer and the reading taken (Frank et al., 2012).

**Statistical Analysis**

Statistical analyses were performed with SPSS for windows, version 15.0 (SPSS, Chicago, IL). Data were expressed as mean and standard deviation. Differences between the glucose levels of different groups of rats were analyzed using one-way ANOVA. A value of *P*<0.05 will be considered statistically significant.

**Result**

**Acute Toxicity Study**

The result of the acute toxicity study of the *M. sativa* showed that there is no death or lesions observed in any of the rats used for the study at doses 200mg/kg, 500mg/kg, 1000mg/kg and 2000mg/g body weight after 14 days *a*s shown in table 1.

**Table 1: Acute Toxicity Study Result of *M. sativa* Aqueous Leaf Extract**

|  |  |  |  |
| --- | --- | --- | --- |
| *M. sativa*  Extract Dosage (mg/kg.bwt) | No. of Rats | No. of Deaths | Lesion |
| 200 | 3 | 0/3 | None |
| 500 | 3 | 0/3 | None |
| 1000 | 3 | 0/3 | None |
| 2000 | 3 | 0/3 | None |

**Effects of *M. sativa* aqueous leaf extract and Metformin on Blood Glucose Levels of Alloxan-induced Diabetic Albino Rats**

Table 2 shows the effects of *M. sativa* leaf aqueous extract and Metformin on blood glucose levels of the alloxan-induced diabetic rats within 21 days of treatments. The results show that induction of diabetes resulted in significant (p<0.05) elevation of blood glucose level in diabetic rats compared to normal control rats. The blood glucose levels of the diabetic rats respectively administered with *M. sativa* leaf aqueous extract (500mg/Kg b.wt and 250mg/kg b. wt.) and metformin (250mg/Kg b.wt.) were significantly reduced (P<0.05) compared to the diabetic rats that were neither administered with the  *M. sativa* leaf extract nor metformin. The extract at dose of 500 mg /Kg b.wt significantly (p<0.05) reduced blood glucose concentration of the diabetic rats within 7 days of treatment. However, administration with 250 mg /Kg b.wt dose of *M. sativa* leaf aqueous extract significantly reduced the blood glucose levels of the diabetic rats after 14days of treatment. The result revealed that the reduction in blood glucose levels of the diabetic rats by the extract at dose of 500 mg /Kg b.wt was comparable to that of the standard drug (metformin) after 21 days of treatment. The extract at dose of 500 mg /Kg b.wt showed considerably higher efficacy than the 250 mg/Kg b.wt dose of the extract as seen in table 2. Hence, the hypoglycemic effect of *M. sativa* leaf aqueous extract is dose dependent. The observed reversal of hyperglycaemia in diabetic rats by the aqueous extract of *M. sativa* leaf is indicative of its potent antidiabetic properties.

**Table 2 Blood glucose concentration of alloxan-induced diabetic rats treated with *M. sativa* leaf aqueous extract and Metformin**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Days** | **Baseline (0hr)** | **24hrs** | **48hrs** | **7days** | **14days** | **21days** |
| **NC** | 94.33 ±  8.02a | 97.00 ±  8.00a | 100.33 ±  9.87a | 109.67±  13.80a | 101.67 ±  2.00a | 91.00±  2.00a |
| **DC** | 397.00 ±  2.65a | 403.67 ±  15.53a | 358.67±  24.28b | 342.00 ±  15.72b | 299.67 ±  2.52c | 277.33±  12.01c |
| **STD**  **(Metformin) 250mg/kg b.wt** | 379.33 ±  11.02a | 372.33±  10.07a | 322.00 ±  27.40b | 288.67±  10.60c | 184.67 ±  9.87d | 140.33±  4.51d |
| **Treatment 1**  **(250mg/kg b.wt extract)** | 386.00±  8.19a | 381.00 ±  18.25a | 368.00±  23.07a | 328.33 ±  24.58b | 262.33 ±  10.79c | 241.67±  22.55c |
| **Treatment 2**  **(500mg/kg b.wt extract)** | 390.33±  20.50a | 394.00±  9.54a | 318.00±  19.97b | 248.33 ±  6.66c | 189.00 ±  20.00d | 144.33±  9.29d |

Results are mean ±standard deviation of five (5) determinations. Values with different superscript across rows are significantly different (p<0.05).NC: Normal Control, DC: Diabetic Control, STD: Standard

NC DC STD T1 T2

400-

350-

300-

250-

200-

150-

100-

50-

mg/dl

**NC**: Normal Control Group

**DC**: Diabetic Control Group

**STD**: Group treated with Standard drug (Metformin)

**T1**: Group treated with 250mg/kg b.wt of *M. sativa*

**T2**: Group treated with 500mg/kg b.wt of *M. sativa*

**Fig. 1: Effect of *Medicago sativa* and Metformin on blood glucose level of the diabetic rats at baseline**

NC DC STD T1 T2

mg/dl

400-

350-

300-

250-

200-

150-

100-

50-

**NC**: Normal Control Group

**DC**: Diabetic Control Group

**STD**: Group treated with Standard drug (Metformin)

**T1**: Group treated with 250mg/kg b.wt of *M. sativa*

**T2**: Group treated with 500mg/kg b.wt of *M. sativa*

**Fig. 2: Effect of *Medicago sativa* and Metformin on blood glucose level of the diabetic rats after 24 hours**

NC DC STD T1 T2

400-

350-

300-

250-

200-

150-

100-

50-

mg/dl

**NC**: Normal Control Group

**DC**: Diabetic Control Group

**STD**: Group treated with Standard drug (Metformin)

**T1**: Group treated with 250mg/kg b.wt of *M. sativa*

**T2**: Group treated with 500mg/kg b.wt of *M. sativa*

**Fig. 3: Effect of *Medicago sativa* and Metformin on blood glucose level of the diabetic rats after 48hours**

NC DC STD T1 T2

400-

350-

300-

250-

200-

150-

100-

50-

mg/dl

**NC**: Normal Control Group

**DC**: Diabetic Control Group

**STD**: Group treated with Standard drug (Metformin)

**T1**: Group treated with 250mg/kg b.wt of *M. sativa*

**T2**: Group treated with 500mg/kg b.wt of *M. sativa*

**Fig. 4: Effect of *Medicago sativa* and Metformin on blood glucose level of the diabetic rats after 7 days**

NC DC STD T1 T2

400-

350-

300-

250-

200-

150-

100-

50-

mg/dl

**NC**: Normal Control Group

**DC**: Diabetic Control Group

**STD**: Group treated with Standard drug (Metformin)

**T1**: Group treated with 250mg/kg b.wt of *M. sativa*

**T2**: Group treated with 500mg/kg b.wt of *M. sativa*

**Fig. 5: Effect of *Medicago sativa* and Metformin on blood glucose level of the diabetic rats after 14 days**

400-

350-

300-

250-

200-

150-

100-

50-

mg/dl

**NC**: Normal Control Group

**DC**: Diabetic Control Group

**STD**: Group treated with Standard drug (Metformin)

**T1**: Group treated with 250mg/kg b.wt of *M. sativa*

**T2**: Group treated with 500mg/kg b.wt of *M. sativa*

NC DC STD T1 T2

**Fig. 6: Effect of *Medicago sativa* and Metformin on blood glucose level of the diabetic rats after 21 days**

**Discussion and Conclusion**

This study assessed the antidiabetic properties of *M. sativa* leaf aqueous extract on alloxan-induced diabetes in albino rats. Medicinal plants such as *M. sativa* leaf have been studied as an alternative source of antidiabetic agents (Esmaiel et al*.,* 2015). They form a quick intervention in the traditional management of diabetes and associated complications (Esmaiel et al.*,* 2015). Medicinal plants are useful in the management of diseases due to the rich battery of phytochemicals associated with their tissues (leaves, stem, roots, seeds) (Esmaiel et al.*,* 2015). These phyto-compounds may directly influence blood glucose concentration in diabetic condition or aid recovery and regeneration of damaged/non-optimal pancreatic function (Esmaiel et al.*,* 2015).

The oral acute toxicity study on *M. sativa* leaf aqueous extract as shown on Table 1, revealed that administration of the aqueous extract of *M. sativa* leaf up to the dosage of 2000 mg/Kg b.wt resulted in no death of the experimental rats 14 days after dosing, and did not produce any noticeable sign of toxicity in the rats. Hence, the aqueous extract of *M. sativa* leaf is considered generally safe for animals’ consumption up to the dose of 2,000 mg/kg b.wt. The result of our findings corroborates with previous studies on the acute toxicity of *M. sativa* leaf hydroethanol extract. Anele et al*.,* (2024), in their study using wistar rats, showed that *M. sativa* leaf extract was non-lethal in doses up to 5000 mg/Kgb.wt. Raeeszadeh et al., (2022), reported the acute toxicity of *M. sativa* leaf extract to be above 2000 mg/kg body weight.

“The result of antidiabetic study of aqueous extract of *M. sativa* leaf on alloxan-induced diabetic rats showed that the extract had significant (p<0.05) hypoglycaemic effect. The blood glucose levels of the normal control rats were normal throughout the period of the experiment. The blood glucose of the diabetic control rats was significantly elevated after injection of alloxan monohydrate. Treatment with *M. sativa* aqueous leaf extract (250mg/kg b.wt and 500mg/kg b.wt) significantly reduced the elevated blood glucose of the diabetic rats. Also, treatment with 250mg/kg b.wt of Metformin significantly reduced the blood glucose level. The extract at dose of 500 mg/Kg b.wt significantly (p<0.05) reduced blood glucose concentration of diabetic rats within 7 days of treatment, while administration of 250 mg /Kg b.wt dosage of *M. sativa* leaf aqueous extract significantly reduced blood glucose after 14days of treatment. The antidiabetic property of *M. sativa* leaf aqueous extract was found to be dose dependent, as the 500 mg/Kg b.wt showed a higher hypoglycemic effect after 21 days of treatment than the 250 mg/Kg b.wt. The result of the study agrees with previous reports that *M. sativa* leaf possess antidiabetic potentials; the extract have been shown to stimulate insulin secretion of β-TC-6 cells in hyperglycemic conditions *in vitro”* (Paun et al., 2024). “In another study by Khalaf et al. (2021), the aqueous extracts of *M. sativa* was found to improve glycaemic index and other normalized deranged biochemical parameters in alloxan-diabetic rats. **According to Ma et al (2024), *in vivo* studies have shown that *Medicago sativa* polysaccharides (MSP-II-a) significantly reduced fasting blood glucose levels in HFD/STZ-induced diabetic mice.** Medicinal plants have been postulated to mediate their hypoglycaemic effect via restoration of insulin production by pancreatic beta cells; or thorough immune-stimulation and anti-inflammatory effect” (Habtemariam, 2020). This various mechanisms are attributable to the action of bioactive phytochemicals in the plant (Alfalfa). The antidiabetic properties of *M. sativa* leaf extract maybe attributed to a number of active mechanisms such as enhancement of glucose uptake, stimulation of insulin secretion from pancreatic β- cells and inhibition of pancreatic amylase (Habtemariam, 2020). Conclusively, the observed reversal of hyperglycaemia in diabetic rats by aqueous extract of *M. sativa* leaf is indicative of its potent antidiabetic properties. Aqueous extract of *M. sativa* leaf has comparable effectiveness to Metformin for the treatment of Diabetes Mellitus.

**Disclaimer (Artificial Intelligence)**

Authors hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of this manuscript.

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