



## Exploration of Anti-Hyperglycemic and Hypolipidemic Activities of Ethanolic Extract of *Annona muricata* Bark in Alloxan Induced Diabetic Rats

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### ABSTRACT

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for diabetes mellitus. The aim of present study was to demonstrate the hypoglycemic and anti-diabetic activity of the Ethanolic extract of *Annona muricata* stem bark in alloxan induced diabetic animals with a view to explore its use for the treatment of diabetes mellitus in humans. The Ethanolic extract of *Annona muricata* (*Annonaceae*) stem was investigated for its anti-hyperglycemic and anti-hyperlipidemic effects in male albino rats. Diabetes was induced in the albino rats by administration of a single dose of alloxan monohydrate (150 mg/kg, bwt, i.p) and the Ethanolic bark extract of *A. muricata* was administered daily at single doses of 150 and 300 mg/kg, p. o to diabetes-induced rats for a period of 14 days. The effect of Ethanolic extract of *A. muricata* bark on blood glucose level was measured in the diabetic rats. Serum lipid profiles [total cholesterol, triglycerides, phospholipids (low density, very low density and high density lipoprotein)] were also determined. The activities were also compared to the activity produced by a standard anti diabetic agent, Glibenclamide (500 µg/kg). The present investigation established pharmacological evidence to support the folklore claim that Ethanolic extract of *Annona muricata* stem bark is an anti-diabetic agent.

**Keywords:** Alloxan, *Annona muricata*, Glibenclamide, Hyperglycemia.

### INTRODUCTION

Diabetes mellitus is a metabolic syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting in impaired glucose metabolism and other energy-yielding fuels such as lipids and protein.<sup>1</sup> Dyslipidemia is a frequent complication of DM and is characterized by low levels of high density lipoprotein-cholesterol (HDL-C) and high levels of low density lipoprotein-cholesterol (LDLC) and triglyceride (TG). Several groups of hypoglycemic drugs are currently available to treat DM<sup>2</sup>. Different types of oral hypoglycemic agents such as biguanides and sulphonylureas are available along with insulin for the treatment of diabetes mellitus, but have side effects associated with their uses<sup>3</sup>. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low costs.

*Annona muricata* is a plant, which belongs to the family Annonaceae. It is a medicinal plant that has been used as a natural remedy for a variety of illnesses. Several studies by different researchers demonstrated that the bark as well as the leaves had anti-hypertensive, vasodilator, anti-spasmodic (smooth muscle relaxant) and cardio depressant (slowing of heart rate) activities in animals<sup>4</sup>.

*Annona muricata* is also known as guayabano, lakshmanaphalam. The bark, leaves and roots are considered sedative, anti spasmodic, hypoglycemic, hypotensive, smooth muscle relaxant and nervine<sup>5</sup>. Bark has anti fungal properties. Stem bark yielded one acetogenin, solamin, and two triterpenoids, stigmaterol & sitosterol<sup>6</sup>.

*A. muricata* has a long history of usage in herbal medicine in the tropical areas of South and North America, as well as in West Africa, especially in Western Nigeria. Although all the morphological parts of the plant have been claimed to be useful in traditional medicine, no scientific studies have been carried out to establish the hypolipidemic and antioxidant effects on the stem bark. Therefore, the present study was undertaken to investigate the hypoglycemic, hypolipidemic properties of *Annona muricata* stem bark alcoholic extract in alloxan induced diabetic rats.

### MATERIALS AND METHODS

#### Plant materials

The stem bark of *Annona muricata* was collected during December- February 2013 from Jalluru, East Godavari Dist, Andhrapradesh. The stem bark of *Annona muricata* was identified and authenticated by the Taxonomist Dr. T. V. Raghavarao, Maharani College, Peddapuram.

#### Drugs and chemicals

Alloxan monohydrate was procured from LOBA CHEMIE laboratory reagents and fine chemicals, Mumbai. Glibenclamide was gifted sample from TABLETS INDIA PVT LTD; Mumbai. Enzymatic kits for the estimation of lipid profile were obtained from CHEMA DIAGNOSTICA (INDIA).

#### Preparation of plant extract

The freshly collected barks were cleaned from dirt and they were dried under shade and then coarsely powdered manually. The powder was macerated in ethanol for a period of 7 days and then subjected to hot percolation for



8 hrs. Then the solution was filtered, concentrated and then dried.

### Experimental Animals<sup>7</sup>

This study was carried out in healthy, male young adult, albino rats (150-220gms). The animals were housed under standard laboratory conditions of light, temperature and humidity. Before and during the experiment, rats were fed with standard diet. After randomization into various groups and before initiation of experiment the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours ad libitum.

### Experimental Protocol

#### Acute study in normal rats

Animals were divided into 4 groups of 3 rats each.

**Group I:** Rats served as normal-control and received the vehicle (0.5 ml distilled water/day/rat)

**Group II:** Rats (normal) were administered Ethanolic extract of *Annona muricata* bark (150 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

**Group III:** Rats (normal) were administered Ethanolic extract of *Annona muricata* bark (300 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

**Group IV:** Rats (normal) were administered *Glibenclamide* (500µg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

**Blood Sugar Estimation on normal rats:** The normal animals were randomized to the following groups of 3 rats each: group I served as normal control, groups II and III received graded doses of the extract at 150, and 300 mg. kg<sup>-1</sup> bwt respectively by gavages. Group IV received glibenclamide (500µg. kg<sup>-1</sup> bwt). Blood samples were collected by tail vein puncture just prior to drug administration i. e. at 0 hr and at 1, 2, 4, and 6 hrs. The blood glucose was estimated by Accu check glucometer<sup>8</sup>.

#### Acute study in diabetic rats

Animals were divided into 4 groups of 3 rats each.

**Group I:** Rats served as diabetic-control and received the vehicle (0.5 ml distilled water/day/rat)

**Group II:** Rats (diabetic) were administered Ethanolic extract of *Annona muricata* bark (150 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

**Group III:** Rats (diabetic) were administered Ethanolic extract of *Annona muricata* bark (300 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

**Group IV:** Rats (diabetic) were administered *Glibenclamide* (500µg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

**Induction of Diabetes in Experimental Animals:** Rats were fasted for 16 hours and were induced with alloxan monohydrate, 150 mg/kg body weights (bwt), intraperitoneally (ip)<sup>9</sup>. Hyperglycaemia was confirmed when elevated blood glucose level was ≥200 mg. dL<sup>-1</sup> after 72 hours of injection<sup>10</sup>.

**Blood sugar estimation on diabetic rats:** The diabetic animals were randomized to the following groups of 3 rats each: group I served as diabetic control, groups II and III received graded doses of the extract at 150, and 300 mg. kg<sup>-1</sup> bwt respectively by gavages. Group IV received glibenclamide (500µg. kg<sup>-1</sup> bwt). Blood samples were collected by tail vein puncture just prior to drug administration i. e. at 0 hr and at 1, 2, 4, and 6 hrs. The blood glucose was estimated by Accu check glucometer<sup>11</sup>.

#### Chronic study in diabetic rats

**Group I:** Rats served as normal-control and received the vehicle (0.5 ml distilled water/day/rat)

**Group II:** Rats served as diabetic-control and received the vehicle (0.5 ml distilled water/day/rat)

**Group III:** Rats (Diabetic) were administered Ethanolic extract of *Annona muricata* bark (150 mg/kg b. wts. /day) in distilled water as a fine aqueous suspension orally.

**Group IV:** Rats (Diabetic) were administered Ethanolic extract of *Annona muricata* bark (300 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

**Group V:** Rats (diabetic) were administered *Glibenclamide* (500µg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

All the rats were fasted for 16 hr. Before experimentation, but allowed free access to water.

**Chronic Study<sup>11</sup>:** All the rats received treatment for 14 days in all groups.

**A. Body weight:** Body weight was measured at the time of alloxan-dosing. After 5 days of alloxan-dosing, the body weight of all the rats was measured once a week for 14 days treatment.

**B. Collection of Blood Sample and Blood Glucose Determination:** The diabetic animals were randomized to the following groups of 3 rats each: group I served as normal while group II was diabetic control, groups III and IV received graded doses of the extract at 150, and 300 mg. kg<sup>-1</sup> bwt respectively by gavages. Group V received Glibenclamide (500µg. kg<sup>-1</sup> b.wt). Blood samples were drawn from tail tip of rat at weekly intervals till the end of study (i. e., 2 weeks). Fasting blood glucose estimation and body weight measurement were done on day 0, 7, and 14 of the study. Blood glucose estimation can be done by Accu check glucometer using glucose test strips. The results were expressed in terms of mg/dl of blood.

Percentage change in body weight =

[Weight<sub>initial</sub>: measurement on the first day (D0);



Weight  $n$ ; measurements at end of D2, D4... D14] <sup>12</sup>

### Estimation of biochemical parameters

On day 14, Serum cholesterol<sup>13</sup> and triglycerides<sup>14</sup> were estimated on final day of experiment of each model by CHOD –POD method and enzymatic colorimetric method (GPO which is highly influenced by level of fasting). HDL<sup>15</sup> cholesterol was determined by using LDLC<sup>16</sup> was derived from cholesterol and triglycerides values, VLDL cholesterol value was derived from cholesterol and triglycerides value was derived from cholesterol and HDL values.

### Statistical analysis

All values were expressed as mean  $\pm$  standard error of mean and the statistical significance between treated and control groups were analyzed by means of Student's t-test.  $P < 0.05$  was considered significant.

## RESULTS

### Acute study

#### 1. Effect of Ethanolic AMBE on fasting blood glucose in normal rats

The results of effect of oral administration of the plant extract on normal rats were shown in Table 1 and figure 1. 150 mg/kg b.wt dose of *Annona muricata* bark did not cause any significant change in blood sugar levels. But 300 mg/kg b. wt dose of *Annona muricata* bark shows significant change in blood sugar levels.

#### 2. Effect of Ethanolic ambe on fasting blood glucose in alloxan induced diabetic rats

The results of effect oral administration of the plant extract on diabetic rats were shown in Table 2 and figure 2. 150 and 300 mg/kg b. wt dose of Ethanolic *Annona muricata* bark extract exhibited significant change in blood sugar levels.

### Chronic study

#### 1. Effect on Body weight

The body weight changes of diabetic animals treated and the untreated is indicated in Figure 3. Animals exhibited decrease in appetite and weight depreciation after alloxan induction. In the untreated group, progressive weight decrease occurred while in the extract/Glibenclamide treated, there was weight appreciation after few days of treatment as well as showed increase in appetite.

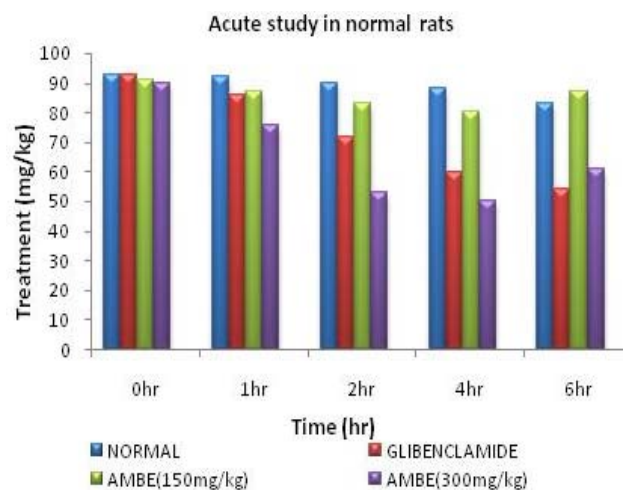
#### 2. Effect on Fasting Blood Glucose (FBG) Levels

The Table 3 and figure 4 demonstrate the levels of FBG in alloxan induced diabetic rats. The administration of both doses of Ethanolic extract of *Annona muricata* bark to diabetic rats resulted in a significant decrease in the levels of fasting blood glucose. In Ethanolic extract of *Annona muricata* bark treated rats, although a significant anti-hyperglycemic effect was evident from the 7 day onwards; the decrease in FBG was highly pronounced on

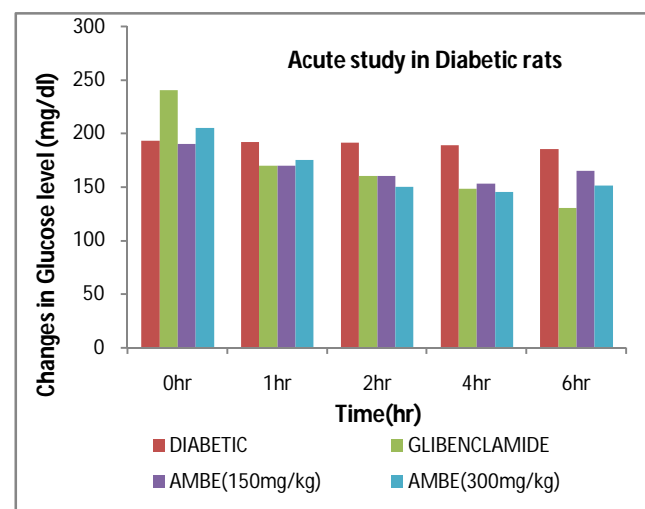
15 day and moved towards resettlement to the normal level.

### Effect of Ethanolic Bark Extract of *Annona Muricata* on the Serum Lipid Profile

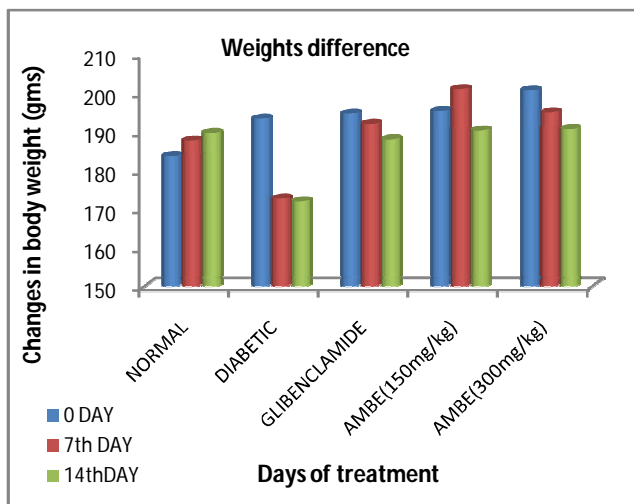
Tables 4 illustrate the effects of Ethanolic extract of *Annona muricata* bark on the levels of total cholesterol, triglycerides, HDLC, LDLC, VLDLC in the serum of experimentally induced diabetic rats. The levels of total cholesterol, triglycerides LDL-C and VLDL-C were significantly ( $p < 0.05$ ) increased in diabetic rats whereas the level of HDL-C were significantly ( $p < 0.05$ ) reduced in diabetic rats when compared to the control normal rats. Administration of Ethanolic extract of *Annona muricata* bark to ALLOXAN induced diabetic rats restored all these changes to near normal levels by significant ( $p < 0.05$ ) reduction of the level of total cholesterol, triglycerides, LDLC and VLDLC of diabetic rats and significant increase in the level of HDL-C.



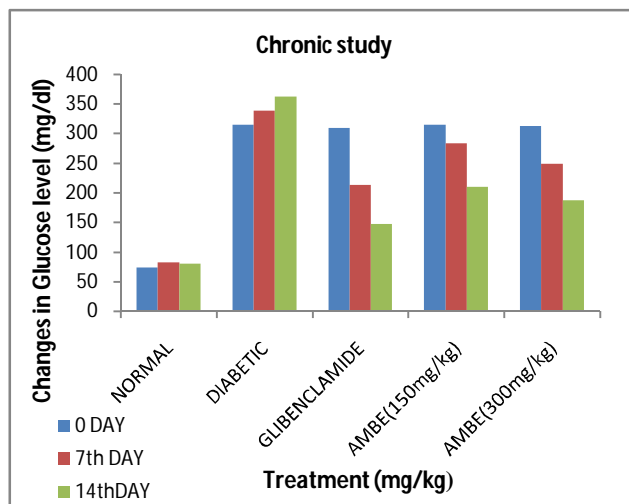
**Figure 1:** Variation in blood glucose levels after oral administration of Ethanolic bark extract of *A. muricata* in normal rats in acute study.



**Figure 2:** Variation in blood glucose levels after oral administration of Ethanolic bark extract of *A. muricata* in alloxan-induced diabetic rats in acute study.



**Figure 3:** Effects of treatment with Ethanolic bark extract of *A. muricata* on body weight in rats.



**Figure 4:** Variation in blood glucose levels after oral administration of Ethanolic bark extract of *A. muricata* in alloxan induced diabetic rats in chronic study.

**Table 1:** Variation in blood glucose levels after oral administration of Ethanolic bark extract of *A. muricata* in normal rats in acute study.

Treatment (mg/kg)	Changes in Glucose level (mg/dl)				
	0hr	1hr	2hr	4hr	6hr
Group I: Normal (control)	93.9±1.6	92±1.5	90.4±1.2	88±1.2	83±1.26
Group II: AMBE (150 mg/kg b.w)	91±11.0	87±8.5	83.5±10.5*	80±8.2**	87±2.0
Group III: AMBE (300 mg/kg b.w)	90±6.0	76±2.0*	53.6±8.5**	50±7.2***	61.5±6
Group IV: Glibenclamide (500 µg/kg )	93±1.2	86±2.7	72±7.6*	60±7.8**	54±8.2***

n = 3 rats in each group; Values of BGL are given in mean ± S.E.M; \*P < 0.001 when compared with control (no drug) animals.

**Table 2:** Variation in blood glucose levels after oral administration of Ethanolic bark extract of *A. muricata* in alloxan-induced diabetic rats in acute study.

Treatment (mg/kg)	Changes in Glucose level (mg/dl)				
	0hr	1hr	2hr	4hr	6hr
Group I: Normal (control)	193.33±5.7	192±6.3	191±6.2	189±5.9	185±5.7
Group II: AMBE (150 mg/kg b.w)	190.1±5.6	170±4.9*	160±5.0**	153±6.2***	165±2.6**
Group III: AMBE (300 mg/kg b.w)	205.1±1.9	175±2.0*	150±2.4**	145±3.2***	151±2.6**
Group IV: Glibenclamide (500 µg/kg )	240.12±1.5	170±2.4*	160±2.1**	148±0.5***	130±0.8***

n = 3 rats in each group; Values of BGL are given in mean ± S.E.M; \*P < 0.001 when compared with control (no drug) animals.

**Table 3:** Effects of Ethanolic bark extract of *A. muricata* on Fasting blood glucose (FBG) in alloxan induced diabetic rats in chronic study.

Treatment (mg/kg)	Changes in Glucose level (mg/dl)		
	Initial day	7 <sup>th</sup> day	14 <sup>th</sup> day
Group I: Normal (control)	74.00 ± 1.00	83.33±4.16	81.00±1.00
Group II: Diabetic (control)	315.00±5.00	339.00±9.00	362.33±5.69
Group III: AMBE (150 mg/kg b.w)	315.33±3.51	283.33±5.13	210.33±4.51
Group IV: AMBE (300 mg/kg b.w)	312.67±2.52	249.0±7.57	187.33±6.43
Group V: Glibenclamide (500 µg/kg )	309.33±3.06	213.00±3.61	148.0 ± 1.0

n = 3 rats in each group; Values of BGL are given in mean ± S.E.M; \*P < 0.001 when compared with control (no drug) animals.



**Table 4:** Effect of Ethanolic bark extract of *A. muricata* on biochemical profiles of the control and treated animals in the chronic study

Treatment (mg/kg)	Changes in lipid parameters(mg/dl)				
	TC	TG	HDL	LDL	V LDL
Group I: Normal (control)	76.16 ± 2.39	62.30 ± 2.14	43.50 ± 2.52	16.33 ± 1.33	14.83 ± 1.83
Group II: Diabetic (control)	94.33 ± 2.38	96.16 ± 2.82	30.3 ± 1.96*	21.5 ± 1.22*	21.1 ± 3.42*
Group III: Glibenclamide (500 µg/kg)	80.33 ± 2.59**	72 ± 2.59**	50.6 ± 1.45**	20 ± 1.56**	14.8 ± 2.16*
Group IV: AMBE (150 mg/kg b.w)	95.33 ± 3.79	83.67 ± 3.2	58.0 ± 2.0	20.59 ± 1.43	16.73 ± 3.69
Group VI: AMBE (300 mg/kg b.w)	89.00 ± 3.0	77.33 ± 5.03	66.0 ± 4.0	15.46 ± 1.20	14.36 ± 2.61

n = 3 rats in each group; Values are given in mean ± S.E.M; \*P < 0.001 when compared with control (no drug) animals.

## DISCUSSION

Diabetes mellitus is a major global health problem, which is becoming an epidemic. Diabetes mellitus is associated with profound alteration in the serum lipid and lipoprotein profile with an increased risk in coronary heart disease<sup>17</sup>. It is a metabolic disease characterized by high-blood glucose levels resulting from defects in insulin secretion, insulin action, or both<sup>18</sup>. Among many forms of Diabetes mellitus, Type II (Non -Insulin -Dependent Diabetes mellitus) occurs predominantly and affects major population, i. e 90% of diabetic patients<sup>19</sup>. Increased triglycerides and reduced HDL-C levels are the key characteristics of dyslipidemia in type 2 diabetes<sup>20</sup>. Hypertriglyceridemia in type 2 diabetes can result from an increased hepatic very low-density lipoprotein (VLDL), overproduction and impaired catabolism of triglyceride rich particles. The function of lipoprotein lipase, the key enzyme in removal and degradation of triglycerides is attenuated by both insulin deprivation and insulin resistance.

For various reasons, in recent years, the popularity of alternative medicine has increased. Surveys conducted in Australia and the United States indicate that almost 48.5% and 34% of the respondents, respectively, had used at least one form of unconventional therapy, including herbal medicine<sup>21</sup>. The WHO Expert Committee on Diabetes has also recommended further evaluation of the folkloric methods of managing this disease because of the high mortality and morbidity arising from its attendant complications and problems associated with the use of conventional anti-diabetic agents<sup>22</sup>. This has led to the increasing demand for herbal products with anti-diabetic activity and fewer side effects<sup>23</sup>.

In modern medicine, the beneficial effects of standard medications on glycemic levels are well documented; the preventive activity of these medications against the progressive nature of diabetes and its complications produce reasonable result but they are not always effective. Insulin therapy affords glycemic control in type 1 diabetes, yet its shortcomings, such as ineffectiveness on oral administration, short shelf life, the requirement of constant refrigeration, fatal hypoglycemia in the event of excess dosage, and the reluctance of patients to take insulin injections, and above all the resistance due to

prolonged administration, limit its usage<sup>24</sup>. Similarly, treatment of type 2 diabetes patients with sulfonylureas and biguanides is always associated with side effects<sup>25</sup>. Hence the search for a drug with low cost, greater potential and no adverse side effects is underway in several laboratories around the world.

Medicinal plants have been used for centuries in the treatment of diabetes mellitus. The need to evaluate the toxicity profile of Ethanolic *Annona muricata* bark extract (AMBE) was prompted by the increasing awareness and interest in medicinal plants and their preparations commonly known as herbal medicine.

In oral acute toxicity studies, no untoward clinical signs were observed in the rats. The extract was safe up to a dose of 5000 mg/kg<sup>26</sup>.

In oral acute studies, no untoward clinical signs were observed in the rats at two doses studied (150 and 300 mg/kg). There were no changes in the nature of stool, urine and eye colour. No mortality was observed at two dose levels from the critical 24 hours post administration to the end of the fourteen day.

In chronic studies, all rats used for the study appeared normal before, during and post-treatment. Mortality was not recorded at two dose levels used for the study; 150, and 300 mg/kg b. wt. The results of the effect of the extract on the body weight of the animals compared with vehicle are as shown in Figure 3.

The result of this present study clearly shows that Ethanolic bark extract of *A. muricata* has a lipid lowering effects on serum triglycerides, total cholesterol and low-density lipoprotein cholesterol of Alloxan induced diabetic rats. Ethanolic bark extract of *A. muricata* treatment also increase the serum level of High-density lipoprotein cholesterol termed as "good cholesterol". There is a substantial evidence that lowering the total cholesterol, particularly LDL-C level will lead to a reduction in the incidence of coronary heart disease which is still the leading cause of death in diabetic patients.

Hypercholesterolemia and Hypertriglyceridemia have been reported to occur in diabetic rats<sup>27</sup> and significant increase in total cholesterol and triglycerides observed in the present experiment was in accordance to these

studies. Furthermore, increase in circulatory VLDL and their associated triglycerides are largely due to defective clearance of these particles from circulation<sup>28</sup>. The increase and fall in the individual lipoprotein levels is a reflection of the serum total cholesterol levels i.e. The levels of HDL-C, LDL-C and VLDL-C increase or decrease with the levels of total serum cholesterol, and it is their ratio that determines the pathophysiology of lipoprotein metabolism<sup>29</sup>. As there is a close relationship between elevated serums total cholesterol level and occurrence of atherosclerosis, the ability of the Ethanolic bark extract of *A. muricata* in the selective reduction of total cholesterol through the reduction of LDL and VLDL components could be beneficial in preventing atherosclerotic conditions and thereby reduce the possibilities of coronary heart disease in general. Considering the effect of extract of Ethanolic bark extract of *A. muricata* on serum HDL, the result of this study clearly show that the level of this lipoprotein fraction increased with this treatment.

Some phytochemical compounds such as polysaccharides<sup>30</sup>, terpenes, tannins<sup>31</sup>, steroids<sup>32</sup>, and alkaloids<sup>33</sup> have been implicated in the anti-diabetic activities of plants.

## CONCLUSION

Currently, insulin and synthetic oral hypoglycemic agents like sulfonylureas and biguanides are the major players in management of Diabetes mellitus. Despite the availability of synthetic drugs, there is an ever-increasing demand of anti-diabetic herbal options. Through present work, Ethanolic bark extract of *A. muricata* seems to be useful in controlling elevated blood glucose levels in diabetes induced by alloxan in rats. And also lowers hypertriglyceridemia and hypercholesterolemia in alloxan-induced diabetic rats. These results indicate that it is worth undertaking further studies on possible usefulness of the Ethanolic extract of the bark of *A. muricata* in diabetes mellitus.

## REFERENCES

1. El-Soud NHA, Khalil MY, Hussein JS, Oraby FSH, Farrag FAR. Anti-diabetic effects of fenugreek alkaloid extract in streptozotocin induced hyperglycemic rats. *J Appl Sci Res*, 3, 2007, 1073-1083.
2. D. O. Adeyemi, O. A. Komolafe, S. O. Adewole, E. M. Obuotor: Anti Hyperlipidemic Activities of *Annona Muricata* (Linn). *The Internet Journal of Alternative Medicine*. 7(1), 2009, DOI: 10. 5580/293b.
3. Rajesh Kumar Gupta, Achyut Narayan Kesari, Geeta Watal, P. S. Murthy, Ramesh Chandra, Kapil Maithal and Vibha Tandon, Hypoglycaemic and antidiabetic effect of aqueous extract of leaves of *Annona squamosa* (L.) in experimental animal, *CURRENT SCIENCE*, 88, 25 APRIL 2005, 1244.
4. D. O. Adeyemi, O. A. Komolafe, S. O. Adewole, E. M. Obuotor: Anti Hyperlipidemic Activities of *Annona Muricata* (Linn). *The Internet Journal of Alternative Medicine*. Volume 7, Number 1, 2009, DOI: 10. 5580/293b.
5. Holdsworth D. K "Traditional medicinal plants of Rarotonga, cook islands". Part 1Int J. crude drug, 28(3), 1990, 209-218.
6. B. Ahalya, K. Ravishankar and P. PriyaBandhavi, EVALUATION OF IN VITRO ANTI OXIDANT ACTIVITY OF *ANNONA MURICATA* BARK, *IJCBS*, 3(2), 2013, 406-410.
7. Mohammed Fazil Ahmed, Syed Mohammed Kazim, Syed Safiullah Ghorri, Syeda Sughra Mehjabeen, Shaik Rasheed Ahmed, Shaik Mehboob Ali, and Mohammed Ibrahim. Antidiabetic Activity of *Vinca rosea* Extracts in Alloxan-Induced Diabetic Rats, *International Journal of Endocrinology*, 2010, Article ID 841090, 6 pages doi:10.1155/2010/841090.
8. Hemant Pareek, Sameer Sharma, Balvant S Khajja, Kusum Jain and GC Jain, Evaluation of hypoglycemic and anti-hyperglycemic potential of *Tridax procumbens* (Linn.), *BMC Complementary and Alternative Medicine* 2009, doi:10.1186/1472-6882-9-48.
9. MBAKA, GO. , ADEYEMI, OO. , ANUNOBI, CC., NORONHA, CC. And OKANLAWON, OA. Anti- hyperglycemic effects of ethanol leaf extract of *Sphenocentrum jollyanum* in normal and alloxan induced diabetic rabbits, *Global Journal of Pharmacology*, 2, 2008, 46-51.
10. MBAKA, GO. , ADEYEMI, OO. , NORONHA, CC., ANUNOBI, CC. And OKANLAWON, AO. Anti-hyperglycemic and hypoglycemic effects of ethanol root extract of *Sphenocentrum jollyanum* in normal and alloxan-induced diabetic rabbits. *Brazilian Journal of Morphological Sciences*, 26, 2009, 123 -127.
11. Mbaka, GO. 1, Ogonnia, SO.2 and Banjo, AE. 3, Activity of *Raphia hookeri* root extract on blood glucose, lipid profile and glycosylated haemoglobin on alloxan induced diabetic rats, *J. Morphol. Sci.*, 29, 2012, 214-222.
12. Arthur, F. K. N. ,a Woode, E. ,b Terlabi, E. O. a and Larbie, C. a\* Evaluation of acute and subchronic toxicity of *Annona Muricata* (Linn. ) aqueous extract in animals *European Journal of Experimental Biology*, 1 (4), 2011,115-124.
13. P. Roeschlau, E. Bernt, and W. Gruber, "Enzymatic determination of total cholesterol in serum," *Zeitschrift für klinische Chemie und Klinische Biochemie*, 12, 1974, p. 226.
14. P. H. Mueller, R. M. Schmuelling, and H. M. Liebich, "A fully enzymatic triglyceride determination," *Journal of Clinical Chemistry and Clinical Biochemistry*, 15, 1977, 457-464.
15. C. C. Allain, L. S. Poon, and C. S. G. Chan, "Enzymatic determination of total serum cholesterol," *Clinical Chemistry*, 20, 1974, 470-475.
16. W. T. Friedewald, R. I. Levy, and D. S. Fredrickson, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge," *Clinical Chemistry*, 18, 1972, 499-502.
17. D. O. Adeyemi, O. A. Komolafe, S. O. Adewole, E. M. Obuotor: Anti Hyperlipidemic Activities of *Annona Muricata* (Linn). *The Internet Journal of Alternative Medicine*. 7, 2009, DOI: 10. 5580 / 293b.
18. D. O. Adeyemi<sup>1</sup>, O. A. Komolafe<sup>1</sup>, O. S. Adewole<sup>1</sup>, E. M. Obuotor<sup>2</sup>, A. A. Abiodun<sup>1</sup>, T. K. Adenowo<sup>3</sup>, Histomorphological and morphometric studies of the pancreatic islet cells of diabetic rats treated with extracts of *Annona muricata*. *Folia Morphol.* 69, No. 2, 92-100.

19. Urmila C. Kumavat, Shraddha N. Shimpi,<sup>1</sup> and Sandesh P. Jagdale, Hypoglycemic activity of *Cassia javanica* Linn. In normal and streptozotocin-induced diabetic rats *Journal of Advanced pharmaceutical Technology and Research*, 3(1), 2012, 47–51.
20. Lehto S, Haffner SM, Pyörälä K, Kallio V, Laakso M. Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with NIDDM; *Diabetes* 1997; 46, 1354–1359.
21. Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL Unconventional medicine in the United States. Prevalence, costs, and patterns of use. *N Engl J Med*, 328, 1993, 246–252.
22. Chattopadhyay RR A comparative evaluation of some blood sugar lowering agents of plant origin. *J Ethnopharmacol*, 67, 1999, 367–372.
23. Kim JD, Kang SM, Park MY, Jung TY, Choi HY, Ku SK, Ameliorative antidiabetic activity of Dangnyosoko, a Chinese herbal medicine in diabetic rats. *Biosci Biotechnol Biochem*, 71, 2007, 1527–1534.
24. Kasiviswanath R, Ramesh A, Kumar KE Hypoglycemic and antihyperglycemic effect of *Gmelina asiatica* LINN in normal and in alloxan induced diabetic rats. *Biol Pharm Bull*, 28, 2005, 729–732.
25. Gandhipuram PSK, Palanisamy A, Durairaj SK, Sorimuthu PS, Anti diabetic activity of fruits of *Terminalia chebula* on streptozotocin-induced diabetic rats. *J Health Sciences*, 52, 2006, 283–291.
26. Arthur, F.K.N., Woode, E., Terlabi, E.O. and Larbie, C., Evaluation of acute and subchronic toxicity of *Annona muricata* (Linn.) aqueous extract in animals, *Euro. J. Exp. Bio.*, 1(4), 2011, 115-124.
27. Chakrabarti S, Biswas TK, Rokeya B, Ali L, Mosihuzzaman M, Nahar N, Azad-Khan AK. Mukherjee B. Advanced studies on the hypoglycemic effect of *Caesalpinia bonducella* F. in type 1 and 2 diabetes in long evens rats"; *J. Ethnopharmacol*, 84, 2003, 41–46.
28. Segal P, Bachorik PS, Rifkind BM, Levy RI. Lipids and lipoproteinemia; in *Clinical diagnosis and management by laboratory methods* (ed.) Henry, J.B. (Philadelphia: W B Saunders), 1984, 180–203.
29. Anti Hyperlipidemic Activities of *Annona muricata* (Linn) D.O. Adeyemi, O.A. Komolafe, S.O. Adewole, E.M. Obuotor: Anti Hyperlipidemic Activities Of *Annona muricata* (Linn). *The Internet Journal of Alternative Medicine*. 7, 2009, DOI: 10.5580/293b.
30. Tomoda, M., Shimada, K., Konno, C., Hikino, H. Structure of Panaxan B, A hypoglycaemic glycan of *Panax ginseng* roots. *Phytochemistry*, 24, 1985, 2431-2433.
31. Reher G., Slijepcevic M., Krans L., Hypoglycaemic activity of triterpenes and tannins from *Sarcopoterium spinosum* and two *Sanguisorba* species. *Planta. Med.* 57, 1991, A57-A58.
32. Karawya MS, Wahab SA., Diphenylamine, an antihyperglycaemic agent from onion and tea. *J. Nat. Prod.* 47, 1984, 775-780.
33. Mohammed Fazil Ahmed, Syed Mohammed Kazim, Syed Safiullah Ghorri, Syeda Sughra Mehjabeen, Shaik Rasheed Ahmed, Shaik Mehboob Ali, and Mohammed Ibrahim. Antidiabetic Activity of *Vinca rosea* Extracts in Alloxan-Induced Diabetic Rats, *International Journal of Endocrinology*, Volume 2010 (2010), Article ID 841090, 6 pages doi:10.1155/2010/841090.

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