

RESEARCH ARTICLE

Hypoglycemic, Hypolipidemic, Renal Protective and Antioxidant Activity of *Annona muricata* in Streptozotocin-Induced Diabetic Rats

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ABSTRACT:

Annona muricata, an herbal plant commonly used in traditional medicine to manage numerous diseases, diabetes as other diseases could be managed with herbal medicine. This study was designed to be investigated the antidiabetic, hypolipidemic, renal protective, and antioxidant effects of aqueous extracts of *Annona muricata* as used alone or combined with metformin in streptozotocin (STZ)-induced diabetic rats. Methods: the study was involved twenty adult Wister albino rats in four groups (five rats in each) and designated as groups, control group (1), and experimental groups (2, 3, 4). Diabetes was induced in experimental groups by 60 mg/kg intravenous streptozotocin injection. Group 2: serves as a diabetic control group, Group 3: diabetic rats treated with oral administration of 100 mg/kg of *Annona muricata* aqueous extract, Group 4: diabetic rats treated with combination (100 mg/kg aqueous extract of *Annona muricata* + 50 mg/kg metformin). The treatment continuous daily for 4 weeks to determine the levels of blood glucose and biochemical analysis. Result: aqueous extract of *Annona muricata* was reduced the serum glucose level effectively in streptozotocin-induced diabetic rats, by 48% and 55% after 28 consecutive days of treatment when used alone and with metformin, respectively. These compared to the preliminary values and the reduction was statistically significant compared to a diabetic control group. Daily oral administration of 100 mg/kg aqueous extract of *Annona muricata* for 4 weeks to streptozotocin-induced diabetic rats significantly reduced the level of total cholesterol, urea, creatinine, and MDA, whereas the reduction was non-significant in triglyceride and VLDL-cholesterol levels as compared to the non-treated diabetic group. However, the reduction is more significant in streptozotocin-induced diabetes rats that were treated with a combination of *Annona muricata* and metformin when compared to the diabetic control group. Conclusion: Aqueous extracts of *Annona muricata* have anti-diabetic action through their hypoglycemic, hypolipidemic, renal protective, and antioxidant effects in streptozotocin-induced diabetic rats. Thus, can be used alone or with anti-hyperglycemic drugs as metformin in the management of DM. The combination is preferred in severe hyperglycemic cases with more hypoglycemic effect requirements.

KEYWORDS: Diabetes Mellitus, Aqueous extracts of *Annona muricata*, Metformin, Streptozotocin (STZ), blood glucose level.

1. INTRODUCTION:

Diabetes Mellitus (DM), a heterogeneous metabolic syndrome, described as a chronic hyperglycemia condition due to an imbalance of pancreatic β -cells function. It referred to high blood glucose levels and its complications cause organ damage¹⁻⁴.

The incidence of DM increased in worldwide to act as a major problem in public health. It's probably expected the percentage will rise to reach about 72% in 2025^{2,5}. Nowadays, Type 2 DM is more common and the prevalence increased in past decades. This condition is characterized by hyperglycemia, hypercholesterolemia, and hypertriglyceridemia^{3,6}. As well, the evidence of oxidative stress augmented in DM that predominately associated with a high level of lipid peroxidation biomarkers as malondialdehyde (MDA)⁷.

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Although oral hypoglycemic drugs as metformin can be controlled early diabetes's manifestation, the complications may appear in some patients related to many side effects such as peripheral edema, abdominal pain, lactic acidosis, and others⁸⁻¹⁰. Hence, the scientific works continue to assess the antidiabetic effects of raw materials and extracts of natural compounds, despite the continuous efforts in the scientific community to the production of new drugs and their validation on scientific principles^{2, 6, 11, 12}

One of the natural plants from the Annonaceae family called *Annona muricata* (Ann. Muricata), and well-known as Soursop, the leaves, fruit and other parts of its tree had an outdated use in traditional medicine for a long history^{13, 14}. *Annona muricata* has a wide range of ethnopharmacological uses in the management of various conditions involving rheumatism, pain, fever, hypertension, diabetes, vomiting, worm, diarrhea, and heart or liver disease^{15, 16}. This also acts as an anti-inflammatory and anti-cancer mediator with high effects on the immune system¹⁶. Additionally, *Annona muricata* possesses antibacterial, antimicrobial^{16, 17}, and antiviral effects¹⁸.

With regards to previous literature, *Annona muricata* has positive effects in several body systems such as anti-hypertensive, anti-spasm, vasodilatation, cardiovascular depression^{19, 20}, and Suppress seizure with anxiolytic action²¹. Although, several investigators were observed that *Annona muricata* exhibits toxic effects on both the liver and kidney, the low dose of it has a protective effect^{22, 23}.

Moreover, the leaves of *Annona muricata* were contained biological activity against plasmodium, parasite, protozoa, and anti-anthelmintic in internal and external worms^{13, 16}. Likewise, it has beneficial effects in controlling the diabetic patient as a hypoglycemic, hypolipidemic, and antioxidant effect^{7, 20, 24}. The alleviating properties of *Annona muricata* as a result of its content. In fact, the extracts of *Annona muricata* contain more than 200 phytochemical constituents with pharmacological activities such as alkaloids, phenol, acetogenins, tannins, flavonoids, coumarins, terpenoid, ellagic acid, stearic acid, and other components^{23, 25}. The present study investigates the anti-diabetic, hypolipidemic, antioxidant, and renal protective effects of aqueous extract of *Annona muricata* on diabetic rats after their induction by streptozotocin (STZ) (STZ-induced diabetic rats) as used alone or combined with hypoglycemic drugs (metformin).

2. MATERIALS AND METHODS:

2.1 Animals:

Twenty healthy adult male Wister albino rats, (2-3)

months old were used in this study, with a weight between 160 and 190 gm. These animals were collected in animals' houses in the college of pharmacy, University of Basra, the animals were kept under the appropriate laboratory conditions, in a 12 hr. environmental light/ dark cycle at room temperature (25±1) C and humidity (55±5%). The animals were fed a commercial diet, pellets, and water *ad libitum*. All measures were engaged according to the guidelines (National Institutes for health USA publication, 1985).

This study was approved via the Animal Ethics Committee in the college of pharmacy/ Basra University.

2.2 Plant materials:

The leaves of *Annona muricata* were collected in March 2020; the plant was identified in the Pharmacognosy Department, College of Pharmacy, Basra University, Iraq. It dried in the air at room temperature then grind to powder manually. According to the traditional method obtained aqueous extract of powder through the process in which the powder dissolved in distilled water (D.W) within a ratio of 1:3 (1kg of *Annona muricata* with 3 ml D.W) at room temperature for 48 hrs. The oven at 40 C is used to evaporate the filtrate to achieve the desired dose for therapeutic testing²⁶.

2.3 The procedure design:

In this experiment, the healthy animals were randomly distributed into four groups with five animals in each one to investigate the effects of aqueous extract of *Annona muricata* on streptozotocin (STZ) induced diabetic rats.

2.4 Experimentally Induction of diabetes mellitus in rats:

Diabetes condition induced in animals during 2-4 days after injected of the three groups of rats with streptozotocin (STZ) intravenously (I.V), the dose was used 60 mg /kg dissolved in 0.9% sodium chloride solution. While the control group was received normal saline intravenously at this time. The animals fasted before streptozotocin administration for 16 hrs.

After 72 hr. of diabetes induction, blood samples were engaged from the tail vein rats to measure Blood Glucose level (BG). the glucose level in each blood was measured by using the Glucose Oxidase Method²⁷. If the fasting blood glucose of the animals more than 200 is considered diabetic conditions²⁸.

After that, the animals were kept under careful observation for 2 weeks without treatment to stabilize the diabetic conditions and re-measuring the parameters on the last day to confirm the rising of blood glucose level after streptozotocin induction²⁰.

2.5 Oral administration of *Annona muricata*:

After 2 weeks of diabetic stabilizing, the experimental rats were separated into four groups (5 animals in each one) for treatment:

- Group (1) control group: (non-diabetic induction rats) were treated with distilled water (D.W) 1 ml/kg only.
- Group (2) STZ- induced diabetic rats (diabetic control rats) were treated with D.W (1 ml/kg) only.
- Group (3) STZ-induced diabetic rats were treated orally with aqueous extract of *Annona muricata* at a dose of 100mg/kg/day dissolved in D.W
- Group (4) STZ-induced diabetic rats were treated orally by a combination of aqueous extract of *Annona muricata* (100mg/kg) dissolved in D.W and metformin with a dose of 50 mg/kg dissolved in 0.9 sodium chloride solution.

The experimental rat’s therapy continued for 28 days sequentially via oral gastric intubation, the blood sample was pulled from the tail vein of the rats to monitor the experimental parameters and blood glucose level.

2.6 Blood samples and Biochemical analysis:

Blood samples were obtained from the animals on various days and all the results are recorded. The readings of blood glucose levels were taken in (day 0 before treatment, day 3, 14, 21, and 28 after treatment) as shown in table 1.

At the beginning and end of the treatment, the blood collected in standard tubes then centrifuged in (Genex, Florida, USA) at 3000 rpm for 15 minutes to obtain serum for biochemical analysis (total cholesterol (TC), triglyceride (TG), serum creatinine (S Cr), blood urea and MDA). The parameters were measured by using a spectrophotometer (Integra machine, Roche Germany) following the instruction of the commercial diagnostic kits.

2.7 Statistical analysis:

The results of the study were analyzed by using the statistical system (spss) version 20. Parameters values were expressed as mean ± standard error of the mean. The statistical significance was determined by using

one-way analysis of variance (ANOVA), then Tukey test. P-value < 0.05 was reflected statistically significant.

3. RESULTS:

3.1 Effect on blood glucose level:

The intravenous administration of Streptozotocin results in increased levels of blood glucose significantly during the period of the study in three experimental groups as compared to the normal group, as shown in table 1. The values of blood glucose showed significantly reduced (p-value < 0.05) after the daily administration of aqueous extract of *Annona muricata* alone and with metformin compared with the diabetic non-treated group on day 14. Then on day 21, the blood glucose levels were reduced by 42.1% for the group that received *Annona muricata* extract, and 53.5 % for the group provided with aqueous extract of *Annona muricata* and metformin, and the reduction was statistically significant compared with a diabetic control group. After 4 weeks of management, the reduction in the glucose level in blood was 48% and 55 % for *Annona muricata* extract-treated group and *Annona muricata* extract plus metformin-treated group, respectively, these compared to the preliminary values, and the reduction was statistically significant as compared to the non-treated diabetic group.

3.2. Effect on serum lipid profile.

The serum lipid profile in experimental diabetic rats showed significantly high levels in total cholesterol, triglycerides, and VLDL-cholesterol as compared to the normal control group (p-value < 0.05). After 4 weeks of treatment with an aqueous extract of *Annona muricata*, the total cholesterol level has shown a significant decrease (20%) when compared with the non-treated diabetic rats (p-value < 0.05). As well, the administration of aqueous extract of *Annona muricata* with metformin to STZ-induced diabetic rats reduced the total cholesterol level significantly (14%) as compared to the non-treated diabetic group. Whereas, the reduction in triglycerides and VLDL cholesterol levels did not reach the statistical significance level (p > 0.05), as shown in table 2.

Table 1: Effect of aqueous extract of *Annona muricata* on levels of blood glucose in STZ induced diabetic rats.

Treatment groups	Blood Glucose levels (mg/dl)				
	Day(0)	Day (3)	Day (14)	Day (21)	Day (28)
Normal control	106.2 ±6.1	104.2±7.9	105.2±4.2	105±6.04	103.8±8.7
Diabetic control	231.4 ±3.9*	231.8±2.5*	219.6±3.7*	225.8±2.6*	225.6±6.7*
<i>Annona muricata</i> 100 mg/kg/day	234.2 ±4.2*	230.6±3.4*	142.2±3.3 [#] *	135.6±3.4 ^{#**}	121.8±3.6 [#]
<i>Annona muricata</i> + metformin	234.8 ±2.5*	225.4±4.1*	127.4±3.8 [#] *	109.2±2.9 [#]	105.7±2.5 [#]

Data are expressed as mean ± SEM, n=5

* P < 0.05 significantly different as compared to the normal control group.

P < 0.05 significantly different as compared to the diabetic control group.

Table 2: effect of aqueous extract of *Annona muricata* on serum lipid profile after four weeks of treatment.

Lipid Parameters	Control group	Diabetic control group	<i>Annona muricata</i> extract100 mg/kg/day group	<i>Annona muricata</i> extract+metformin group
Total cholesterol (mg/dL)	105.4±8.7	139.2±5.1*	111.3± 6 [#]	119.7±4.1 [#]
Triglycerides (mg/dL)	75.1±7.4	110.3±10.7*	94.5±8.7	102.4±6.2*
VLDL- cholesterol (mg/dL)	15.1±1.4	22.04±2.1*	18.8±1.7	20.4±1.3

Data were expressed as mean ± SEM, n=5

* P-value < 0.05 significantly different as compared to the normal control group.

P-value < 0.05 significantly different as compared to non-treated diabetic group.

Table 3: Effect of aqueous extract of *Annona muricata* on renal function after four weeks of treatment.

Renal function Parameters	Control group	Diabetic control group	<i>Annona muricata</i> extract100 mg/kg group	<i>Annona muricata</i> extract+metformin group
Creatinine (mg/dL)	1.1±0.1	1.85±0.2*	1.07±0.1 [#]	0.92±0.1 [#]
Urea (mg/dL)	23.4±1.2	52±6.8*	24.4±1.7 [#]	21.6±2.5 [#]

Data expressed as mean ± SEM, n = 5

* P-value < 0.05 significantly different as compared to the control group.

P-value < 0.05 significantly different as compared to the diabetic control group.

3.3. Effect of aqueous extract of *Annona muricata* on Renal function in experimental diabetic rats.

Table 3 illustrated the significant increase in Urea and Creatinine levels in STZ induced diabetic group as compared to the normal control group. The treatment for four weeks with 100 mg/kg aqueous extract of *Annona muricata* alone and with a combination of *Annona muricata* extract and metformin result in a significant reduction in the Urea and Creatinine levels (p-value < 0.05) when compared to the non-treated diabetic group.

3.4. Effect of *Annona muricata* as an antioxidant:

The level of MDA was elevated significantly in experimental diabetic rats when compared with the control group (p< 0.05), the daily treatment with plant extracts decrease the MDA levels significantly (68.6%) as compared with the non-treated diabetic group. Also, the group treated with *Annona muricata* extract and metformin show a significant reduction in the MDA level (52.7%) after 28 days of treatment. Table 4.

Table 4: Effect of aqueous extract of *Annona muricata* on MDA level after four weeks of treatment

Biomarker	Control group	Diabetic control group	<i>Annona muricata</i> extract100 mg/kg group	<i>Annona muricata</i> extract+metformin group
MDA (nmol)	0.87±0.2	2.83±0.4*	0.89±0.1 [#]	1.34±0.2 [#]

Data expressed as mean ± SEM, n=5

* P-value < 0.05 significantly different as compared to the control group.

#P value < 0.05 significantly different as compared to the diabetic control group

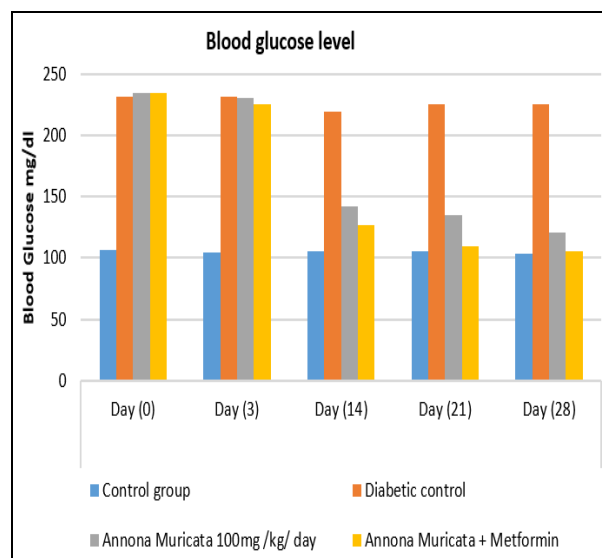


Figure 1: Effect of Aqueous Extract of *Annona muricata* on levels of blood glucose in Streptozotocin-induced diabetic rats.

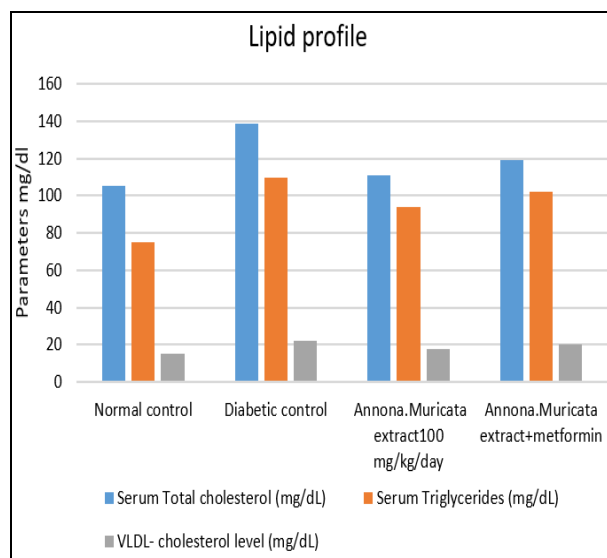


Figure 2: effect of aqueous extract of *Annona muricata* on serum lipid profile after four weeks of treatment.

4. DISCUSSION:

Diabetes Mellitus, one of the main global health problems, which associated with abnormalities in glucose hemostasis and lipid profile^{3,29,30}. The high level of blood glucose can result from the impairment in secretion and/or effect of insulin^{3,31,32}. Scientists are ongoing to search for plant extracts having a protective role and effectiveness in the management of diabetic animals and humans^{33,34}. The necessity to use alternative antidiabetic compounds to overcome the undesirable effects of the current therapy. Thus, the controlling and treatment of DM need exploring of low toxicity, inexpensive, and antioxidant natural compounds with inhibitory enzymatic activity^{6, 25, 34-36}. One of these herbal extracts is *Annona muricata*; the present study demonstrates the effects of the aqueous extracts of *Annona muricata* in improving the hyperglycemic, hyperlipidemia, and renal function in streptozotocin-induced diabetic rats.

Generally, streptozotocin could destruct the β - cell in Langerhans islet in the pancreatic tissue that induces experimental DM after 3 days of administration^{28, 37}. Our results have been shown; the continuous daily treatment of aqueous extract of *Annona muricata* for 28 days in STZ- induced diabetic groups significantly reduces the blood glucose levels, which consistently manages the diabetes condition after induction by streptozotocin. The levels of blood glucose in experimental diabetic rats dropped within 14 days after treatment and continuously lowered until reach a normal level. This is attributed to the aqueous extracts of *Annona muricata* contain hypoglycemic phytochemical constituents as flavonoid, alkaloid, tannin, triterpenoids, and other secondary metabolites^{14, 16, 38}. Additionally, the previous researchers suggested that *Annona muricata* can exert a hypoglycemic effect through its stimulated insulin secretion, enhanced the repairing of β cell, and increased the activity of both insulin and adrenalin^{20, 39, 40}. Our findings were similar to the results of previous studies^{20, 40}, which found that the administration of 100mg/ kg of *Annona muricata* to diabetic rats renormalized the blood glucose level and improved glycemic control. These also in agreement with Gavamukulya et al⁴¹ who identified that *Annona muricata* has the traditional uses and pharmacological properties as the anti-hyperglycemic effect. Besides, The work done by Vidona et al⁴² observed that there are beneficial effects of *Annona muricata* aqueous pulp extracts on diabetic animals by reducing body weight, serum glucose levels, and hyperglycemia symptoms. The glucose level in blood is significantly more reduced in the STZ- induced diabetic group treated with a combination of aqueous extract of *Annona muricata* and metformin compared to the STZ- induced diabetic group treated with *Annona muricata* alone as a result of using

both compounds with a hypoglycemic effect., this is similar to the finding of Michael et al⁴³. While the study of Vidona et al⁴² demonstrated that *Annona muricata* has a moderate effect when used alone compared with metformin in diabetic rats. So the combination is recommended in severe cases of hyperglycemia as a synergistic effect and reduces the complication⁴².

Induction of diabetes by streptozotocin could be elevated the lipid profile as triglyceride (TG), total cholesterol, atherogenic index, LDL-cholesterol, VLDL-cholesterol, and reduced HDL-cholesterol²⁹. The increasing of TG level in DM is attributed to the high production of very-low-density lipoprotein, defect in its catabolism, and attenuating the activity of lipoprotein lipase enzyme which responsible for triglyceride degradation⁴⁴. The results of the present study revealed that the administration of *Annona muricata* aqueous extracts alone or combined with metformin in STZ-induced diabetic for 4 weeks significantly decrease the level of serum total cholesterol as compared to a diabetic control group that ascribed to the anti-hyperlipidemia and anti-hyperglycemic properties of *Annona muricata*^{25, 44}. While the non-significant reduction of the TG and LDL-cholesterol levels after treatment probably due to an inadequate dose of plant or required prolonging the duration of treatment. Additionally, increased the circulatory of TG and VLDL- cholesterol may result from defective of their clearance from blood circulation^{44, 45}. These results agree with the conclusion of previous studies^{20, 44} which observed that *Annona muricata* reduced the lipid profile in diabetic conditions. Hence, the aqueous extracts of *Annona muricata* could have possibly decreased the risk of cardiovascular disease in DM and death^{44, 46}.

On the other hand, both serum creatinine and urea were elevated significantly in experimental diabetes rats, which indicates the end stage of kidney disease and extracellular dehydration⁴⁷. The daily use of the *Annona muricata* extract in treating experimental groups could significantly lower these markers through its protective effects in low dose, thus, may be an improved renal function^{22, 48}. These findings were in line with Florence et al²⁰, who suggested that *Annona muricata* significantly reduced the serum creatinine level.

Based on the previous evidence that streptozotocin influenced pancreatic injury by stimulating the free radical and oxidative stress. Alteration of enzymatic and non-enzymatic antioxidant systems with enhanced lipid peroxidation is the most appearance of oxidative stress in diabetic status^{7, 49}. The MDA increased in the diabetic group, which acts as a biomarker of oxidative stress that is raised in hyperglycemia. The administration of aqueous extract of *Annona muricata*

for four weeks significantly reduced the level of MDA, that caused by the *Annona muricata* has protective activity against oxidative stress^{25, 41}. The antioxidant activity of *Annona muricata* due to contains flavonoids, which reduce the MDA level either directly through neutralizing the toxicity of free radicals by donating hydrogen ions or indirectly through elevating the endogenous antioxidant gene expression²⁶. Furthermore, the protective effect and antioxidant properties of *Annona Muricata* were observed in previous studies that highly agreeing with the results of the current study^{7, 20}.

5. CONCLUSION:

Based on the results of this study, the aqueous extracts of *Annona muricata* have anti-diabetic action through their hypoglycemic, hypolipidemic, renal protective, and antioxidant effects in streptozotocin-induced diabetic rats. So this plant is widely presented with low cost in a different geographical area. Thus, can be used alone or with anti-hyperglycemic drugs as metformin in the management of DM. The combination is preferred in severe hyperglycemia cases with more hypoglycemic effect requirements. These results indicate that need further studies on the beneficial effect of a combination of *Annona muricata* with hypoglycemic drugs in controlling DM.

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