IN-VIVO, ANTI-HYPERGLYCEMIC AND ANTI-HYPERLIPIDEMIC ACTIVITY OF ANNONA SQUAMOSA (Linn.) LEAVES, COLLECTED FROM SOUTHERN ODISHA

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ABSTRACT
The present study was aimed to evaluate the anti-hyperglycemic and anti-hyperlipidemic activity of ethanolic extracts of Annona squamosa (Linn.) leaves (AS), collected from southern Odisha. Alloxan induced, Streptozotocin-Nicotinamide (STZ-NA) induced diabetic models and Triton (WR 1339) induced hyperlipidemic models in rats were used in the said experiment. Treatment with AS (400 mg/kg) to alloxan treated rats for 10 days significantly (p<0.001) attenuated the blood glucose level (141.30 ± 11.56 mg/dl) of test animals when compared to alloxan control (293.16 ± 7.32 mg/dl). Similarly, in STZ-NA induced diabetic model, significant (p<0.001) reduction in blood glucose level (152.61 ± 6.83 mg/dl) in test animals were observed, following the treatment of AS (400 mg/kg) for 28 days. In Triton (WR 1339) induced hyperlipidemia (48 hour model), AS (400 mg/kg) treatment exhibited significant (p<0.001) attenuation in serum total cholesterol (70.92 ± 3.46 mg/dl), triglyceride (125.67 ± 6.54 mg/dl) and LDL (10.42 ± 6.77 mg/dl) with significant (p<0.05) elevation in serum HDL (35.36 ± 5.32 mg/dl) levels. Statistical significance of data was assessed by One-way ANOVA followed with Tukey-Kramer Multiple comparison between different groups.
The data obtained from the study suggested, the anti-diabetic and lipid lowering activities of Annona squamosa (Linn.) leaves.
KEYWORDS: antidiabetic, anti-hyperlipidemic, custard apple.

INTRODUCTION
There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are used widely, even when their biological active compounds are unknown. The World Health Organization (WHO) approves the use of natural drugs for different diseases, including diabetes mellitus. Therefore, it is useful to know the efficacy, safety, dosage and mechanism of action of these herbal drugs.

Diabetic dyslipidemia is a well-recognized manifestation of uncontrolled diabetes mellitus. It is due to the fact that, insulin has important regulatory effects on lipid metabolism. Diabetes mellitus (DM) is characterized by increased fasting and post prandial blood sugar levels, associated with significant abnormalities in lipoprotein metabolism resulting from either insulin insufficiency or dysfunction [1]. DM associated with dyslipidemia elevates the risk of cardiovascular diseases and patients with diabetes are at increased risk for all the manifestations like atherosclerosis, coronary artery disease, cerebrovascular events, hypertension and peripheral vascular diseases [2].

The treatment of DM is based on oral hypoglycemic agents and insulin. However, DM is also treated in Indian traditional medicine using anti-diabetic medicinal plants [3]. However, there is a constant need of newer herbal anti-diabetic medications with pronounced therapeutic effectiveness and comparatively lower side effects. In this regard, Annona squamosa (Linn.) (AS) belonging to the family Annonaceae, commonly known as Sitaphal (local name) custard apple, sugar apple etc. It is native to West-Indies and now cultivated throughout India [4]. AS leaves are used as insecticide, anthelmintic, styptic [5]. Unripe and dried fruit of AS are used as antidysernetic, bark is used as tonic astringent, antidysernetic and vermifuge [5]. Numerous Phytochemical and pharmacological studies have been carried out on AS [6]. Ayurvedic practitioners use stem and leaf extracts of AS as indigenous uterotonic drug [7]. Postcoital antifertility activity (seed extract) [8], cardiotonic activity (leaf extract) [9] and anti-cancer activity (leaf extract) [10] were reported for AS. The present study was aimed to evaluate the antidiabetic and anti hyperglycemic activity of ethanolic extract of leaves of Annona squamosa Linn. in different models of hyperglycemia and hyperlipidemia.
MATERIALS AND METHODS

Chemicals
Alloxan, Triton (WR 1339), Streptozotocin and Nicotinamide were procured from Hi-media laboratories Pvt. Ltd. (India). Atorvastatin (Storvas 20; Ranbaxy Laboratories Ltd., India), Metformin (Glyciphage 500; Franco-Indian Ltd., India) and Gliclazide (Glyred 40; Novartis-Sandoz Ltd., India) were purchased from local market. Total cholesterol, HDL cholesterol Triglycerides and Glucose assay kits were procured from Span Diagnostics Ltd. (India).

Animals
Albino rats of either sex weighing between 150 – 200 gm were used for the experiment. Animals were housed in a group of six in polypropylene cages at controlled room temperature 25 ± 2ºC, relative humidity 55% and 12 hrs. light: dark cycle. They were fed with standard chow diet and water ad libitum during the experiment. The experimental protocols were approved by CPCSEA and cleared by Institutional Animal Ethical Committee (IAEC) at College of Pharmaceutical Sciences, Mohuda, Ganjam, Odisha (IAEC Regd. No: 1170/ac/08/ CPCSEA).

Plant Materials and Preparation of Extracts
Leaves of Annona squamosa (Linn.) were collected from local regions near Mohuda (Berhampur, Odisha, India) in the month of October – November. Plant material was authenticated by Prof. S.K.Dash, Dept. of Biological Sciences, CPS, Mohuda, Berhampur, Odisha, India. Extract was prepared using successive extraction method. After collection, AS leaves were shade dried at room temperature for one week. Dried leaves were crushed to fine powder by using mechanical grinder. Powder was weighed and extracted successively with different solvents. The fraction extracted with taking ethanol (90%) was evaporated at room temperature to obtain test extract. The extract was stored at 2 - 8°C for further uses.

Acute oral toxicity study was performed as per the OECD guidelines \[11\]. Following the oral toxicity study, screening of anti-diabetic and anti-hyperlipidemic activities were carried out at a fixed dose of 200 and 400 mg/kg body weight of AS leaf extract.

Alloxan induced Diabetes
30 rats were divided into five groups each comprising six animals. Diabetes was induced by a single intraperitoneal (i.p) injection of alloxan dissolved in freshly prepared 0.9 % normal saline, at a dose of 160 mg/kg body weight \[12\].
Experimental groups:

**Group 1:** Normal rats, treated with 0.3% w/v Sodium-CMC (5 ml/kg of b.w.; p.o) once daily

**Group 2:** Diabetic control rats (Alloxan 160 mg/kg)

**Group 3:** (Standard group) diabetic rats treated with Metformin (11.3 mg/kg of b.w.; p.o) once daily

**Group 4:** (Test group) diabetic rats treated with AS leaf extract (200 mg/kg of b.w; p.o) once daily

**Group 5:** (Test Group) diabetic rats treated with AS leaf extract (400 mg/kg of b.w; p.o) once daily

The treatment duration was of 10 days [13].

**Streptozotocin Nicotinamide induced Diabetes**

30 rats were divided into five groups each comprising six animals. Diabetes was induced by a single intraperitoneal (i.p) injection of NA (120 mg/kg of b.w) followed with (i.p) injection of STZ (50 mg/kg of b.w) freshly prepared in citrate buffer (pH: 4.5) [14].

The experimental groups:

**Group 1:** Normal rats, treated with 0.3% w/v Sodium-CMC (5 ml/kg of b.w.; p.o) once daily

**Group 2:** Diabetic control rats (NA 120 mg/kg followed STZ 50 mg/kg. b.w; i.p)

**Group 3:** (Standard group) diabetic rats treated with Gliclazide (25 mg/kg of b.w.; p.o) once daily

**Group 4:** (Test group) diabetic rats treated with AS leaf extract (200 mg/kg of b.w; p.o) once daily

**Group 5:** (Test Group) diabetic rats treated with AS leaf extract (400 mg/kg of b.w; p.o) once daily

The treatment duration was of 28 days [15].

**Oral glucose tolerance test (OGTT) in normoglycemic rats**

36 rats were divided into six groups each comprising six animals. Rats were fasted overnight before OGTT.

**Group 1:** Normal rats, treated with (treated with demineralized water 10 ml/kg and glucose 2 gm/kg)

**Group 2:** Vehicle Control rats, treated with 0.5 % w/v Sodium-CMC (5 ml/kg of b.w.; p.o)

**Group 3:** Received Metformin (11.3 mg/kg of b.w. in 0.5% CMC; p.o)

**Group 4:** Received Gliclazide (25 mg/kg of b.w. in 0.5% CMC; p.o)
Group 5: Received *Annona squamosa* Linn. extract (200 mg/kg of b.w in 0.5% CMC; p.o)
Group 6: Received *Annona squamosa* Linn. extract (400 mg/kg of b.w in 0.5% CMC; p.o)

After 30 minutes of said treatments, rats of each group were administered with glucose (5 gm/kg of b.w in distilled water; p.o). Blood samples from each test animals were drawn at 0, 1st, 2nd and 3rd hours and blood glucose level was determined[16].

**Triton Induced Hyperlipidemia**

30 rats were divided into five groups each comprising six animals. Hyperlipidemia was induced by single intraperitoneal (i.p) injection of Triton WR-1339 (200 mg/kg of b.w in 0.5% NaCl solution)[17].

**Group 1:** Normal rats, treated with 0.3% w/v Sodium-CMC (5 ml/kg of b.w.; p.o) once daily
**Group 2:** Lipidemic control rats, treated with Triton WR-1339 (200 mg/kg of b.w, i.p)
**Group 3:** (Standard group) lipidemia induced rats, treated with Atorvastatin (7.2 mg/kg of b.w.; p.o) once daily
**Group 4:** (Test group) lipidemia induced rats, treated with AS leaf extract (200 mg/kg of b.w; p.o) once daily
**Group 5:** (Test Group) lipidemia induced rats, treated with AS leaf extract (400 mg/kg of b.w; p.o) once daily

The treatment duration was of 2 days[17].

Blood glucose (GOD/POD method)[18], total cholesterol, HDL cholesterol, triglyceride levels were estimated by using commercially available biochemical kits in standard procedures[19-20]. Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol were calculated by Friedewald’s formula: VLDL cholesterol = Triglyceride/5 and LDL cholesterol = Total cholesterol – (VLDL + HDL cholesterol)[21].

**STATISTICS**

Results are expressed as mean ± SEM. Statistical significance of data was assessed by One-way ANOVA followed with Tukey-Kramer Multiple comparison between different groups.

**RESULT**

**Alloxan induced Diabetes**

Table 1 constitutes the blood glucose levels and change in body weight of test animals, observed in alloxan induced diabetic model, following the treatment with AS (200 and 400 mg/kg of b.w, p.o) for 10 days. Alloxan treated rats showed significant change in body
weight (-3.5 ± 1.52 gm; p<0.05) and elevation in blood glucose levels in all test days as compared to the normal group, where blood glucose level on 10\textsuperscript{th} day being the highest (293.16 ± 7.32 mg/dl; p<0.001). Rats treated with Metformin (11.3 mg/kg b.w) showed significant weight gain (3.25 ± 1.87 gm; p<0.05) and marked differences in blood sugar levels on 4\textsuperscript{th}, 7\textsuperscript{th} and 10\textsuperscript{th} day, where the value of blood glucose on 10\textsuperscript{th} day being the lowest (86.54 ± 4.09 mg/dl; p<0.001) when compared with alloxan control group. Treatment groups (AS 200 and 400 mg/kg) showed progressive reduction in blood glucose levels. The glucose levels in AS 200 and 400 mg/kg treated rats on 10\textsuperscript{th} day were found to be (179.64 ± 10.34) and (141.30 ± 11.56) mg/dl respectively which are significantly (p<0.001) less than that of alloxan control group.

**Streptozotocin Nicotinamide induced Diabetes**

Table 2 constitutes the blood glucose levels and change in body weight of test animals, observed in STZ-NA induced diabetic model, following the treatment with AS (200 and 400 mg/kg of b.w, p.o) for 28 days. STZ-NA treated rats showed significant (p<0.001) change in body weight (-6.41 ± 1.28 gm) and elevation in blood glucose levels in all test weeks as compared to the normal group, where blood glucose level on 4\textsuperscript{th} week being the highest (376.94 ± 3.04) gm/dl. Rats treated with Gliclazide (25 mg/kg b.w) exhibited significant (p<0.001) weight gain (11.53 ± 3.12) gm and attenuation of blood glucose levels on 2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th} week when compared with STZ-NA control group, where the value of blood glucose on 4\textsuperscript{th} week being the lowest (112.11 ± 10.34) mg/dl. Significant changes in body weights were observed in AS (200 mg/kg), (5.97 ± 1.51 gm; p<0.05) and in AS (400 mg/kg), (9.05 ± 3.44 gm; p<0.01) treatment groups when compared to the STZ-NA group. The glucose levels in AS 200 and 400 mg/kg treated rats on 4\textsuperscript{th} week were found to be 176.94 ± 3.12 and 152.61 ± 6.83 mg/dl respectively which are significantly (p<0.001) less than that of STZ-NA control group.

**Oral glucose tolerance test (OGTT) in normoglycemic rats**

Oral glucose tolerance test (OGTT) data for AS extracts are cited in Table 3. Normal group rats (treated with demineralized water 10 ml/kg and glucose 2 gm/kg) exhibited an elevation in blood glucose level up to 2\textsuperscript{nd} hour (142.36 ± 2.13) mg/dl, followed with a decrease in 3\textsuperscript{rd} hour (117.36 ± 3.03) mg/dl. Treatment with vehicle (0.5% w/v sodium CMC; 5 ml/kg) has no alternating effects on blood glucose levels of test rats. Vehicle control group followed the same increase/ decrease pattern in blood glucose levels as that of normal group. From the
data obtained in Metformin (11.3 mg/kg) and Gliclazide (25 mg/kg) treatment groups, it can be inferred that, the selected reference drugs attenuated blood glucose levels significantly (p<0.001) as compared to the normal and vehicle control group on 3rd hour. Similarly, AS (200 and 400 mg/kg) treatments reduced blood glucose levels in test rats significantly (p<0.001) when compared to normal and vehicle control group on 3rd hour.

**Triton Induced Hyperlipidemia**

Table 4 constitutes the data of effect of AS (200 and 400 mg/kg b.w.) on change in body weight, serum total cholesterol, triglyceride, LDL and HDL levels in Triton-induced hyperlipidemia in rats. Triton-induced hyperlipidemia is prominent in triton treated rats at 48th hour as, serum levels of total cholesterol (122.57 ± 6.35) mg/dl and LDL (69.34 ± 1.59) mg/dl were elevated significantly (p<0.001) with a net weight gain of 7.29 ± 0.54 gm and reduced serum HDL level (18.75± 2.63) mg/dl as compared to the normal rats. Weight gain (2.75 ± 0.82 gm; p<0.01) in Atorvastatin (7.2 mg/kg b.w) treated rats were observed to be significantly less than the Triton-control group. Serum cholesterol (64.17 ± 5.05) mg/dl and LDL (6.06 ± 4.35) mg/dl levels were attenuated significantly (p<0.001) with a elevation in serum HDL level (41.53 ± 4.14) mg/dl in Atorvastatin treated rats as compared to the Triton-control group. Change in body weight (3.04 ± 1.18) gm in AS (400 mg/kg b.w) treated rats were found to be significantly (p<0.01) less as compared to the Triton control group. AS (200 mg/kg) treatment group showed, reduction in serum total cholesterol (103.71 ± 2.63 mg/dl; p<0.05) and LDL (37.58 ± 2.07 mg/dl; p<0.001) levels and elevation in serum HDL (34.86 ± 3.17 mg/dl; p<0.05) level when compared with triton-control group. Similarly, AS (400 mg/kg) treatment group exhibited significant (p<0.001) decrease in serum total cholesterol (70.92 ± 3.46) mg/dl, triglyceride (125.67 ± 6.54) mg/dl and LDL (10.42 ± 6.77) mg/dl levels and elevation in serum HDL (35.36 ± 5.32 mg/dl; p<0.05) level when compared with triton-control group.

**DISCUSSION AND CONCLUSION**

The number of people with diabetes mellitus worldwide is increasing rapidly. Presently, there are more than 150 million people with diagnosed disease and 314 million with impaired glucose tolerance, a prediabetic state[22]. Dyslipidemia, in both type 1 and type 2 diabetes, plays a significant role in the manifestation and development of premature atherosclerosis leading to cardiovascular (CV) disease, and together, they are the major cause of CV morbidity and mortality in diabetic patients[23].
Table 1: Effect of *Annona squamosa* Linn. on Blood glucose levels in alloxan induced diabetic rats:

<table>
<thead>
<tr>
<th>Group</th>
<th>Change in body weight (gm)</th>
<th>Blood glucose level on 0 day (gm/dl)</th>
<th>Blood glucose level on 4th day (gm/dl)</th>
<th>Blood glucose level on 7th day (gm/dl)</th>
<th>Blood glucose level on 10th day (gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4.30±0.36</td>
<td>76.81±3.07</td>
<td>80.50±4.13</td>
<td>77.41±5.64</td>
<td>75.33±3.41</td>
</tr>
<tr>
<td>Alloxan</td>
<td>-3.5±1.52*</td>
<td>268.45±7.93***</td>
<td>281.39±5.96***</td>
<td>290.16±11.32***</td>
<td>293.16±7.32***</td>
</tr>
<tr>
<td>Std (Metformin)</td>
<td>3.25±1.87*</td>
<td>271.33±10.39**</td>
<td>156.95±11.08***</td>
<td>140.00±7.09***</td>
<td>86.54±4.09***</td>
</tr>
<tr>
<td>AS (200 mg/kg)</td>
<td>1.80±2.31**</td>
<td>240.51±9.59**</td>
<td>238.00±13.77*</td>
<td>201.66±11.26***</td>
<td>179.64±10.34***</td>
</tr>
<tr>
<td>AS (400 mg/kg)</td>
<td>2.14±1.32**</td>
<td>262.71±7.34**</td>
<td>214.50±11.41**</td>
<td>182.35±9.38***</td>
<td>141.30±11.56***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6.

* P≤0.05, ** P≤0.01, *** P≤0.001, ns: non-significant.

Vehicle control Vs Alloxan Control, Alloxan Control Vs. all Treatment groups.

Table 2: Effect of *Annona squamosa* Linn. on Blood glucose levels in STZ-NA induced diabetic rats:

<table>
<thead>
<tr>
<th>Group</th>
<th>Change in body weight (gm)</th>
<th>Blood glucose level on 0th week (gm/dl)</th>
<th>Blood glucose level on 2nd week (gm/dl)</th>
<th>Blood glucose level on 3rd week (gm/dl)</th>
<th>Blood glucose level on 4th week (gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>14.23±2.23</td>
<td>77.00±3.89</td>
<td>80.39±2.32***</td>
<td>79.29±2.43</td>
<td>76.94±3.32</td>
</tr>
<tr>
<td>STZ-NA</td>
<td>-6.41±1.28***</td>
<td>287.00±10.38***</td>
<td>302.22±14.89***</td>
<td>347.63±18.46***</td>
<td>376.94±3.04***</td>
</tr>
<tr>
<td>Std (Gliclazide)</td>
<td>11.53±3.12***</td>
<td>292.00±9.72**</td>
<td>180.39±2.02***</td>
<td>174.29±5.35***</td>
<td>112.11±10.34***</td>
</tr>
<tr>
<td>AS (200 mg/kg)</td>
<td>5.97±1.51*</td>
<td>299.50±7.85**</td>
<td>234.39±17.34**</td>
<td>203.46±10.93**</td>
<td>176.94±3.12**</td>
</tr>
<tr>
<td>AS (400 mg/kg)</td>
<td>9.05±3.44**</td>
<td>302.22±14.89**</td>
<td>217.22±12.21**</td>
<td>180.96±3.06**</td>
<td>152.61±6.83**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6.

* P≤0.05, ** P≤0.01, *** P≤0.001, ns: non-significant.

Vehicle control Vs STZ-NA Control, STZ-NA Control Vs. all Treatment groups.
Table 3: Effect of *Annona squamosa* Linn. on Blood glucose levels in oral glucose tolerance test in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose level on 0(^\text{th}) hour (gm/dl)</th>
<th>Blood glucose level on 1(^\text{st}) hour (gm/dl)</th>
<th>Blood glucose level on 2(^\text{nd}) hour (gm/dl)</th>
<th>Blood glucose level on 3(^\text{rd}) hour (gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>72.54±3.19</td>
<td>99.62±5.09</td>
<td>142.36±2.13</td>
<td>117.36±3.03</td>
</tr>
<tr>
<td>Vehicle</td>
<td>79.00±5.41 ns</td>
<td>97.51±3.67 ns</td>
<td>151.04±4.66 ns</td>
<td>124.33±1.51 ns</td>
</tr>
<tr>
<td>Std1 (Metformin)</td>
<td>70.47±9.61 ns, ns</td>
<td>75.35±6.03 b, d</td>
<td>73.95±6.03 c, f</td>
<td>66.41±3.93 c, f</td>
</tr>
<tr>
<td>Std2 (Glicazide)</td>
<td>69.83±3.20 ns, ns</td>
<td>75.41±2.17 b, d</td>
<td>81.55±4.29 c, f</td>
<td>78.35±2.30 c, f</td>
</tr>
<tr>
<td>AS (200 mg/kg)</td>
<td>81.25±7.33 ns, ns, ns</td>
<td>89.55±6.10 ns, ns, ns</td>
<td>80.47±5.73 c, f, ns, ns</td>
<td>85.31±5.07 c, f, *, ns</td>
</tr>
<tr>
<td>AS (400 mg/kg)</td>
<td>75.33±4.01 ns, ns, ns</td>
<td>84.00±2.69 ns, ns, ns</td>
<td>76.31±6.19 c, f, ns, ns</td>
<td>80.51±4.55 c, f, ns, ns</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6.

Normal Vs all groups: a: \( P\leq0.05 \), b: \( P\leq0.01 \), c: \( P\leq0.001 \).

Vehicle Vs Metformin, Glicazide, EEAS (200 mg/kg) and EEAS (400 mg/kg): d: \( P\leq0.05 \), e: \( P\leq0.01 \), f: \( P\leq0.001 \).

Metformin Vs AS (200 mg/kg) and EEAS (400 mg/kg): *: \( P\leq0.05 \), **: \( P\leq0.01 \), ***: \( P\leq0.001 \).

Glicazide Vs AS (200 mg/kg) and EEAS (400 mg/kg): #: \( P\leq0.05 \), #: #: \( P\leq0.01 \), #: #: #: \( P\leq0.001 \).

ns: non-significant.
Table 4: Effect of Annona squamosa Linn. on Triton (WR-1339) induced hyperlipidemic model (data observed at 48th hour)

<table>
<thead>
<tr>
<th>Group</th>
<th>Change in body weight (gm)</th>
<th>Total cholesterol (gm/dl)</th>
<th>Triglyceride (gm/dl)</th>
<th>LDL (gm/dl)</th>
<th>HDL (gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.20 ± 0.57</td>
<td>65.18 ± 3.72</td>
<td>78.51 ± 3.20</td>
<td>9.30±2.48</td>
<td>40.17±2.55</td>
</tr>
<tr>
<td>Triton</td>
<td>7.29 ± 0.54***</td>
<td>122.57 ± 6.35***</td>
<td>172.44 ± 2.54**</td>
<td>69.34±1.59***</td>
<td>18.75±2.63**</td>
</tr>
<tr>
<td>Std (Atorvastatin)</td>
<td>2.75 ± 0.82**</td>
<td>64.17 ± 5.05***</td>
<td>82.86 ± 5.69**</td>
<td>6.06±4.35***</td>
<td>41.53±4.14**</td>
</tr>
<tr>
<td>AS (200 mg/kg)</td>
<td>5.75 ± 0.52 ns</td>
<td>103.71 ± 2.63*</td>
<td>156.31 ± 2.145 ns</td>
<td>37.58±2.07***</td>
<td>34.86±3.17*</td>
</tr>
<tr>
<td>AS (400 mg/kg)</td>
<td>3.04 ± 1.18**</td>
<td>70.92 ± 3.46***</td>
<td>125.67 ± 6.54***</td>
<td>10.42±6.77***</td>
<td>35.36±5.32*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6.

*: P≤0.05, **: P≤0.01, ***: P≤0.001, ns: non-significant.

Vehicle control Vs Triton Control, Triton Control Vs. all Treatment groups.
The present study was an effort to investigate the hypoglycemic and anti-Hyperlipidemic activity of the ethanolic extract of *A. squamosa* leaves in alloxan and STZ-NA induced diabetic animals and Triton induced hyperlipidemic rats. The continuous treatment of the extracts of AS for a period of 10 (in alloxan induced diabetes) and 28 (STZ-NA induced diabetic) days respectively produced a significant decrease in the blood sugar levels of diabetic rats. These results confirmed the use of AS leaves of traditional practice as an anti-diabetic.\[24\] The standard drug, Metformin and Gliclazide has been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic cells\[25\]. It may be suggested that the mechanism of action of AS is similar to gliclazide. The possible mechanism by which AS leaves brings about a decrease in blood sugar level may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β-cells of the islets of Langerhans or its release from the bound form. A number of other plants have been reported to exert hypoglycemic activity through insulin release-stimulatory effects \[26-27\]. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of the hypoglycemic effect of AS leaves. Several researchers have reported that the lipid lowering effect of different plants may be due to mobilization of cholesterol from extra hepatic tissues to the liver where it is catabolised. It was also reported that several plants due to presence of some phytoconstituents such as (glycosides, saponins and steroids) activate the rate limiting step in cholesterol catabolism, that is, cholesterol 7-α-hydroxylase thereby stimulating the conversion of cholesterol to bile acid, an important pathway in the degradation of cholesterol \[28\]. Additionally, the phytochemical screening of AS leaves reveled similar phytoconstituents which may produce significant fall in serum total cholesterol and triglyceride levels, by the above said mechanism, which indicating profound lipid-lowering activity.

The study suggested significant anti-hyperglycemic and anti-Hyperlipidemic activity of ethanolic extract of *Annona squamosa* (Linn.) *in-vivo*.

**REFERENCES**


